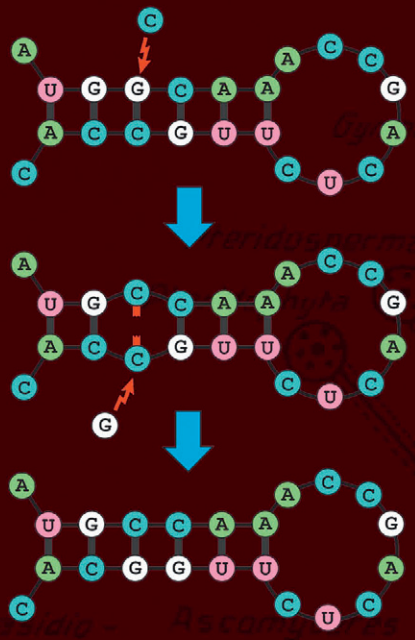

The Encyclopedia of
EVOLUTIONARY BIOLOGY

Edited by
RICHARD M. KLIMAN

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ENCYCLOPEDIA OF
EVOLUTIONARY BIOLOGY

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EDITOR IN CHIEF

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Richard M. Kliman, PhD, is Professor of Biological Sciences at Cedar Crest College in Allentown, Pennsylvania. He received his BA from Colby College in biology and music. His graduate work at Wesleyan University focused on quantitative genetics of circadian rhythms and photoperiodism in the Djungarian hamster, *Phodopus sungorus*. As a postdoctoral fellow at Rutgers University and Harvard University, he studied molecular evolution and population genetics. Prior to Cedar Crest College, he taught at Radford University in Virginia and Kean University in New Jersey. He has also served as a program director in the Division of Environmental Biology at the US National Science Foundation (NSF).

Kliman's research interests center on questions in molecular evolution, including the evolution of codon usage bias in a variety of organisms; speciation and natural history; and ecology and conservation. Much of this work has relied on population genetics/genomics and bioinformatics approaches. He has also collaborated with Cedar Crest colleague John Cigliano on an Earthwatch-supported "before-after-control-impact" study on the effects of a new marine reserve in Belize on queen conch populations. His research in evolutionary and ecological genetics has been supported by the US National Institutes of Health and by Conservation International.

Kliman has served on the editorial boards of *Genetica* and *The Journal of Molecular Evolution*. He has been deeply involved in evolution education, helping to coordinate "Undergraduate Diversity at SSE/SSB," an NSF-supported program to bring a diverse group of undergraduates to the annual Evolution research conference. He was a lead editor of population/quantitative genetics and evolutionary genetics for *Nature Education/Scitable* at its inception. He is a member of the Education and Outreach Committee of the Society for the Study of Evolution, and editor of the society's peer-reviewed educational resource, the *EvoEd Digital Library*.

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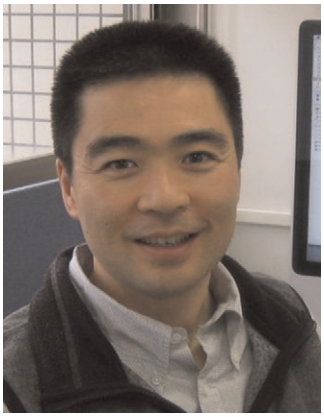


Hiroshi Akashi is a Professor of Evolutionary Genetics at the National Institute of Genetics, Japan. He worked with Marty Kreitman for his PhD in Ecology & Evolutionary Biology from the University of Chicago (1996) and with John Gillespie as a postdoctoral fellow at UC Davis. He has been a faculty member at the University of Kansas (1998–2000), Penn State University (2000–2008), and NIG (2009–present). Akashi's research focuses on inferring causes of genome evolution, especially weak selection, from within and between species sequence variation. His studies of codon usage employed population genetic methods to detect natural selection acting at its limit of efficacy and identified a phenotypic basis of natural selection (translational accuracy) from sequence comparisons in *Drosophila*. Extensions of this work revealed constraints related to biosynthesis that act globally on compositional properties of microbial proteins. The interplay of weak evolutionary forces appears to shift frequently among closely-related species and current interests include tests of adaptive changes in protein/DNA composition.

Tim Coulson's primary interest is in creating better links between the fields of ecology and evolution. He does this by developing theory, parameterising models for field and laboratory systems, making predictions from these models, and, where possible, testing these predictions with experiments. He works on a range of systems, from bulb mites within the laboratory, to guppies living in streams in Trinidad, to wolves in Yellowstone. His motivation to do this comes from observations that ecological and evolutionary change can be observed occurring on similar time scales, yet ecological theory typically ignores evolutionary processes and vice versa.

Tim was awarded his PhD in plant ecology from Imperial College, London, in 1994. He moved on to research genotype-by-environment interactions as Natural Environment Research Council (NERC)-funded post-doc at the Institute of Zoology in London. He remained at the Institute on a fellowship where he developed models to investigate the economic and life history consequences of a range of population management strategies. In 2000 he moved to the University of Cambridge, where he briefly lecturered in the Zoology department. In 2004 he moved back to Imperial College London as a senior lecturer where he started developing models that allow the simultaneous investigation of the dynamics of life history, populations, and quantitative characters. In 2007 he became Professor of Population Biology at Imperial College London. He left Imperial in 2013 to take up his current position as Professor of Zoology at the University of Oxford. He is also a Professorial fellow of Jesus College, Oxford.

Andrew Forbes

Rosemary Gillespie is a Professor at the University of California, Berkeley, where she also holds the Schlinger Chair in Systematics. She is Past President of the *International Biogeography Society* and Trustee and Fellow of the *California Academy of Sciences*, and serves as Associate Editor for *Molecular Ecology*. Gillespie was born and educated in Scotland, receiving her BSc in Zoology from Edinburgh University in 1980. She came to the US to conduct graduate work on the behavioral ecology of spiders at the University of Tennessee. After her PhD she spent several months at the University of South in Tennessee, and then started work at the University of Hawaii in 1987, initially as a postdoc, and then in 1992 as Assistant Professor in Zoology and Researcher in the Hawaiian Evolutionary Biology Program. It was during her first year in Hawaii that she discovered an adaptive radiation of *Tetragnatha* spiders. She left Hawaii in 1999 to join the faculty at the University of California in Berkeley, where she continues her research focus on the islands of the Pacific, Hawaii in particular, using islands of known age and isolation to assess the combined temporal and spatial dimension of biogeography and determine patterns of diversification, adaptive radiation, and associated community assembly.

David Guttman received his PhD from Stony Brook University in 1994 working with Daniel Dykhuizen on questions related to the role and importance of recombination in structuring genetic diversity in bacterial populations. He followed this with a postdoc in molecular evolution with Brian and Deborah Charlesworth at the University of Chicago, and a second postdoc at the University of Chicago with Jean Greenberg to gain experience in the fields of molecular plant pathology and plant-microbe interactions. He started his faculty position at the University of Toronto in 2000, and is currently a Professor in the Department of Cell & Systems Biology (CSB). He is also the Associate Chair for Research in CSB, founder and Director of the University of Toronto Centre for the Analysis of Genome Evolution & Function, and Canada Research Chair in Comparative Genomics. He has served as the Chair of the American Society for Microbiology, Division R (Evolutionary and Genomic Microbiology), and was the *PLoS Pathogens* Section Editor for Bacterial Evolution & Genomics.

Dr. Guttman runs a highly diverse research program generally focused on bacterial evolutionary genomics, with three major foci: (1) the evolution of host specificity and virulence in plant pathogenic bacteria; (2) microbial comparative genomics; and (3) studies of the human and plant-associated microbiome. He is best known for elucidating and linking evolutionary and mechanistic processes that determine the course and fate of bacterial infections, and characterizing the impact of genetic variation on the balance between disease and immunity.

Norman A. Johnson, the section editor for Applied Evolution, is an evolutionary geneticist and author. He received his PhD from the University of Rochester in 1992 and did postdoctoral research at the University of Chicago. His research interests have generally focused on aspects of speciation, specifically those related to the genetics and evolution of hybrid incompatibility: sterility, inviability, or other reduction of fitness in hybrids between species. Dr. Johnson, an adjunct professor in the Biology Department at the University of Massachusetts at Amherst, has taught classes there, as well as at Hampshire College, the University of Texas at Arlington, and the University of Chicago.

Dr. Johnson also has a long-standing commitment toward improving the communication of science in general and evolutionary biology in particular to other scientists, educators, and the public at large. He is the author of *Darwinian Detectives: Revealing the Natural History of Genes and Genomes* (Oxford University Press: 2007), a book geared to general audiences that shows how biologists use DNA sequence data to make inferences about evolutionary processes. He also was the lead organizer for a working group on communicating human evolution at the National Evolutionary Synthesis Center (NESCent).

Laura Kubatko received a PhD in Biostatistics from The Ohio State University (OSU) in 1999. After seven years on the faculty at the University of New Mexico, she returned to OSU in the Fall of 2006, and is now Professor of Statistics and of Evolution, Ecology, and Organismal Biology at OSU. Laura served as an Associate Director of the Mathematical Biosciences Institute at OSU from 2013–2015. At OSU, she is a Faculty Affiliate of the Initiative in Population Research, and a Faculty Affiliate in Translational Data Analytics (TDA@OSU). She holds appointments as an Affiliate Faculty Member at the Battelle Center for Mathematical Medicine at Nationwide Children's Hospital in Columbus and as an Adjunct Research Scientist at Lovelace Respiratory Research Institute in Albuquerque, NM. Laura's research interests are in statistical genetics, with a focus on the development of statistical methods for inferring phylogenies from molecular data. Her recent work in this area concentrates on bridging the gap between traditional phylogenetic techniques and methodology used in population genetics analyses, primarily through the application of coalescent theory to species-level phylogenetic inference. She develops and distributes several software packages for phylogenetic inference, and has been an active member of the *Society of Systematic Biologists*. She has served as an Associate Editor for the journal *Systematic Biology* since 2007.

Amy Litt has been studying plant evolution and diversity since her PhD on floral structure and evolution in the neotropical plant family Vochysiaceae, known for its beautiful but unusual flowers many of which have only one petal and one stamen. While completing her PhD in plant systematics and morphology in the joint City University of New York/New York Botanical Garden Plant Sciences program under Scott Mori and Dennis Stevenson, she became interested in the molecular basis of plant diversity. She did her post-doc in the developmental genetics lab of Vivian Irish at Yale University on the evolution of a family of transcription factors involved in flower development, and she continues to study the functional evolution of this gene family currently. After one year on the faculty of University of Alabama, she moved back to The New York Botanical Garden as Director of Plant Genomics, where she developed her research program studying the evolution of plant form along two paths: studying evolutionary changes in genes to see how those changes affected flower and fruit form; and identifying the genes that underlie differences in form among closely related species. Dr. Litt also served as a program director in Plant, Fungal, and Microbial Development and Evolutionary Development at the National Science Foundation. She recently moved to the University of California at Riverside, where she continues to study the genetic basis of plant diversity.

Maria E. Orive is a professor of evolutionary genetics in the Department of Ecology and Evolutionary Biology at the University of Kansas. Her research in theoretical population genetics aims to develop mathematical models that provide a conceptual framework for exploring important questions in evolutionary biology and analytical tools for demographic and genetic data. Her work has considered levels of selection and mutation in organisms that reproduce both sexually and asexually, the relationship of population structure and life-history attributes to gene flow and genetic diversity, and models of within- and between-host pathogen and symbiont population dynamics. Orive received her BS from Stanford University and her PhD from the University of California at Berkeley. After spending two years as a postdoctoral researcher in genetics at the University of Georgia, she was an NSF-NATO Postdoctoral Fellow at the University of Edinburgh. Her research has been funded by multiple grants from NSF and NIH. In 2007–2008, she was the Carl and Lily Pforzheimer Foundation Fellow at the Radcliffe Institute for Advanced Study (Harvard University), and has served as the University Faculty Ombudsman for the University of Kansas since 2007.

Daniel Ortiz-Barrientos is an Associate Professor in evolutionary genetics in the School of Biological Sciences at The University of Queensland, Brisbane, Australia. During his scientific career he has investigated the ecological and genetic basis of speciation both in plants and animals. His current research program explores the early stages of speciation, the molecular basis of parallel speciation, and the interplay between recombination and natural selection during the origin of new species. His research funds come from The Australian Research Council. He is married to Antonia Posada, and is the father of three energetic and beautiful kids.

Claudia Russo was born in Leeds, England, but has lived in Rio de Janeiro, Brazil since she was two years old.

Claudia has an academic major in Ecology from Universidade Federal do Rio de Janeiro completed in 1989, and finished her Master's thesis in 1991 on population genetics of two actiniid species of sea anemones with different reproductive strategies, under the supervision of Associate Professor Antonio Mateo Sole-Cava. Her PhD dissertation was on the diversification of drosophilids and on the use of a known phylogenetic tree to estimate the reliability of tree building methods. The dissertation was completed in 1995 under the supervision of the Evan Pugh Professor Masatoshi Nei who recently received the prestigious Thomas Hunt Morgan Medal. Her graduate degrees were obtained as a student at the Genetics Program from the Universidade Federal do Rio de Janeiro and as a visiting scholar at the Pennsylvania State University (1992–1995).

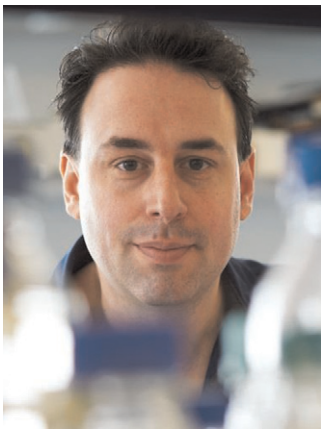
Claudia is currently the Head of the Genetics Department at the Federal University of Rio de Janeiro, having been a member since 1997. Claudia has supervised 13 Master's dissertations, eight PhD theses and seven post-docs, of which eight are now Assistant Professors at universities in Brazil and abroad. She has published 42 academic papers that have been cited over 1,200 times. Her *h-index* is 14. Since 2012, Claudia has been a member of the editorial board, and an associate editor of the *Molecular Biology and Evolution* journal. Since 2012 she has been a council member for the Pan American Association of Computational Interdisciplinary Sciences and since 2009 for the Brazilian Association for the Advancement of Science.

Claudia's general academic interests are on key aspects of animal phylogenetics, including their diversification patterns in time and space. She has worked with various metazoans groups but more prominently on marine sponges, sea anemones, arthropods, passerine birds, and mammals. Claudia has also published on the use of known phylogenetic trees to estimate the efficiency of phylogenetic methods in recovering and rooting those trees. More recently, she has developed some interesting *hands-on* educational tools for evolutionary biology practices in the classroom.

Karen E. Sears is an evolutionary developmental biologist whose primary research goal is to determine how developmental variation within a species produces congenital malformations in humans, and among species generates new evolutionary forms in mammals. Dr. Sears earned her PhD from the University of Chicago, did postdoctoral research at in the Howard Hughes Medical Institute (HHMI) lab of Dr. Lee Niswander, and joined the faculty of the University of Illinois at Urbana-Champaign. At Illinois she holds positions as an Associate Professor in the Department of Animal Biology, a Faculty Member in the Institute of Genomic Biology, and an Affiliate of the Program in Ecology, Evolution and Conservation Biology and the Department of Cell and Developmental Biology. She is also the President of the Pan American Society for Evolutionary Developmental Biology. She has authored or co-authored over 35 publications including first-authored publications in *Nature*, *Proceedings of the National Academy of Sciences*, and *Evolution*. She has served as a principal investigator on multiple, nationally-funded research projects, and presented invited seminars at more than 30 institutions and symposia. She is routinely ranked among the top 10% of Illinois professors for her teaching, and was a featured scientist in the PBS/HHMI documentary "*Your Inner Fish*."

Vassiliki "Betty" Smocovitis is Professor of the History of Science in the Department of Biology and in the Department of History at the University of Florida. Her areas of expertise include the history of evolutionary biology, genetics and systematics and the history of botany. She is best known for her contributions to understanding the historical event known as the "evolutionary synthesis" and in gaining greater understanding of the origins of the discipline of evolutionary biology. She has published extensively on both the intellectual and social aspects of the history of evolutionary biology including a history of the Society for the Study of Evolution, a history of the Darwin Centennial of 1959, and the integration of botany, genetics, and anthropology into the evolutionary synthesis. She was the contributor to the *Oxford Bibliographies* entry on Charles Darwin at over 25,000 words and the entry on the modern synthesis. She is the author of *Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology* (Princeton: Princeton University Press, 1996).

Nina Wedell is a professor of evolutionary biology with research interests focused on the evolutionary ecology of sex. She has worked extensively on various aspects of sexual selection and sexual conflict, in particular on the role of selfish genetic elements in reproductive biology. Nina is the Academic lead for the Behaviour research group at the University of Exeter.



Jason Wolf is Professor of Evolutionary Genetics in the Department of Biology & Biochemistry and The Milner Centre for Evolution at the University of Bath. His research is unified with a special focus given to understanding the influence that frequently ignored or under-appreciated sources of genetic variation have on the genotype-phenotype relationship and how this, in turn, influences evolutionary processes. He integrates theoretical, computational and empirical quantitative and population genetic techniques to achieve this goal. He is particularly interested in understanding the evolutionary consequences of various types of interactions, including gene interactions (epistasis), parent-offspring interactions and social interactions. He received a PhD from the University of Kentucky, after which he was a postdoctoral researcher at Indiana University and a US National Science Foundation Postdoctoral Fellow at Washington University School of Medicine. Prior to moving to the University of Bath he held positions at the University of Tennessee and the University of Manchester. He won the Dobzhansky Prize from the Society for the Study of Evolution, a Young Investigator's Prize from the American Society of Naturalists and the Scientific Medal from the Zoological Society of London.

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Alphabetical Contents List

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

All articles within the encyclopedia have an extensive list of cross-references which appear at the end of each article, for example:

Adaptive Mutation Controversy

> *See also*: Directional Selection and Adaptation. Microbial Experimental Evolution. Mutation, Population Genetic Models of. Natural Selection, Introduction to. Recombination in Bacterial Populations

3. Subject Classification List

A subject classification list has been developed by the editors to group articles under broad topics. This index is useful for making broad connections between articles and locating the relevant article for a topic that is covered in more than one article.

4. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

5. Contributors

A full list of contributors appears in the front mater of each volume.

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Plant/Fungus Diversification

Population Genetics

Quantitative Genetics

Sex, Recombination, and Mating Systems

Speciation and Hybridization

PREFACE

The *Encyclopedia of Evolutionary Biology* was developed to provide an authoritative overview of the current state of evolutionary biology. It was an ambitious goal, especially given that the field did not pause for the two and a half years needed to complete the project. The encyclopedia's 15 section editors collaborated to ensure that content gaps were kept to a minimum, and their efforts show. When the project was completed, we had compiled 256 entries, covering a broad range of topics selected by the editors to ensure a comprehensive resource. It was a privilege to read every one of these entries, and I was truly humbled by the collective efforts of hundreds of authors to communicate the excitement and sophistication of a field of study that touches on every conceivable topic in biology today.

There are many ways to envision an encyclopedia of evolution, and we had to choose an approach that would lead to a cohesive resource. Readers will note that, in the more organismal-focused entries (edited by David Guttman, Amy Litt, and Claudia Russo), there is an emphasis on *diversification* of life. We did not set out to provide an overview of the diversity of life, as such a goal would be untenable; rather, we focused on the evolutionary processes and key events responsible for diversity. Numerous entries deal with speciation, life history evolution, evolutionary biogeography, and coevolution. These entries (edited by Daniel Ortiz-Barrientos, Tim Coulson, Rosemary Gillespie, and Andrew Forbes) bring to light how the evolution and diversification of life is intimately entwined with ecology. Of course, there is extensive coverage of population genetics, quantitative genetics, evolutionary developmental biology, the evolution of sex and mating systems, molecular/genome evolution, and phylogenetic analysis (edited by Maria Orive, Jason Wolf, Karen Sears, Nina Wedell, Hiroshi Akashi, and Laura Kubatko), all fundamental to our understanding of evolutionary processes. And as thematic bookends, several entries (edited by Betty Smocovitis and Norman Johnson) cover the history of evolutionary biology and applications of evolutionary biology.

Readers of the encyclopedia will find that entries are generally pitched at a somewhat advanced level, although with great effort by authors to make entries as accessible as possible to a broad audience. Encyclopedias, like living organisms, are compromises. If all entries could be readily understood in their entirety by first-year university students, this encyclopedia would be of limited value to experts. At the other extreme, if entries were extremely technical – and our authors were undoubtedly capable of producing such entries – the encyclopedia might be inaccessible to students. While there is, by necessity, variation among entries in this regard, we settled on a general target: the majority of an entry should be accessible to a motivated, advanced undergraduate. Readers are, of course, directed to additional resources, with authors providing bibliographies and lists of further reading.

As with any undertaking of this scale, there are many individuals who should be recognized for their roles in the development of this encyclopedia. Special thanks go to Norman Johnson for early discussions that helped us develop the general structure of the encyclopedia. The dedicated and distinguished team of section editors deserves the credit for drafting the table of contents, recruiting authors, and working extensively with authors to ensure the highest quality product. It should go without saying that the high quality of this encyclopedia ultimately reflects the efforts of the editors and authors. Finally, the project management and development teams at Academic Press were always ready to assist, and while it is not possible to name everyone who contributed to the effort, I am particularly indebted to Simon Holt, Will Bowden-Green, Paula Davies, and Justin Taylor.

Richard Kliman
Editor in Chief

FOREWORD

What is life, how did it originate, and what accounts for its great diversity? These are fundamental scientific questions that have and will always be the source of endless fascination and wonderment. Charles Darwin and Alfred Russel Wallace provided an answer to the latter question through the grand idea of evolution and the process of natural selection. Darwin also speculated on the where question of the origin of life by hypothesizing it originated long ago in a warm lagoon. Most importantly, however, Darwin shattered the notion that the natural world is static and replaced it with a biology that is dynamic and continually changing. Species are not fixed, typological entities. Rather, they are related by common descent in a great tree of life. Analogous to tracing one's ancestors back in time in a pedigree, one can climb down the tree along its branches and boughs that connect species in a hierarchy of phylogenic relationships until reaching the base of its trunk and the common ancestor of us all. One can also climb up the tree and quickly realize that evolution produces a seemingly endless array of new forms (and sometimes extinction). Thus, as the tree of life grows, populations are continuously evolving and diverging from one another, creating novel varieties and races (showing slight differences) that eventually evolve into new species (separated by distinct gaps). And natural selection – the differential survival of individuals in populations possessing heritable traits favorable for their survival and reproduction – is the primary materialistic process causing evolutionary change and the origin of new species.

The *Encyclopedia of Evolutionary Biology* chronicles our current state of understanding of the dynamics of evolution and its product, Darwin's great tree of life. A diversity of seminal topics are covered including overviews of the history of the field, the origin of life, the history of life (including the phylogentic methods used to reconstruct life's history), the myriad ways and means (including mechanisms other than natural selection) that evolution is affected, and the important roles that conflict versus cooperation, and mergers and acquisitions, occurring within across varying levels of biological organization, play in the narrative of life. In so doing, the *Encyclopedia* highlights the grandeur in Darwin's view of life. We are not separate, but rather a twig along a branch of life, a twig that has evolved the ability to comprehend the existence of and our connectedness to the tree, and climb around its branches to see what has been and think about what may come. It is a wonder of life that it can look at and understand the meaning of itself.

But Darwin's grand view has even larger ramifications, going beyond providing a materialist basis for organismal change and putting us in our place. The reality of evolution also answers the question of what life is. If pressed to define life, most of us would reply with a list of the things that living organisms do. For example, living organisms metabolize, grow, develop, move, behave, mutate (are variable), and reproduce with inheritance. One can investigate these different characteristics of life separately and discern the mechanistic basis for the different processes that constitute life – the "how" of life. And such studies represent the basis for many fields of the life sciences. However, these are only the components of life and, in isolation, produce a static view of the natural world. Rather, the seminal insight is that populations of living beings possessing these characteristics have the emergent property that they evolve. Darwin's "*On the Origin of Species*" therefore not only describes how populations evolve, and as a logical extension how new species form, but also conveys the essence of what life itself is – evolution. Thus, as Theodosius Dobzhansky famously stated "nothing makes sense except in the light of evolution." The Encyclopedia wonderfully brings this view of life to light, providing the reader with the breadth of knowledge and overview of the current state of the field of evolution needed to appreciate and participate in the next major ongoing synthesis in our understanding of life, the so-called "Omics Revolution."

The study of evolution is in an accelerated phase of discovery brought about by major technical advances in our ability to DNA sequence whole genomes (genomics), and to generate profiles of mRNA transcription (transcriptomics), protein levels and enzymatic activity (proteomics), and metabolic products (metabolomics) at varying stages in the life cycle and development of organisms. This "Omics Revolution" may not change foundational evolutionary principals, per se. Our understanding of evolution has been heightened by a series of such advances in the past, including the "Modern Synthesis" when Mendelian genetics was wedded to Darwinian thinking and the "Molecular Revolution" in which genetic technology increasingly allowed allele frequencies in natural populations to be analyzed. The Omics Revolution is an extension of these pervious advances, but one in which the workings of whole organismal systems and the composition of entire communities can be gleaned at once.

Perhaps, the most important discoveries in Omics will come from linking an understanding of the process occurring at the cellular and microevolutionary level with large scale patterns and trends at the macroevolutionary scale. Previously, processes occurring within and interactions occurring among cells could be studied in at least some detail. Omics is providing an opportunity to fully understand how all of these processes interact simultaneously to result in the development and functioning of integrated, multicellular systems of life. At the other end of the spectrum, fossils attest to the evolution of new life forms through time and the creation of great and observable morphological diversity. Genomic sequencing is providing a powerful means to help accurately place these fossils within the framework of a fully resolved molecular phylogenic tree to better understand the history of life, including major trends, themes, and variation in the tempo and mode of evolutionary change. But it is the middle of the micro and macro at the branching points in the tree of life that Omics may prove most insightful. Now it is possible to not only DNA sequence large numbers of individuals within populations, but to equate these genetic differences within and between populations to morphological, physiological, and behavioral phenotypes, and discern the developmental

and physiological mechanisms by which these genetic changes produce organismal variation, diversity, and reproductive isolation – the stuff of evolution and speciation itself. Thus, we will be able to not only understand the everyday processes responsible for how the tree of life grows, but be able to translate this into a mechanistic appreciation of how these processes result in new branches on the tree forming and others dying out, giving shape to the history of being on our planet.

The field of evolution is currently inundated with a mass of Omics data and the bottleneck is the development of bioinformatic analytical tools to edit, analyze, and interpret the results. However, it is clear that many new insights are on the horizon, and even if they do not affect the root principles of evolution, soon a deep connection of the how and why of life will emerge to help forge a truly integrative evolutionary biology: The *Encyclopedia of Evolutionary Biology* is an excellent guide to prepare readers to assimilate these new findings, keeping bioinformatics grounded in the bio and providing a valuable source for seeing the tree through the forest to understanding the grand synthesis of life that is flowering.

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A

Adaptation, History of

A Larson, Washington University, Saint Louis, MO, USA

Glossary

Acquired characteristics Traits obtained by an organism during its lifetime, such as hypertrophy of muscles through exercise. Lamarck's hypothesis that such traits could be passed to offspring by altering heredity underlay his evolutionary mechanism for adaptation.

Adaptation (as a character) An organismal trait that evolved gradually by natural selection for a biological role retained by its possessors, and superior to contrasting traits present in the evolutionary history of the population.

Adaptation (as a process) In Darwinian and neo-Darwinian theory, evolution by natural selection causes a population to accumulate favorable characters that arise among its members and to discard less favorable alternatives.

Adaptationist program Term used by Stephen Jay Gould and Richard Lewontin to criticize an idealistic research program prevalent in the late 1960s and 1970s that uncritically assumed widespread adaptation of characteristics that had simpler historical explanations. An analogistic approach equating, for example, behavioral variation in a bird species to family conflicts in humans, was a widespread methodology criticized as having no basis in testable historical hypotheses.

Additive variance in fitness In Ronald Fisher's genetic theory of natural selection, the critical condition for evolution by natural selection and the condition ultimately destroyed by the resulting evolutionary change. It constitutes populational variation in traits influencing the expected numbers of offspring produced by individuals of a given genotype relative to those of contrasting genotypes.

Aptation A trait that provides a useful biological role to its possessors not present in contrasting traits present in the evolutionary history of the population.

Behavioral drive Allan Wilson's term for a learned behavior that changes evolutionary adaptation in a population; for example, discovery of a novel food source could lead to evolution by natural selection to adapt the population to use the novel food.

Burden Rupert Riedl's term for a trait that becomes indispensable to a population following its evolutionary origin; for example, presence of a notochord early in development is indispensable for viability in chordates despite absence of this characteristic in other animals.

Developmental constraint An evolved bias in the phenotypes or morphologies capable of being produced by a developmental process; disturbance of development by mutation or an environmental stimulus typically produces a small set of possible outcomes. Although perceived as a contrast to adaptation, it was later recognized as important for evolution by natural selection of complex traits.

Developmental module A semiautonomous pattern of development of interrelated subparts of an organismal structure. For example, mutations as well as environmental stimuli can cause the metathorax of a fruit fly to develop the typical appendage, a bulb-like balancer, or an alternative developmental sequence producing a wing. Switching of development between contrasting developmental modules can be tied to genetic or developmental switches.

Exaptation A trait that performs a biological role other than one for which it evolved by natural selection and contributes greater utility than contrasting traits present in the evolutionary history of the population. Presence of feathers in birds, for example, is an exaptation for flight because feathers preceded flight in avian evolution, yet feathers contribute utility in flight not possible from the scales from which feathers evolved.

Function A biological role through which a character evolved by natural selection. For example, the hypothetical role for feathers in avian evolution was as insulation for a homeothermic animal.

Genetic assimilation A developmental process or phenotype initially induced by an environmental stimulus reveals additive variance in the population capable of being selected to stabilize expression of the novel phenotype. The concept was proposed by Conrad Waddington to explain experimental evolution in fruit flies.

Genetic drift Random change in the frequency of a genetic trait caused by sampling error in a finite population. By chance alone, the gene pool of a population is not identical to the gene pool of the same population in earlier generations.

Gradualism Darwin's theory that only small, quantitative changes in organismal form would be potentially favored

by natural selection, and that large changes in form could evolve only through a succession of small steps across generations. Gradual evolution by natural selection is critical for selection to be judged the creative force underlying adaptation rather than just a sieve that retains adaptations produced by mutation or development.

Homology Equivalence relationship among characters that descend from an equivalent characteristic of a common ancestor. Hypotheses of homology are critical for testing hypotheses that a trait constitutes an adaptation evolved by natural selection.

Idealism In science, a pejorative term used for explanations that fail to provide potential falsification through testing of hypotheses. Lewontin criticized the adaptationist program for arbitrarily constructing organismal traits and perceived utilities that could not withstand historical testing.

Lamarckism General term for an evolutionary theory that postulates hereditary transmission of acquired characters as the basis of evolution. The term 'neo-Lamarckism' is often used for theories from the late 1800s onward that purged Lamarck's original notion that organismal volition played a major role in evolution of adaptation.

Modern Evolutionary Synthesis Progression of evolutionary theory from the 1920s through 1940s toward favoring neo-Darwinian explanations of gradual evolution of adaptation through natural selection, generally discrediting alternative theories based on Lamarckian inheritance, saltation, or orthogenesis.

Mutation Term proposed by de Vries around 1900 for large genetic changes that can produce new species and adaptations by saltation rather than by gradual evolution. Mutation is now used more broadly to denote any genetic change regardless of its consequences for organismal phenotype.

Natural Selection Darwin's primary mechanism for evolution of adaptation, in which organisms possessing the traits most favorable for using environmental resources have higher rates of survival and reproduction than individuals with contrasting traits. The population thus accumulates the most favorable characteristics across generations and discards less favorable characteristics.

Neo-Darwinism Darwin's evolutionary theory as modified by August Weismann's purging of its Lamarckian elements in the late 1800s. Mendelian genetics and the chromosomal theory of inheritance would become the genetic basis of neo-Darwinism in the 1900s.

Neutral evolution Evolutionary change explained by the interaction of mutation and genetic drift and not requiring a selective explanation. Most evolutionary change measured in genes and proteins is likely neutral. Neutral evolution is nonetheless a useful null hypothesis whose rejection leads investigators to discover adaptive evolutionary change at the molecular level.

Orthogenesis General term for disparate theories popular in the decades around 1900 that considered evolutionary change directed by biases in the production of new variation rather than by Darwinian natural selection. Although the hypothesis of a 'momentum' in production of variation would be discredited by Mendelian genetics, orthogenetic theories often used arguments similar to those of 'developmental constraint' that would become established in the 1980s by evolutionary developmental biology.

Phenocopy Environmentally induced change in development that produces a phenotype characteristic of a known genetic mutation in the species. For example, Waddington's treatment of fly eggs with ether caused some genetically wild type individuals to express the phenotype characteristic of the Bithorax mutation.

Phenotypic accommodation West-Eberhard's observation that an organism's altered use of body parts often causes those parts to undergo atypical development that enhances the new use. For example, a goat with paralyzed forelimbs used the hind limbs for bipedal locomotion and caused non-characteristic development of the associated skeletal and muscular structures. This could expose additive variance in the population for evolutionarily stabilizing the new phenotype.

Phylogeny Empirical reconstruction of the history of common descent of species.

Saltation Occurrence of a discontinuous phenotype suddenly within a single generation. Darwin argued that such variants would be too harmful to contribute to evolution by natural selection.

Selfish DNA An intragenomic process analogous to natural selection in which particular DNA sequences use the cellular processes of gene replication and expression to make extra copies of themselves to be inserted into the genome. This process has no inherent advantage to the organism, but it can produce populational variation that leads to evolution of adaptation by natural selection.

Volition A notion that an organism's choices, sometimes but not necessarily conscious ones, influence its adaptive evolution. Lamarck's evolutionary theory put strong emphasis on organismal volition. In Darwinian theory, an organism's choice of a favored habitat among various alternatives can influence how natural selection acts on the population to produce adaptation.

Pre-Darwinian Concepts and Lamarck's Theory

Perhaps the greatest challenge to evolutionary theory from its beginnings was to explain the origins of organismal characteristics that appeared structured to fit their possessor's needs. The implied teleological argument that an organism's needs were somehow assessed prior to its formation, and its characteristics constructed to meet preconceived needs, was at odds with a materialistic explanation of life. The problem of 'adaptation' in organismal form has long dominated the study of evolution.

In the most noteworthy pre-Darwinian theory of evolution, Jean-Baptiste de Lamarck (1809) credited animals with volition that could direct acquisition of useful characteristics, which would then be transmitted by heredity to their offspring.

Differential use versus disuse of body parts, for example, would influence corresponding hereditary changes in body form and thus direct adaptive evolution. Lewontin (1983) applies the term 'transformational' to evolutionary theories like Lamarck's in which organismal change during development is the hypothesized mechanism of evolutionary change. Allen (2014) makes a critical distinction between Lamarck's notions of organismal volition versus inheritance of characteristics 'acquired' during an organism's lifetime. Although a role for volition was widely discredited in early criticism of Lamarck's theory, Lamarck's notion of inheritance of acquired characters persisted at least as a minor component of evolutionary theory from Darwin (1859) to contemporary evolutionary genetics (Jablonka and Raz, 2009). The Darwinian tradition following Weismann (1886) nonetheless largely discredited Lamarckian transformational arguments entirely as being inconsistent with hereditary mechanisms and thus unable to explain adaptation. Evolution by natural selection is indispensable to neo-Darwinian explanations of adaptation, although evolution by natural selection does not guarantee adaptation.

Darwin's Theory of Evolution by Natural Selection

Darwin's insight in explaining adaptation is that variation among individuals within populations is the material from which adaptive characteristics arise. This theory is thus 'variational' in its evolutionary mechanism in contrast to Lamarck's 'transformational' mechanism (Lewontin, 1983). Darwin (1859) had no concepts of genes or gene mutations, both of which emerged following rediscovery of Mendel's work in 1900 (Allen, 2014). Darwin's selection theory required that variation be produced at random with respect to an organism's needs, and that the variation be 'heritable' in the sense that organisms resemble their parents or grandparents more closely than they do individuals sampled randomly from the population. Darwin considered saltations, large changes in organismal form arising within a generation, inevitably harmful to their possessors. Only changes of small effect could include new forms potentially beneficial and thus potentially leading to adaptation. Adaptation as a process thus required gradual change in organismal form. Accumulation of small, gradual changes over hundreds or thousands of generations could construct new adaptations. Such adaptations would have no reasonable chances of occurrence except by natural selection increasing the frequency in a population of many small, individually favorable subparts, and thus combining them to construct a novel organismal form.

Mayr (1985) separates the observations and logical inferences underlying Darwin's theory of natural selection. The first major inference is a struggle for existence among organisms within populations, resulting from what would later be called 'superfecundity': populations produce large numbers of offspring each generation, with a biological potential for exponential growth in population size, but most individuals perish prior to adulthood because resources are insufficient to support them. Combined with the observations of heredity and variation as described in the preceding paragraph, Darwin argued that organisms whose characteristics are most favorable for survival and reproduction contribute disproportionately large numbers of offspring to the next generation. The favorable characteristics that permit an organism to survive and to reproduce thus increase in frequency in the population. Favorable characteristics that arose in different individuals in different generations have a high probability of being combined in the same organism when those characteristics are common in the population. Such combinations of favorable characteristics gradually construct new adaptations. Dennett (1995) characterizes evolution by natural selection as an algorithm that operates by chance (random variation) to produce order (adaptations). Adaptations of greater complexity become possible using previously evolved adaptations as modular subunits.

Criticism of Natural Selection as the Mechanism of Adaptation

Critics of natural selection as the source of adaptation focus on the ability of randomly produced, gradually accumulated changes to construct new features. 'Mivart's dilemma' argues that the incipient stages of an adaptation would provide insufficient utility for natural selection to accumulate them. Darwin (1859) anticipated this challenge in arguing that the initial stages of a structure often served biological roles different from ones acquired later following evolution of a minimum level of complexity. Feathers in birds clearly have utility for flight, although feathers preceded flight in avian evolution. The initial evolutionary stages of feathers likely provided insulation for a homeothermic animal, with some feathers being modified to form flight structures much later by co-opting a thermoregulatory device for a new role. The concept of exaptation, as formalized by Gould and Vrba (1982), is clearly expressed but not distinguished from adaptation in Darwin's *On the Origin of Species*.

The integrity of natural selection as a means for creating highly improbable new structures lay in its coupling with gradualism. Only by bringing together many variant forms, each one arising in a different individual in a different generation, could natural selection actually construct something new. If a new form occurred abruptly in a single individual and natural selection served merely to increase its frequency to become the prevalent form, natural selection would fail to explain how the new form was constructed. That explanation would be sought either in the process that generated the saltational variation (mutation) or by developmental properties of the organism that governed how a new genetic mutation would alter organismal form. The mutation theory of de Vries (1901–03) would emphasize the former explanation; theories of orthogenesis (reviewed by Bowler, 1983) would emphasize the latter, coupled with a notion that 'momentum in variation' would direct evolution in linear trends, sometimes but not always adaptive ones. The saltational theories of Goldschmidt (1948) would include both of these criticisms of natural selection, judging it incapable of explaining evolution of the characteristics that distinguish higher taxa of animals.

As in many biological controversies, both sides contributed critical parts to our current understanding of adaptation. Pharyngeal jaws of many cichlid fishes show hypertrophied skeletal and muscular elements considered useful in crushing hard prey, such as snails. This 'molariform' morphology differs

from the contrasting and presumably ancestral form, the 'papilliform morph,' in which the homologous skeletal and muscular components are much smaller. There is no obvious reason why the hypertrophied molariform structures could not have evolved gradually from the papilliform structures; nonetheless, these contrasting morphs are expressed as discrete alternatives within species (Liem and Kaufman, 1984). Switching of development between these discrete developmental pathways might be genetic in some cases, although it occurs facultatively in some species depending on the dietary environments of young fishes. The contrasting molariform and papilliform morphs are sufficiently different to qualify as saltational change should the change arise within a generation, as it appears to do. Perhaps the simplest explanation is that the molariform morph and its associated developmental processes evolved gradually by natural selection for an ability to process hard food, such as snails, and became tied to a developmental 'switch' that could activate this module or the contrasting papilliform one depending on genetic and environmental cues at a critical stage of development. A population that normally does not express the molariform morph might retain an ability to do so under the critical conditions. Gradual evolution in the past would make possible later saltational evolution by activating a latent developmental pathway whose result is potentially beneficial.

Saltational change mediated by developmental modularity also can occur by expressing a module that evolved gradually in one part of the body at a new location in which the module is potentially useful. A major characteristic of geckos is presence of adhesive toepads, modified scales on the ventral tips of the digits. Some members of genus *Lygodactylus* exhibit such toepads also at the tip of the tail (reviewed by West-Eberhard, 2003, p. 257), conceivably evolved by a saltational change that substituted toepad development for the ventral scales at the tip of the tail.

Evolution of developmental modularity fits Dennett's (1995) analogy of evolution by natural selection to construction of buildings by cranes; construction of smaller cranes makes possible construction of larger cranes, which make possible construction of large buildings. Construction of developmental modules that are semiautonomous of their surroundings, such as developmental of a lizard scale in the form of an adhesive toepad, can form the building blocks of new characters that would be unlikely without such modularity.

Historical Development of the 'Adaptationist Program'

Much of what now constitutes evolutionary developmental biology (West-Eberhard, 2003; Wagner, 2014) was largely missing from evolutionary theory in the 1950s–70s. Also absent was a precise means for constructing phylogenetic relationships among species or even patterns of gene flow among populations within species. Molecular genetic data provided the critical means for measuring the past history of populations and estimating the historic origins of characteristics considered adaptations. One cannot overstate the increased precision of hypotheses of adaptation made possible by robust hypotheses of homology and phylogenetic relatedness of species (Baum and Larson, 1991; Larson, 2009).

Adaptation and Natural Selection by George C. Williams (1966) was perhaps the most influential work of its time on adaptation. It formalized the meaning of adaptation as a structure or behavior that evolved by natural selection for a particular biological role. Williams was instrumental in discrediting group selection as a source of either adaptation, or of characteristics that would favor other organisms at the expense of their possessor. Apparently, altruistic behaviors received special focus because Darwin claimed that natural selection could not evolve a trait primarily for the benefit of others rather than its possessor. Explanations that such traits arose as group-level adaptations rather than as organismal-level adaptations declined following Williams (1966). Williams simultaneously introduced a reductionist principle that adaptive explanations should focus on the lowest biological level of complexity capable of explaining the data, specifically the genic level. From this precedent, Dawkins (1976) popularized the notion that organisms are merely vehicles for the propagation of selfish genes. Williams (1989) would extend this argument to claim that human genomes are a blueprint for selfish behavior, an evil force that human society must combat. This genic-level determinism of organismal characters and adaptations was widely criticized as unfounded and inconsistent with the very fluid relationship between genotype and phenotype on an evolutionary timescale. Waddington's genetic-assimilation experiments (summarized in Waddington, 1975) already had revealed the complexity of mapping genotype onto phenotype. Williams dismissed Waddington's genetic-assimilation theory because the experiments used saltational traits that were clearly nonadaptive. Waddington's process nonetheless later emerged as a general one from later discoveries in evolutionary developmental genetics, whereas the selfish-gene model yielded no comparable insights on the developmental relationship between genotype and phenotype.

A naïve mixing of gradualism and natural selection in the absence of precise tests of phylogeny produced what Gould and Lewontin (1979) criticized as the 'adaptationist program' (see also Lewontin, 1985). Extant characteristics were often considered adaptive by default, perhaps optimally so, using an argument that the population otherwise would be extinct. Natural selection would act each generation to eliminate structural deviations from the optimal form. Adaptationism thus became an idealism in which organismal form was arbitrarily partitioned into characters with no precise tests of character homology, and each such character was assumed optimally adaptive for reasons to be revealed by adaptationist study (see critique by Lewontin, 1985). Perhaps the most extreme version of adaptationism and the one that stimulated the criticisms of the 'adaptationist program' was sociobiology (Wilson, 1975). Lewontin (1985) used Wilson's claim that indoctrinability was a characteristic of human populations evolved by natural selection and encoded by our genes to illustrate the idealism of apaptationist arguments. How could such conjectures be tested and potentially refuted by data?

Idealism of the adaptationist program is perhaps most evident in analogistic approaches to studying human behavioral evolution by finding 'unrelated' species having behaviors judged equivalent to human ones; studies of selective consequences of those behaviors then would explain human evolution (Alcock, 2001). For example, Alcock (2001:196–200)

interprets behaviors of individuals in 'durable and sometimes less than harmonious families' of white-fronted bee-eaters (*Merops bullockoides*) as sharing selective explanations with analogous behaviors in humans, both driven by the ultimate goal of contributing as many surviving copies of their genes as possible to their respective populations. The author doubts that the avian study has any relevance for understanding human social behavior, or even that the human behaviors being addressed demand a biological explanation as adaptation.

Fortunately, advances in molecular phylogenetics and population genetics combined with evolutionary developmental biology provided a precise means for testing hypotheses of adaptation by reconstructing the historical sequence of events antecedent to the population being studied. Lineage-specific developmental constraints on variation reveal the contrasting morphological and behavioral character states most likely to arise and thus to be discriminated by natural selection.

Developmental Constraints and Adaptation

Central to Gould and Lewontin's (1979) criticisms of the adaptationist program is the claim that any characteristic sufficiently explained by physico-chemical causes or the inherent limitations of organismal development need not be burdened by the less parsimonious claim that it evolved by natural selection. In such cases, no contrasting traits could reasonably be expected to have occurred in the past evolutionary history of the population being studied, thus precluding a selective explanation. Ellstrand (1983) makes this argument effectively and humorously in a parody of adaptationism called "Why are juveniles smaller than their parents?" The parody considers a number of different but not mutually exclusive explanations for the ubiquitous occurrence among living forms of juveniles at birth being smaller than their parents. The parody makes passing reference to a possibility that juvenile small size (JSS) is a historical or physical constraint. A phylogenetic approach to this question would reveal in its first step that JSS arose with the origin of life, and remained a fundamental physical constraint within which all subsequent life evolved. There would thus be no need to investigate separate but analogous selective explanations of JSS in all species.

Newman and Müller (2000) present what is probably the most expansive argument that inherent physical properties and dynamics of cellular matter provide the building blocks of and constraints on organismal form. Genetic evolution consists largely in placing control constraints on which among alternative possible forms becomes stabilized and produced with fidelity at a particular developmental stage in a particular population lineage. Evolution by natural selection thus increases the frequency of particular forms over counterparts by ensuring that evolved genic functions make the favored form much more likely to occur than contrasting ones within the environmental conditions experienced by a population. In some cases, a particular developmental event becomes evolutionarily 'burdened' (Riedl, 1978); its occurrence becomes critical for later developmental events and viability of the organism following subsequent evolution. Group-specific developmental constraints thus evolve from the more basic physical constraints shared by living matter.

An extreme case of a burdened structure is the notochord of chordate animals, including all vertebrates. Only chordates have a notochord in their development, and most chordates do not retain this structure intact into their adult morphology. The notochord most likely evolved by natural selection as a cartilaginous and cellular rod serving to support attachment of muscles, a simple skeleton. This condition characterizes *Branchiostoma*, but most chordates depart from it. Most animals never had a notochord in their evolutionary history and never will, but chordates are incapable of survival without development of a notochord at gastrulation, because developmental induction of the central nervous system, and of vertebrae in vertebrates, depends upon the notochord. The notochord is thus a developmental constraint of chordates; separate adaptive explanations for a notochord in the diverse species of chordates in the manner of the adaptationist program would be superfluous and misleading. Such explanations would imply incorrectly that presence versus absence of a notochord is a polymorphism subject to evolution by natural selection within chordate populations.

Developmental constraints, unlike simple physical ones, are thus group-specific evolved features. These features usually would have adaptive origins, but their role in extant species may bear little relationship to the role for which the character evolved by natural selection. The notochord in adult vertebrates is reduced to a series of intervertebral discs located on the ventral side of the spinal column; the inferred adaptive role of the notochord as a skeletal rod enabling movement no longer pertains.

The notion of developmental constraints initially met hostility from many evolutionists who perceived it to diminish the role of adaptation in evolution. This conclusion would hold only in a nonhierarchical theory of evolution. Wagner (1988) used corridor models to examine evolution by natural selection of complex morphological structures, ones in which constraints on variation in some interactions among the component parts are required for adaptive condition of the composite structure. Mathematically, developmental constraints on evolution perpendicular to an adaptive corridor enhance evolution by natural selection along the corridor. The corridor models could be applied, for example, to evolution of molar dentition in mammals. Mammalian molars have large, modulated grinding surfaces that make contact with corresponding molars of the other jaw. The molar structure likely evolved from an ancestral condition in which the teeth contacted at pointed tips, similar to those of canine teeth. Evolution by natural selection likely expanded the surface of contact between upper and lower teeth in the molar region, producing an undulating surface useful for grinding food. It is unlikely that such a surface could have evolved if genetic variation in the area and ridges of the contacting surfaces were accompanied by extensive variation in alignment of upper and lower teeth, heights of upper and lower teeth, anchoring of the teeth in their respective jaws, hardness of the teeth, strength of the enamel, and presumably many other characteristics that remained largely stable as the grinding surface became elaborated. A larger or more nuanced grinding surface would have no consistent selective advantage if it were associated, for example, with decreased tooth height such that the upper and lower teeth did not contact

with the mouth closed, or if the alignment were off, or the teeth subject to being crushed or displaced from the jaw. Directional selection on the nuances of the molar surface would require developmental constraints evolved by stabilizing selection on other dimensions of tooth shape and interaction. Developmental constraints then become the basis for evolution by natural selection of higher-order structures that would be unlikely to evolve without those constraints.

Non-Darwinian Evolution and Selective Neutrality as a Null Hypothesis for Studying Adaptation

Criticism of the adaptationist program in evolutionary morphology and behavior accompanied discoveries in molecular genetics that much of the content and structure of the nuclear genomes of animals and plants could not be explained as adaptations evolved by natural selection for organismal functions.

King and Jukes (1969) used 'non-Darwinian evolution' for their hypothesis that most evolutionary change in proteins occurs without natural selection. It is thus not adaptive. Because population sizes are finite, statistical sampling alone ensures evolutionary change across generations in a polymorphic population. This 'random genetic drift' was important to Sewall Wright's shifting-balance theory but served mainly to influence natural selection (Wright, 1932). The notion that most evolutionary change could be explained by mutation and genetic drift alone, without natural selection, was a new theory, elaborated mathematically by Motoo Kimura in the 1960s and summarized in *The Neutral Theory of Molecular Evolution* (Kimura, 1983). Neutral theory postulates that natural selection mainly discards harmful mutations. Most non-detrimental mutations are selectively equivalent to their predecessors, contributing no populational variance in fitness. Such mutations might increase in frequency by chance alone. Very few mutations would have sufficient benefit for natural selection to drive them to fixation. Neutrality theory predicts that the long-term rate of genic evolution approximately equals the rate of neutral mutation. Adaptively important parts of a gene or protein typically evolve slowly, because new mutations likely impair a highly evolved function.

Following much controversy, neutral evolution became a useful null hypothesis for molecular evolution. Mathematical models of neutral evolution serve to identify cases where data reject neutrality, thus highlighting specific molecular structures that demand an adaptive explanation by natural selection. Because neutral evolution requires only mutation and genetic drift, and because these evolutionary forces occur in all populations regardless of whether selection acts, neutrality is the simplest explanation of evolutionary phenomena that fail to reject its predictions (see review by Templeton, 2006).

In an impressive example, Walter Messier and Caro-Beth Stewart in 1997 demonstrated adaptive evolution of mammalian lysozyme c proteins by statistically rejecting the null hypothesis of neutrality. Foregut-fermenting ungulates and primates evolved stomach expression of lysozyme c to enable them to digest bacteria as a food source. The ancestral role of lysozyme c is to destroy infectious bacteria consumed by white blood cells. Recruitment of lysozyme c as a stomach enzyme is enhanced by amino acid substitutions that enable lysozyme c to operate in the stomach's highly acidic and caustic conditions. Lysozyme c evolved more rapidly than predicted by neutrality on ancestral lineages that evolved foregut fermentation, followed by evolutionary rates much lower than neutral ones, representing selective conservation of adaptations for stomach conditions.

Selfish DNA as a Challenge to and Source of Adaptation

Ford Doolittle and Carmen Sapienza (1980) and Leslie Orgel and Francis Crick (1980) introduced a new theory to explain the paradox that organisms contain more nuclear DNA than needed to guide protein synthesis, and that similar species often differ greatly in the DNA content of their cell nuclei. They postulated that some DNA sequences manipulate cellular processes of gene replication and expression to insert extra copies of themselves in a genome, without any advantage to the host organism. This 'selfish DNA' is essentially a genomic parasite. Because transposable elements are primarily detrimental or neutral to the host organism, their origin is not by natural selection but by an intragenomic analogue of natural selection. Vrba and Gould (1986) provide a detailed explanation of selfish DNA as a lower-level analogue of natural selection. Burt and Trivers (2008) present an encyclopedia of selfish genomic elements in their *Genes in Conflict*.

Retroviruses are the most elaborate selfish DNA elements. Their RNA genome encodes reverse transcriptase (makes a DNA copy of the RNA genome), a capsid protein (encloses the viral genome and reverse transcriptase in a particle), and an envelope protein (recognizes receptor molecules on a host cell, permitting infection). A retrovirus inserts copies of itself into the nuclear genomes of host cells, sometimes including the germ line. Latent retroviruses, capable of evolutionary activation by later infection or recombination, can remain in the genome of a species for many millions of years. Nuclear genomes also typically contain many transposable elements that encode reverse transcriptase but not the capsid or envelope proteins of a retrovirus. Transposable elements multiply within a genome but do not produce infective particles; they spread from one cell to another only through the hereditary process of cell division. Some small selfish DNA elements called SINES encode no proteins, but they have signals that utilize the transcriptases (RNA polymerase III) and reverse transcriptases (encoded by longer transposable elements) to replicate themselves in nuclear genomes.

Because occurrence and movement of selfish DNA elements can impair organismal survival and reproduction, an important class of organismal adaptations consists of mechanisms for suppressing activity of transposable elements (reviewed by Burt and Trivers, 2008).

Genetic changes caused by activity of transposable elements occasionally produce traits favorable to an organism, leading them to be co-opted by natural selection for organismal advantage. Roy Britten (2010) reviews evidence that transposable elements were recruited for adaptive evolution of

gene expression in human evolution. The immune system of vertebrates includes evidence of co-option of transposon activity for the kinds of gene rearrangement that are needed for an organism to develop immunity to unanticipated infectious agents.

Adaptation survives as a critical aspect of evolutionary biology whose operation requires a hierarchical perspective. All of the biological phenomena that were perceived initially as replacing evolution by natural selection (genetic drift, developmental constraint, selfish DNA) provide new opportunities for evolution of adaptation by natural selection.

A Role for Volition in Darwinian Evolution of Adaptation?

Darwinian theory of evolution by natural selection has been criticized, even by some of its advocates, for overlooking a role for organismal volition in evolution of adaptation (Lewontin, 1983). The mathematical theory of neo-Darwinism often treats an organism as a passive object of genetically determined traits and the environmentally determined differences in fitness conferred by those contrasting traits (Alberch, 1989). Notions that an organism's choices of habitat or that characters acquired, perhaps by learning, can influence adaptation are often resisted as inevitably introducing discredited Lamarckian inheritance. Nonetheless, most organisms have at least some means of evaluating and often selecting environmental conditions most appropriate for them from the available alternatives. A monarch butterfly seeks plants of the genus *Asclepias* for depositing its eggs. Seeds of *Ceanothus* bushes remain dormant for many years until a sporadic fire stimulates their germination. Clearly, many organisms have at least some ability to discriminate the habitats most suitable for them from an array of environmental conditions encountered. Three related concepts, genetic assimilation (Waddington, 1975), behavioral drive (Larson *et al.*, 1984), and phenotypic accommodation (West-Eberhard, 2005) provide means by which an organism's choice of environment could influence its population's evolution of adaptation by natural selection.

Genetic assimilation arose from experiments by Waddington (1975) on the relationship between genetic variation and susceptibility of development to environmentally induced changes. He exposed eggs of the fly *Drosophila melanogaster* to ether at a critical stage of development, causing the posterior segment of the thorax to develop the physical appearance of the middle thoracic segment, including a pair of hind wings absent in normal fly development. This environmentally induced phenotype closely resembles one produced by mutations at the *Bithorax* locus and is called a *Bithorax* phenocopy. By selecting flies having the greatest propensity to respond to ether treatment by producing a *Bithorax* phenocopy, Waddington demonstrated that the population contained genetic variance capable of being selected to 'canalize' development toward the Bithorax phenotype. The canalization was strong enough after many generations of selection that the *Bithorax* phenotype became common even in the absence of the ether treatment. In terms of population-genetic theory, the environmental stimulus (ether present at a critical stage) revealed additive variance in fitness in the selection experiment. The genetic variation underlying the additive variance in fitness was part of the *D. melanogaster* population even prior to the experiment, but it did not express itself as additive variance in fitness mediated through contrasting modes of thoracic development prior to the experimental conditions.

West-Eberhard's (2005) model of evolution by phenotypic accommodation has many similarities to genetic assimilation. It differs largely by noting that homeostasis during organismal development can cause an organism to develop favorable responses to environmental challenges through developmental plasticity. She illustrates her claims by citing Slijper's observations on development of a goat whose forelimbs were paralyzed at birth. The goat balanced on its hind limbs only, and by doing so directed its muscular and skeletal development toward a novel form conducive to bipedal walking. In a hypothetical case where environmental conditions favored bipedal gait in a goat population following a stimulus that caused some goats to adopt this posture, preexisting genetic variation contributing additive variance in stabilizing the bipedal habit and associated morphological changes could lead to adaptation. The initial usage of bipedal locomotion and developmental changes induced by the altered musculoskeletal development need not be genetic in origin; the evolutionary process is nonetheless a Darwinian one because stabilization of the new condition is achieved by natural selection on genetic variance in fitness.

Allan C. Wilson proposed the hypothesis of behavioral drive to explain what he perceived as disproportionately higher rates of anatomical evolution in mammals and birds than in other vertebrates. He argued that the greater influence of learned behaviors in these groups would lead them more often to explore new environments and in doing so to subject the population to selection for novel utilities and forms of structures. Among his favored examples was learning by a population of *Cyanistes caeruleus* birds to obtain milk from bottles delivered outside homes in the United Kingdom. The novel source of nutrition undoubtedly would have revealed additive variance in fitness with respect to digesting lactose had this become a primary food for the population. Mammals whose diets contain large numbers of bacteria, such as those that inhabit the rumen of foregut-fermenting ungulates and leaf-eating monkeys, show a series of adaptations to this food source, especially high levels of stomach expression of the bacteriolytic enzyme lysozyme and evolutionary changes in the lysozyme protein structure for optimal functioning in the stomach (Messier and Stewart, 1997).

It is likely that evolution of feeding preferences and other instincts begin as behavioral changes not initially under strong genetic control, but that the new context leads to selection for genotypes most conducive to stabilizing expression of a behavior beneficial under new environmental circumstances. As genetic mechanisms are constructed by natural selection, a particular behavior, such as hygienic behavior in bees (Lapidge *et al.*, 2002), acquires a strong genetic association. Evolution of genetic canalization of a behavior initially not under strong genetic control seems more parsimonious than the contrasting interpretation that genetic mutations initiated the details of the behavioral expression. Bonner (1996) used the term 'gene accumulation' to denote the increasing genetic control of

a particular developmental pathway, and 'gene silencing' to denote evolutionary diminishing of such control. Phenotypic accommodation, behavioral drive, and genetic assimilation provide a stronger mechanism for evolution of new adaptations than do models postulating strong genetic control of particular developmental or behavioral pathways as the initial condition rather than as the product of adaptive evolution.

See also: Darwin–Wallace Theory of Evolution. Synthetic Theory of Evolution, History of

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Adaptive Landscapes

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Glossary

Adaptive landscape The relationship between fitness (dependent variable) and genotype (at one or several loci) or phenotype. Different definitions of adaptive landscapes exist; the two most common being either fitness depicted against individual genotypes or fitness depicted against allele frequencies in the population.

Adaptive peak A gene or trait combination in the adaptive landscape that is connected with high fitness, and tends to drive populations to evolve by natural or sexual selection.

Brownian motion (BM) A popular and widely used model of phenotypic evolution that is often used as a null model or point of departure in phylogenetic comparative studies of adaptation. In BM models, interspecific variance increases linearly over time, due to stochastic processes such as mutation accumulation, and variance is thus unbounded.

Correlational selection Selection for optimal trait (or gene) combinations, typically expected to operate around a fitness peak in a multidimensional adaptive landscape.

Epistasis Interactions between alleles at different loci in shaping phenotypes. A special case, of particular relevance to adaptive landscape theory is fitness epistasis, where the phenotype is fitness which is interactively affected by two or more loci, resulting in adaptive peaks or valleys. Fitness epistasis is conceptually closely related to correlational selection.

Fitness valley Combinations of genes or traits in between adaptive peaks that result in low fitness.

Holey adaptive landscape A special case of adaptive landscapes initially modeled and suggested by theoretical evolutionary biologist Sergey Gavrilets, where large regions of genotype space form selectively neutral 'ridges' of high fitness, separated by low-fitness genotypes. Genotypes are assumed to only take two fitness-values in this model (1 = 'viable' and 0 = 'unviable'). This model of adaptive landscape has inspired researchers interested in the dynamics of molecular evolution.

Ornstein–Uhlenbeck (OU) model An alternative model of phenotypic evolution to Brownian motion in phylogenetic comparative studies of adaptations. In contrast to BM models, in OU models the accumulation of interspecific variance is not unbounded, but constrained. This reflects the effects of a 'pullback' force in BM models, which are usually interpreted as the effects of stabilizing selection as populations and species climb alternate adaptive peaks.

Peak shift The process by which a population moves from one adaptive peak to another in the adaptive landscape, and was traditionally thought to be difficult or impossible, as the population would have to cross a fitness valley in between the peaks.

Shifting Balance Theory (SBT) A controversial model of evolution and the problem of peak shift that was initially suggested by population geneticist Sewall Wright in 1932. It invokes a delicate balance between different evolutionary forces in subdivided populations such as genetic drift, local selection, and gene flow.

Stabilizing selection A conservative form of selection that favors intermediate trait values or heterozygotes (as in overdominant selection). Stabilizing selection is thought to be a common mode of selection when populations are at their evolutionary equilibria, such as when they are close to their adaptive peaks.

Historical Introduction

A Central Position in Evolutionary Theory and Population Genetics

Adaptive landscapes hold a central and special position in evolutionary theory, particularly in population and quantitative genetics, but also in some models of macroevolution. The adaptive landscape concept was originally formulated by the population geneticist Sewall Wright in his now classical paper from 1932 entitled 'The roles of mutation, inbreeding and crossbreeding in evolution' (Wright, 1932; **Figure 1**). An adaptive landscape shows the relationship between fitness (vertical axis) and one or several traits or genes (horizontal axes). An adaptive landscape is therefore a form of response surface, describing how dependent variable (fitness) is causally influenced by one or several predictor variables (traits or genes). Evolution by natural selection in the context of adaptive landscapes can be viewed as a hill climbing process, in which populations climb upwards to the trait or gene combination with the highest fitness ('adaptive peaks'). In between the adaptive peaks, there are regions in phenotype or genotype space of low fitness ('fitness valleys'). Adaptive landscapes have appeared in the popular science literature on evolutionary biology, perhaps most explicitly in Richard Dawkin's book 'Climbing Mount Improbable,' published in 1996 (Dawkins, 1996). Here, Dawkins, like many others, likened evolution by natural selection to a hill climbing process, in which populations and species evolved by climbing the closest adaptive peak, even though it is not always the peak with the highest overall fitness.

In the precomputer era, adaptive landscapes were often visualized as three-dimensional surface plots with only two traits (or genes) as independent variables. Adaptive landscapes then mainly served a heuristic function for qualitative reasoning, rather than quantitative analysis. With the rapid

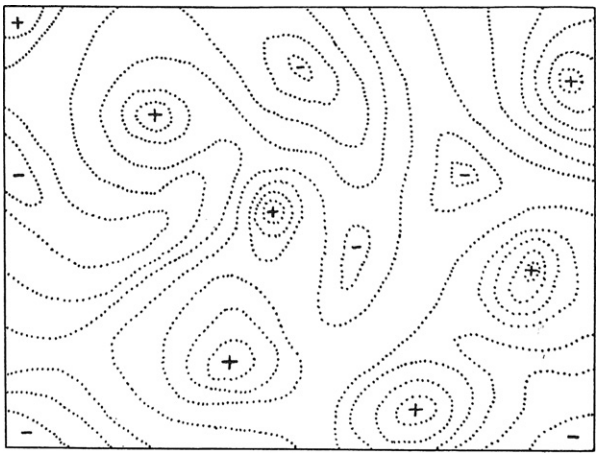

Figure 1 Sewall Wright's classical adaptive landscape figure. The contour plots show various genetic combinations of high fitness ('+' in figure), separated by fitness valleys ('−' in figure). Sewall Wright introduced the concept of the adaptive landscape in his 1932-publication (Wright, 1932). Reproduced from Skipper, R.A., Dietrich, D., 2012. Sewall Wright's Adaptive Landscape: Philosophical reflections on heuristic value. In: Svensson, E.I., Calsbeek, R., (Eds.), The Adaptive Landscape in Evolutionary Biology, Oxford: Oxford University Press, pp. 16–25, with permission from original publisher.

development of evolutionary quantitative genetic theory and increasing computer power, adaptive landscapes are now one of several analytical tools for modern evolutionary biologists. However, the adaptive landscape concept has also generated many criticisms from both philosophers and evolutionary biologists (Kaplan, 2008; Svensson and Calsbeek, 2012).

Influence on Various Fields of Evolutionary Biology

Sewall Wright's main scientific rival at the time when he formulated the adaptive landscape concept was the British mathematical population geneticist Ronald Fisher, who had a different view of the evolutionary process (Provine, 1986; Frank and Slatkin, 1992). Frank and Slatkin (1992) discussed the different views of Sewall Wright and Ronald Fisher, and they argued that whereas Wright's goal was to establish a dynamical theory of evolution, Fisher was more interested in evolutionary equilibria, as reflected in his formulation of the 'Fundamental Theorem of Natural Selection', rather than being interested in adaptive landscapes.

The paleontologist George Gaylord Simpson took the adaptive landscape concept of Sewall Wright into the field of macroevolution of phenotypes, and also discussed so-called quantum evolution – the rapid appearance of novel traits and lineages often seen in fossil record (Simpson, 1944; Kirkpatrick, 1982). The adaptive landscape does still influence paleontological research, particularly those who try to interpret stratigraphic time series in the fossil record (Hunt, 2007; Hunt *et al.*, 2008; Bell, 2012). Unlike Sewall Wright, Simpson thus focused on quantitative phenotypic traits, regardless of their genetic basis, which later influenced evolutionary quantitative genetic theory (Hansen, 1997; Arnold *et al.*, 2001). A major step forward in evolutionary quantitative genetics was achieved by modeling the temporal dynamics of Gaussian phenotype distributions and how these distributions change over time due to natural selection and genetic drift (Lande, 1976). Arnold *et al.* (2001) argued that the adaptive landscape provides a natural conceptual bridge between micro- and macro-evolution. In ecology, the adaptive landscape metaphor has perhaps been most influential in the fields of adaptive radiation and ecological speciation (Schluter, 2000) and in analyses of the evolutionary consequences of competition (Fear and Price, 1998). The adaptive landscape has also been a major influence in experimental evolution studies, particularly in studies on microbes such as *Escherichia coli* (Lenski and Travisano, 1994; Cooper, 2012).

Another field where adaptive landscape theory has been strongly influential are phylogenetic comparative methods, where models are now being developed to test various scenarios of phenotypic evolution and adaptive landscape dynamics, including models for evolutionary stasis, peak shifts, random bursts, and directional selection (Uyeda and Harmon, 2014). An important conceptual insight has been that dynamics of phenotypic traits over evolutionary time might sometimes better be described by an Ornstein–Uhlenbeck (OU) process rather than by the traditional stochastic Brownian motion (BM) model (Hansen, 1997). OU models are thought to capture stabilizing selection around adaptive peaks better than BM models, as there is often a signature of a significant 'pullback force' that is usually interpreted as the signature of stabilizing selection (Hansen, 1997).

There has recently been increasing interest in applying adaptive landscape theory to problems in molecular evolution, such as metabolic networks. One key insight that has stimulated this interest is that multiple genotypes can give rise to the same phenotype(s), implying that they can be more or less neutral with respect to fitness. Thus, there might be multiple genetic solutions to the same ecological problem facing organisms surviving in complex environments. In multidimensional genotypes, there can often be a series of mutational steps connecting genotypes to each other, and populations can therefore traverse extensively in genotype space on nearly-neutral ridges (Gavrilets, 2004; Wagner, 2012). With the rapid development of molecular biology and related fields, the adaptive landscape has also recently emerged at the intersection between evolutionary biology and systems biology (Kogenaru *et al.*, 2009) and has caught the attention of researchers interested in protein evolution (Carneiro and Hartl, 2010).

Analytical Approaches and Methods of Study

There are five traditional major empirical approaches to study adaptive landscapes:

1. Measuring and quantifying the mode of selection in natural populations
2. Experimental manipulations of individual genotypes, phenotypic traits or agents of selection
3. Experimental evolution
4. Phylogenetic comparative methods
5. Inferences from time series in the fossil record.

All these methods have their pros and cons, and in-depth reviews and discussions of each of the approaches can be

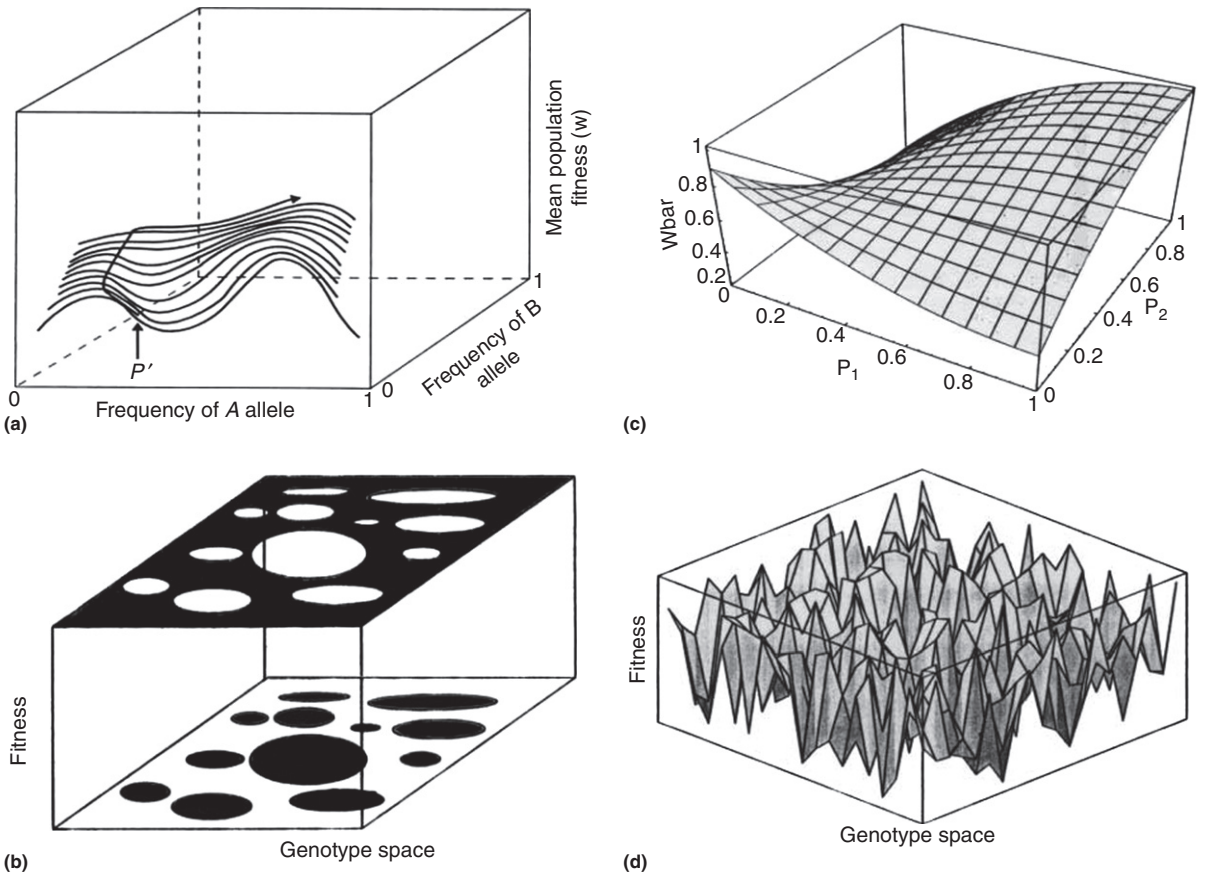

Figure 2 Four different adaptive landscape versions that have grown out from various critiques of Sewall Wright's original adaptive landscape. (a) 'Fisherian' adaptive landscape with a single fitness peak. (b) 'Holey adaptive landscape', where genotypes can either have high or low fitness, as formulated by Gavrilets (2004). (c) 'Wrightian' adaptive landscape with two fitness peaks. (d) 'Rugged adaptive landscape.' Reproduced Skipper, R. A., Dietrich, D., 2012. Sewall Wright's Adaptive Landscape: Philosophical reflections on heuristic value. In: Svensson, E.I., Calsbeek, R., (Eds.), The Adaptive Landscape in Evolutionary Biology, Oxford: Oxford University Press, pp. 16–25, with permission from original publisher.

found elsewhere (Endler, 1986; Harvey and Pagel, 1991; Rose and Lauder, 1996). Methods 1, 4, and 5 are correlative approaches and are inferential, whereas methods 2 and 3 manipulate either phenotypes, selective environments, or both.

The analysis and visualization of adaptive landscapes and fitness surfaces have become increasingly sophisticated with the development of multivariate statistical techniques. Today, evolutionary biologists can describe fitness surfaces both in terms of elevation and curvature and have developed a rigorous formal mathematical framework to quantify adaptive peaks and fitness valleys as well as interactions between traits ('correlational selection') and how they affect fitness (Arnold *et al.*, 2001; Sinervo and Svensson, 2002; Blows, 2007). The development of multivariate statistical methods to analyze fitness surfaces now means that evolutionary biologists are no longer constrained about thinking about adaptive landscapes in only three dimensions, which has been a frequent criticism of adaptive landscapes (Kaplan, 2008). The parameters that are used to quantify fitness surfaces include directional selection gradients (β), curvilinear, and correlational selection gradients (Phillips and Arnold, 1989). Fitness is then modeled as response surface, which is depicted against one or several underlying phenotypic traits (predictor variables).

Different Versions of Adaptive Landscapes

Landscape Structure

Several different types of adaptive landscapes have been developed since Wright (1932), and evolution on these will follow very different dynamics (Skipper and Dietrich, 2012; Figure 2). 'Wrightian adaptive landscapes' are characterized by multiple adaptive peaks, and fitness valleys in between them, reflecting the fitness epistasis that is expected from strong gene interactions. 'Fisherian adaptive landscapes' are smoother than their Wrightian counterparts, and are characterized by a single adaptive peak toward which the population moves through the action of mass selection in large and panmictic populations. In contrast, in the 'rugged adaptive landscapes' envisioned by Wright, populations get stuck on local adaptive peaks for a very long time, being unable to move across fitness valleys. In 'holey adaptive landscapes,' evolution can easily proceed along neutral or semi-neutral ridges that connect genotypes of approximately equal fitness to each other throughout genotype space.

The target model of adaptation is an application of adaptive landscape theory that was initially proposed by Fisher (1930), and formally modeled by Orr (1998) to understand

the genetic architecture of the process of adaptation by natural selection toward a fixed optimum. The target model predicts an exponential size distribution of the fixed factors involved in adaptation, with genes of large effects typically fixed first, followed by genes of smaller effects as the population approaches the optimum (Orr, 1998). The underlying reason for this sequence of events leading to the fixation of factors of differing size is that early on in the population history, genes of major effect are more likely to push the population closer to the fitness optimum, but as the optimum is approached, large-effect mutations are more likely to lead to 'overshoot' of the optimum and hence decrease fitness (Fisher, 1930; Orr, 1998).

Landscape Dynamics

Natural and sexual selection often fluctuate strongly in natural populations when measured across different years or generations. These fluctuating selection pressures strongly suggest that adaptive peaks and valleys are not stable on microevolutionary timescales (Calsbeek *et al.*, 2012). Moreover, adaptive peaks and fitness valleys are expected to be even less stable on macroevolutionary timescales, due to major climatic and biotic changes (Hansen, 2012). The ecological causes and evolutionary consequences of such landscape dynamics are largely unknown and are subject to much discussion among evolutionary biologists. A distinction can thus be made between 'static' and 'dynamic' adaptive landscapes. Major changes in the environment, such as the movement into new 'adaptive zones' are generally considered to be the major explanation for peak movements on macroevolutionary timescales, as envisioned by Simpson (Simpson, 1944; Hansen, 1997; Arnold *et al.*, 2001).

On microevolutionary timescales, density- and frequency-dependent selection have attracted special interest as mechanisms causing landscape dynamics and the movement of adaptive peaks. For instance, under negative frequency-dependent selection (NFDS), a rare morph or genotype will experience high fitness because of its low frequency, but this fitness advantage will disappear as it increases in frequency and climbs the closest adaptive peak. Therefore, a fitness peak will become a fitness trough, over time and a fitness trough will turn into a fitness peak across generations, as the genetic composition of the population changes. The adaptive landscape will therefore be more similar to an 'ocean' with moving waves than a static mountain range. Other ecological interactions and processes such as coevolutionary arms races and 'Red Queen'-dynamics can generate similar effects, and Fisher emphasized this aspect of the evolutionary process in his 'Fundamental Theorem of Natural Selection' (Frank and Slatkin, 1992). Therefore, under NFDS and in similar ecological scenarios, population mean fitness will not necessarily increase, even though the fitness of some genotypes do increase, which points to the crucial distinction between the fitness surface of individuals and the mean fitness of the population (Fear and Price, 1998).

Controversies and Criticisms

The Problem of Dimensionality

One misunderstanding by less mathematically inclined philosophers and historians of science is the frequently repeated claim that adaptive landscapes are only meaningful in three dimensions and that they cease to exist or are meaningless in higher dimensions (Provine, 1986). This early criticism against the adaptive landscape essentially states that since organisms and genotypes are multidimensional, and cannot be restricted to two traits, the adaptive landscape should be abandoned and be replaced with 'formal modeling' (Kaplan, 2008). However, this criticism misses the fact that the phenotypic adaptive landscape in the tradition of evolutionary quantitative genetics does have a rigorous mathematical framework in the form of the visualization of both fitness surfaces through the estimation of parameters quantifying the steepness and curvature of adaptive peaks and valleys (Arnold *et al.*, 2001). This statistical framework is not restricted to only two traits and constitutes exactly the kind of formal modeling that has been requested by critics of the adaptive landscape. With the development of multivariate statistical tools from matrix algebra, such as canonical analysis (CA) and diagonalization techniques, it is now possible to model fitness surfaces in more than three dimensions and to investigate multiple traits simultaneously (Blows and Brooks, 2003). The frequently reiterated criticisms against the simplified three-dimensional fitness surfaces by Sewall Wright (Kaplan, 2008) are therefore no longer valid as modern mathematical, statistical, and computational tools are now regularly used to explore surfaces of increasing dimensionality, both at the phenotypic and genotypic levels (Gavrilets, 2004; Blows, 2007).

Fitness of Individual Genotypes or Allele Frequencies of the Population?

Provine, who published an early and influential in-depth biography of Sewall Wright (Provine, 1986), criticized the adaptive landscape metaphor and pointed to inconsistencies of the two different versions of the adaptive landscape: the genotypic version versus the one showing population allele frequencies. Provine argued these two versions of the adaptive landscape suggested by Wright were 'wholly incompatible,' and that it was only the adaptive landscape of the population allele frequencies that was meaningful. Provine further argued that the adaptive landscape of individual genotypes should be abandoned, as there was no natural way of arranging the discrete genotypes on the axes and essentially this version was meaningless. However, Gavrilets (2004) criticized Provine's view and showed that information about the fitnesses of individual genotypes could be used to deduce fitness landscapes at the population level (but not the other way around). Gavrilets therefore questioned the claim by Provine that these two types of adaptive landscapes were wholly incompatible as they instead are quite naturally connected through each other, as the population mean fitness is obviously influenced by individual fitnesses, but it is not necessarily the other way around (Fear and Price, 1998; Svensson and Calsbeek, 2012).

The adaptive landscape of individual genotypes and the adaptive landscape of allele frequencies of the population have influenced different subfields of evolutionary biology. The adaptive landscape of gene combinations has been particularly important in evolutionary genetics and the understanding of fitness epistasis (Wolf *et al.*, 2000; Phillips, 2008) and in speciation theory, particularly postzygotic speciation

and the development of hybrid incompatibility. In contrast, the adaptive landscape of allele frequencies has played a more prominent role in quantitative genetics and the evolution of phenotypic traits.

The Debate Over the Shifting Balance Theory

Considerable controversy and debate has been focused on the problem of peak shifts and the crossing of fitness valleys in the adaptive landscape. Sewall Wright's suggested solution to the problem of peak shifts and the dilemma for a population that has to traverse through a valley of low fitness was outlined in his Shifting Balance Theory (SBT) of evolution (Wright, 1932; **Figure 1**). Wright argued that in large panmictic populations, peak shifts were unlikely to happen, as mass selection would predominate and the role of genetic drift would be negligible. At the other extreme, in very small populations, genetic drift is stronger and such small populations would potentially go extinct at a high rate. However, in populations of intermediate size that are connected through moderate gene flow, genetic drift could interact with local selection and shape evolutionary outcomes in a different way than would selection operating alone (Wade and Goodnight, 1998). Wright envisioned this ecological setting to be most favorable for peak shifts. SBT, since it was first suggested, has been subject to considerable controversy. For a critical perspective on SBT, where the authors argue it is unlikely to be important in adaptive evolution, see Coyne *et al.* (1997, 2000). For a defense of SBT and Wright's view of peak shifts, see Wade and Goodnight (1998) and Goodnight and Wade (2000). The different views expressed by these different authors illustrate a still existing and strong division between different branches of population genetics, relying either on Sewall Wright's or Fisher's modeling traditions (Provine, 1986; Frank and Slatkin, 1992).

Mathematical Models of Peak Shifts

Several other mathematical models of how peak shifts could happen have been developed. Among these models is the insight that peak shifts could more easily happen when environments fluctuate, so that fitness valleys may temporarily disappear (Whitlock, 1997). Peak shifts can also happen for purely intrinsic reasons, without any environmental change, through increased genetic variance from inbreeding or developmental noise (Kirkpatrick, 1982; Whitlock, 1995). Phenotypic plasticity and subsequent genetic accommodation have also been shown to be able to bring population closer to the domain of attraction of a new adaptive peak (Price *et al.*, 2003).

Peak shifts could also result from correlated responses to selection, if phenotypic traits are genetically correlated with each other, as they typically are (Price *et al.*, 1993). Genetic drift alone has also been shown to be able to cause peak shifts, although the effective population size (N_e) has to become extremely low for this to plausibly happen (Lande, 1985). Finally, the adaptive landscape of the population is influenced by both the relative fitness of different phenotypes and their relative frequency (Kirkpatrick, 1982). As a consequence, the adaptive landscape will 'flatten out' compared to the individual fitness surface, making fitness valleys shallower and potentially easier to cross than one would get the impression of if one only looks at individual fitness surfaces (Fear and Price, 1998). Taken together, these different models have shown that the problem of peak shifts might not be as severe as originally thought, and peak shifts can happen through several different mechanisms. The plausibility of each of these different scenarios is mainly a remaining empirical issue.

Adaptive Ridges and the Non-Existing Problem of Peak Shifts

An alternative perspective on the peak shift debate is that peaks might not exist in real (multidimensional) adaptive landscapes, because all such real adaptive landscapes are likely to have at least one ridge of high fitness between different peaks (Gavrilets, 2004). The increasing number of ridges with increasing dimensionality of the adaptive landscape leads to something which has been coined the 'extradimensional bypass,' relative to the situation for three-dimensional adaptive landscapes (Gavrilets, 2004). This view, therefore, does not deny the existence of the adaptive landscape, as did some early critics of adaptive landscapes, but, according to this view, there will exist large selectively neutral or nearly neutral regions in genotype space that can connect genotypes of approximately equal fitness to each other through networks. Thus, it is therefore the structure of the adaptive landscape, rather than its existence, that is the focus of this and other models of 'holey adaptive landscapes' (Gavrilets, 2004). In these models, the problem of peak shifts therefore disappears, as populations are expected to easily traverse on these adaptive ridges throughout more or less the entire genotype space.

Conclusions and Future Outlook

The adaptive landscape has stimulated a lot of empirical and theoretical research in various subfields of evolutionary biology, including evolutionary and population genetics, quantitative genetics, evolutionary ecology, macroevolution, paleontology, and speciation, to only mention a few such areas. The adaptive landscape has therefore been a very useful concept to evolutionary biologists, judged by the number of investigations and articles that has been published and which are anchored in the adaptive landscape tradition. It is even questionable if certain research fields would have existed at all if the adaptive landscape concept had not been formulated by Wright. These fields include experimental evolution, evolutionary quantitative genetics, and certain branches and models of phylogenetic comparative methods. Judged by this enormous interest, the adaptive landscape must surely be one of the most successful concepts in evolutionary biology. However, the adaptive landscape has also generated considerable controversy and criticisms, resulting in debates of which some have now been settled, whereas others still remain unsolved. Whether these debates have been useful to evolutionary biology or a waste of time is probably a matter of different opinion among different researchers. However, the adaptive landscape is likely to remain in the center of

evolutionary biology for decades to come, and there are signs that it is now attracting the interest of evolutionary biologists working on molecular evolution. Hopefully, future generations of evolutionary biologists can learn from these debates and avoid repeating misunderstandings and past mistakes about adaptive landscapes.

See also: Adaptation, History of. Coevolutionary Fitness Landscapes. Divergence and Diversification, Quantitative Genetics of. Ecological Speciation and Its Consequences. Evolutionary Biology, History of. Evolvability, Quantitative Genetics of. Founder Speciation. Gene Interactions in Evolution. Genotype to Phenotype: Insights from Evo-Devo. Macroevolution, Quantitative Genetics and. Natural Selection, Introduction to. Natural Selection, Measuring. Quantitative Genetic Variation

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https://www.youtube.com/watch?v=4pdiAneMMhU

Adaptive Molecular Evolution: Detection Methods

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Glossary

Balancing selection The maintenance of population genetic polymorphisms (alternative alleles) at a locus by the process of natural selection.

Coding DNA A sequence of DNA that codes for a sequence of amino acids that constitute a protein.

Codon A unit of three adjacent nucleotides that correspond to a specific amino acid. Codons are the units of the genetic code.

Empirical Bayes An approach to statistical inference that employs Bayes' rule in combination with a prior probability distribution estimated from a sample of data. This differs from the standard approach to Bayesian inference where the prior distribution is fixed before observing data.

Evolutionary comparative genetics A field of research where differences between genes or genomes are assessed within a phylogenetic framework.

Fixation event The process whereby one allele at a polymorphic locus increases in frequency until it comprises 100% of that population.

Fixation index A summary statistic applied to population genetic data for the purpose of measuring the magnitude of population genetic subdivisions (e.g., a measure of differences in the genetic composition of geographically separated populations).

Genetic code The relationship between 64 unique triplets of DNA (codons) and a set of 20 unique amino acids. The biochemical basis of protein synthesis within a cell is based on this relationship.

Likelihood ratio test A method of hypothesis testing based on comparing the fit of two competing models to a sample of data. One model represents the null hypothesis. The null model must be a special case of the model that is used to represent the alternative hypothesis.

Linkage disequilibrium Non-random association of allelic polymorphism at different loci within a genome.

Locus In population genetics it is the location of a gene, or a specific sequence of DNA, within a genome.

Markov model A model for a stochastic process of change between states where the probability of an event depends only on the current state, and not on any of the states that may have preceded it.

Maximum likleihood A statistical technique for estimating the unknown value of a population parameter from a sample of data. The value of the parameter that maximizes the probability of observing the sample of data is the maximum likelihood estimate of the unknown value.

Negative selection A type of natural selection that causes alleles with deleterious effects on fitness to decrease in frequency within a population.

Neutral theory A theory of evolution where the majority of genetic polymorphism observed within species, and genetic divergence observed between species, is attributed to the process of random genetic drift.

Neutrality test A test developed for detecting gene sequence evolution that does not fit expectations derived from neutral theory.

Noncoding DNA Any DNA sequence that does not code for a protein product.

Nonsynonymous mutation A mutation in coding DNA that alters the amino acid composition of the encoded protein.

Nonsynonymous substitution rate The rate at which nonsynonymous mutations are fixed within a population.

Polymorphism Within the discipline of population genetics, the existence of alternative forms of a gene (alleles) within a population.

Positive selection A type of natural selection that causes alleles with beneficial effects on fitness to increase in frequency within a population.

Purifying selection An alternative name for negative selection.

Selective sweep The reduction, or complete loss, of genetic polymorphism at loci immediately surrounding the region of a genome where an allele was fixed by positive selection.

Statistical power The probability that a statistical test will detect a signal in the data when that signal actually exists.

Substitution The population genetic process whereby one genetic variant at a locus is completely replaced by an alternative genetic variant at that locus.

Synonymous mutation A mutation in coding DNA that does not alter the amino acid composition of the encoded protein. Mutations within coding DNA can be synonymous due to the redundancy of the genetic code.

Synonymous substitution rate The rate at which synonymous mutations are fixed within a population.

Type-I functional divergence A condition of homologous DNA sequences where sites have different substitution rates within subsets of those sequences due to divergence in their function.

Type-II functional divergence A condition of homologous coding DNA where the amino acids of the proteins encoded by different subsets of sequences have different physiochemical properties. The important distinction from Type-I divergence is that evolutionary rates do not differ between the subsets of sequences.

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Introduction

Models of molecular evolution provide an important tool for investigating how cells and organisms function, and how they have adapted to environments over time. The challenge is to discriminate between the adaptive changes and the potentially large amount of neutral change occurring within a molecule. Knowledge of adaptive molecular change can have practical benefits such as providing insights into the genetic basis of disease susceptibility, evolution of drug resistance, and the spread of pathogens. This has motivated development of sophisticated statistical methods for detecting the signatures of adaptive evolution within DNA sequences. The purpose of this article is to provide a broad introduction to those methods. Because methods can differ greatly according to the type of sequence data, this article is divided into two sections: one is focused on analyzing within-population polymorphism, and the other is focused on analyzing between-species differences. The latter section is further divided according to the type of DNA (codon, amino acid, and noncoding) under study. The goal is to present a general survey of methods, with additional details provided only for the more frequently used approaches. Because several statistical frameworks are frequently used to detect adaptive molecular evolution, formal introductions to those are provided in three 'boxes.' Readers can skip over those boxes, as well as between different sections of this article, without loss of continuity.

Population Genetic Approaches

The availability of genome-scale datasets has transformed evolutionary biology and genomics. Within the field of population genetics, such data are providing an unprecedented opportunity to identify loci, or genomic regions, subject to adaptive evolution (e.g., Pool *et al.*, 2010). Methods for detecting adaptation from population data are most often used to identify a 'selective sweep' event. The selective sweep is initiated by the mutational origin of an advantageous allele. This allele is subsequently driven to fixation by strong positive selection. Neutral polymorphisms in the surrounding genomic region may increase in frequency along with the selected allele, depending on the degree of genetic linkage. Some of these neutral alleles may be 'swept' to fixation (this is the so-called hitchhiking effect) if the linkage is strong. Thus a sweep refers to the tendency for variation to be lost from the genome in the region immediately surrounding the advantageous allele. Completion of a sweep is expected to reduce nucleotide diversity, create an excess of low-frequency polymorphisms, increase homozygosity, and increase linkage disequilibrium (LD) within a localized region of a genome. A variety of statistical methods have been developed to detect these signatures of a selective sweep. As a sweep is driven by strong selection, most of the methods are expected to have reasonable statistical power. Power will be further improved when genome-scale polymorphism data includes rare genetic variants absent from smaller datasets (e.g., Pool *et al.*, 2010).

A class of methods based on detecting generalized departures from neutrality (Tajima, 1989; Fay and Wu, 2000; Fu and Li, 1993) are routinely used to search for selective sweeps. These 'neutrality' tests attempt to detect departures in the allele frequency distribution from expectations derived from a simple neutral model of population evolution (e.g., assuming random mating, no migration, mutation or selection, etc.). A difficulty with these methods is that they are sensitive to any departure from neutrality (selective sweeps, negative selection, or balancing selection). Their interpretability is improved by taking into account the spatial pattern within a genome because the frequency distribution will be most affected in the region around a selective sweep (Nielsen, 2005). However, a further difficulty with these methods is that they are also sensitive to violations of other assumptions about the population, such as constant size and homogeneity. Thus, population demographic processes, as well as genomic variability in the processes of mutation and recombination, may be misinterpreted as positive selection (Nielsen, 2001).

Another class of methods is based on detecting larger than expected levels of population differentiation. When a locus is subject to a selective sweep in a geographically restricted population, alleles in the surrounding genomic region undergo a rapid change in frequency which leads to a localized increase in the signal for population structure. Methods developed to detect such signal often employ a fixation index (F_{st}) to summarize the level of population differentiation (Akey *et al.*, 2002; Weir *et al.*, 2005). However, there are other means of detecting genomically localized population structure (e.g., Beaumont and Balding, 2004; Nielsen *et al.*, 2009; Chen *et al.*, 2010). Just as with the neutrality tests, variability in the process of mutation and recombination, as well as demographic processes, can increase the within-genome variance in the patterns of population differentiation. Comprehensive demographic models can be used to identify and remove variance due to demographic rather than selective processes (Li and Stephan, 2006; Nielsen *et al.*, 2009).

A selective sweep will also increase the association of alleles at different loci in the region surrounding the target of the sweep. When this association is greater than the neutral expectation, it is referred to as LD. Several tests based on a variety of summary statistics have been developed for detecting selection according to LD (Hudson *et al.*, 1994; Kelly, 1997; Depaulis and Veuille, 1998; Andolfatto *et al.*, 1999; Kim and Stephan, 2002; Sabeti *et al.*, 2002; Kim and Stephan, 2003). All these methods exploit the increase in LD that occurs during a selective sweep. Because signal for LD decays as a function of the local recombination rate, this approach will have lower power to detect older events because recombination will have had more time to break down associations created during the sweep. However, the approach is critical to detecting cases where the fixation process is not yet complete (partial sweep) or the strength of selection varies over time (soft sweep).

An alternative to joint analysis of multiple genomic loci is provided by the Poisson random field (PRF) family of methods (Sawyer *et al.*, 1987; Sawyer and Hartl, 1992; Akashi, 1999a; Bustamante *et al.*, 2001, 2003). PRF differs from the above methods because it does not utilize the pattern of variation in the regions surrounding a positively selected locus. It is attractive because it permits inference of selection intensity for different classes of mutations within a principled statistical framework. To accommodate the potential impact of demographic changes, Williamson *et al.* (2005) extended the PRF

approach via a two-step analytical process. In the first step, a selectively neutral class of mutations is used to test for demographics that would lead to rejection of a simple neutral model. In the second step, the parameters of an appropriate demographic model are used to correct for demographic effects on PRF-based tests for selection.

Inferences about selection can be extended by comparing patterns of within-species DNA polymorphisms to divergence between species. This approach allows detection of loci subject to recurrent selective fixations, as well as differentiating between weak and strong purifying selection. The main idea was first proposed by McDonald and Kreitman (1991) in the context of two classes of coding mutations: synonymous (amino acid preserving) and nonsynonymous (amino acid altering). Under neutrality, the ratio of nonsynonymous to synonymous polymorphisms within species should be equal to the ratio between species. Given comparative population data for closely related species, the patterns of polymorphism and divergence are easily summarized as counts within a contingency table (hereafter referred to as the MK method). Thus, it provides an efficient means of detecting non-neutral loci in large datasets (e.g., Bustamante *et al.*, 2005). Unlike many of the above approaches, this method is generally robust to demographic processes (Nielsen, 2001). However, it cannot discriminate between recent and past selective events, and it is not well suited for detecting the focus of a partial or soft selective sweep (Nielsen, 2005). Notwithstanding these limitations, the MK method can provide considerable information about the direction and intensity of selection, and this has led to its extension to several different forms of molecular adaptation (Akashi, 1995, 1999b; Andolfatto, 2005). The analytical framework of the MK method has been widely employed to estimate the historical fraction of substitutions that were fixed by positive selection (see Eyre-Walker (2006) and Fay (2011) for reviews).

Both the PRF and MK methods exploit the information contained within the frequency distribution of population polymorphisms. They differ in that the PRF methods utilize the full polymorphism frequency spectrum, whereas the MK methods collapse that spectrum into counts of just two types of polymorphism. Both approaches, however, depend on the same key assumptions: site independence and the infinite-sites approximation. Although the MK methods should be less sensitive to assumption violations (Desai and Plotkin, 2008), the interpretation of its results is not free from such concerns (e.g., Fay, 2011). It is best to consider the signal detected using either the PRF or MK methods as potentially sensitive to any non-selective processes that impact the polymorphism frequency spectrum (Desai and Plotkin, 2008). Examples of such processes include biased gene conversion and among-site variability in synonymous substitution rates, but other sources are just as plausible (e.g., Fay, 2011). These limitations can be overcome by relaxing the assumptions inherent in the original methods. Readers are refereed to Desai and Plotkin (2008) and the references therein for examples of this line of method development.

Comparative Evolutionary Approaches

In comparative sequence analysis, the differences between sequences are due to one or more substitution events. A substitution event requires that a novel mutation has passed through a period of polymorphism, and become fixed in a population. This contrasts with population data, where variation among sequences is predominantly attributable to genetic polymorphisms within a population. Comparative data are rich in information about the substitution process over deeper timescales, and the purpose of methods developed for such data is to distinguish between a neutral substitution process and a process that has been impacted by natural selection.

The most widely used comparative methods are derived from the general principle that the substitution rate is inversely related to the level of functional constraint (Kimura, 1985). Methods developed explicitly for coding sequences seek to identify significant differences in the rate of amino acid evolution over time or among sites (e.g., Yang, 1998; Gu, 1999; Susko *et al.*, 2002); either of these can be interpreted as evidence of a change in the intensity of natural selection acting on the protein product. The fact that substitution rates can be measured in two distinct ways for coding sequences – synonymous and nonsynonymous – has led to the development of two different analytical strategies. The first scales the nonsynonymous substitution rate according to the inverse of the synonymous (or neutral) rate. This scaling is intended to normalize the nonsynonymous rate relative to the neutral rate, thereby providing a readily interpretable index of the strength and type of natural selection (Kimura, 1985). Being dependent on the synonymous rate, however, means that this approach can't be used for highly divergent sequences where the synonymous rate is difficult to estimate reliably. The second strategy employs the absolute rate of nonsyonymous substitutions. While this is suitable for highly divergent coding sequences, it cannot be used to distinguish among different types of selection pressure. Comparative methods were also developed for noncoding DNA, and they employ the same principle, i.e., the rate on noncoding sequence evolution will be inversely related to the intensity of selective constraint. When applied alongside an analysis of polymorphism data the comparative approaches contribute to a comprehensive assessment of selective pressure that includes both historical and ongoing evolutionary processes (Nielsen *et al.*, 2005).

Codon Analyses

Protein coding sequences can be analyzed with respect to just the changes between nucleotides (i.e., just A, C, G, and T), but this approach will not capture any information related to the process of amino acid substitution. Analyzing evolution at the codon level provides a way forward: (1) evolution can be placed in the context of a specified genetic code, (2) nonindependence of substitutions at different positions within a codon is accommodated, and (3) the processes of synonymous and nonsynonymous substitution can be explicitly separated. Because the rate of synonymous substitution (d_S) is the rate a coding sequence is expected to evolve had there been no selection on the protein product, each coding sequence can be viewed as containing information about its own neutral rate. Because the majority of nonsynonymous mutations are expected to reduce fitness, they will be removed from a

population by selection more frequently than expected under neutrality, and the nonsynonymous rate (d_N) for the same sequence will be lower than d_S. The size of this effect is a function of the intensity of negative selection pressure, with the ratio of those rates ($\omega = d_N/d_S$) taking values less than 1. If, however, the structure or function of a protein becomes unconstrained for any reason, then synonymous and nonsynonymous mutations will be fixed at about the same rate ($\omega \approx 1$). The only way d_N can exceed d_S is by positive Darwinian selection. In this way $\omega = d_N/d_S$ serves as an index of the strength and type of natural selection pressure (Kimura, 1985).

Early codon models were developed for estimating the distance between pairs of sequences in terms of both d_N and d_S (e.g., Miyata and Yasunaga, 1980; Nei and Gojobori, 1986; Yang and Nielsen, 2000). Because the observed differences between a pair of sequences will underestimate the actual divergence as evolutionary time increases, distance methods attempt to correct d_N and d_S so that they are approximately linear with time. Distance methods have the drawback that they produce nonindependent estimates of d_N and d_S for a multispecies alignment. To avoid this, the approach was extended to phylogenetic trees (Nei and Gojobori, 1986). A simple approach to phylogenetic estimation of d_N and d_S was to reconstruct ancestral states for the interior nodes of a phylogeny and count differences between codons across the tree (Messier and Stewart, 1997).

Estimation of d_N and d_S is a complicated task. Early methods employed *ad hoc* corrections for processes of sequence evolution that can negatively impact rate estimates (e.g., Bielawski *et al.*, 2000; Dunn *et al.*, 2001). Maximum likelihood (ML) estimation of the ratio ω under a Markovian model for codon evolution obviates the need for *ad hoc* corrections (Muse and Gaut, 1994; Goldman and Yang, 1994). **Box 1** uses a very simple probability model (binomial) to present a general introduction to the principle of ML. Although codon models represent a considerable increase in complexity, the principles presented in **Box 1** are directly applicable. Most importantly, the *ad hoc* corrections employed by the early distance methods are not necessary under the ML approach, because corrected estimates of d_N and d_S are a natural consequence of the probability calculations carried out under a Markovian model of codon evolution. A further benefit is that the ML approach permits estimation of ω jointly for all the sequences in the alignment, and so permits use of more complex models in a phylogenetic framework. More recently, fully Bayesian methods have been developed (Huelsenbeck and Dyer, 2004; Angelis *et al.*, 2014). Like ML, fully Bayesian methods employ a probability model for codon evolution. However, they also require prior probability distributions for model parameters which are subsequently modified by the sequence data (under the assumed model) to obtain posterior distributions. Rather than seeking to estimate an optimal value for the ω parameter, Bayesian methods seek to estimate a distribution for ω.

The ML and Bayesian methods have several advantages over methods that rely on estimates of ancestral sequences. They take uncertainty about the ancestral states into account by integrating over all possible ancestors, each weighted by their respective equilibrium frequencies. Furthermore, they provide a natural means to estimate the number of hidden substitutions along a branch. They are also amenable to modeling important aspects of DNA sequence evolution, such as transition bias and codon-usage bias, which have proved to be difficult to accommodate without a probability model. These advantages come at the cost of computational complexity. The time required for analyses involving a large number of taxa can be prohibitive without substantial computational resources.

The ML and Bayesian methods assume that the evolutionary process is Markov, meaning that the probability distribution for the identity of a substituting codon at a given site depends only on the identity of the codon currently

Box 1 Maximum Likelihood

A probability model $f(x|\theta)$ is a function that gives the probability of an event x given a vector of model parameters θ. For example, the probability of getting heads x times in three tosses is given by the model

$$f(x|\theta) = \frac{3!}{(3-x)!x!}\theta^x(1-\theta)^{3-x}$$

where θ is the probability of a heads on one toss. The probabilities of various outcomes $x \in \{0,1,2,3\}$ can be computed if θ is known.

There are many scenarios in science where the parameter vector is unknown. In such cases, the outcome of a set of independent trials can be used to estimate θ. Suppose three tosses resulted in $x=2$. Then, treating the data as fixed and the parameter as the variable, the above equation can be written as:

$$L(\theta|x=2) = \theta^2(1-\theta)$$

This equation gives the likelihood of θ given the data. Note that the likelihood function only retains the components of $f(x|\theta)$ that are dependent on the unknown parameter. Given the results $\{x_1,\ldots, x_n\}$ of n independent trials, we can make use of the fact that the probability of a series of independent events is just the product of the probabilities of the individual events and write:

$$L(\theta|\{x_1,\ldots,x_n\}) = \prod_{i=1}^{n} \theta^{x_i}(1-\theta)^{n-x_i}$$

Since it is easier to work with sums than products, it is customary to take the logarithm of both sides of this equation:

$$\ell(\theta|\{x_1,\ldots x_n\}) = \sum_{i=1}^{n} \{x_i \log(\theta) + (n-x_i)\log(1-\theta)\}$$

The maximum likelihood estimate (MLE) for θ is the value $\hat{\theta}$ that maximizes the log-likelihood function $\ell(\theta|x_1,\ldots, x_n)$.

Although probability models for sequence evolution are considerably more complex than the model presented here, the underlying framework is the same. The principle of maximum likelihood is used to infer values for parameters ($\hat{\theta}$) related to the process of codon (e.g., Nielsen and Yang (1998) in Section Codon Analyses), amino acid (e.g., Gaston *et al.* (2011) in Section Amino Acid Analyses), and noncoding evolution (e.g., Wong and Nielsen (2004) in Section Analysis of Noncoding Sequences).

occupying that site. This property is captured by a rate matrix Q with elements q_{ij} that specify the instantaneous rates at which the i^{th} codon is replaced by the j^{th} codon. In Nielsen and Yang (1998), for example, elements of Q were defined for codons i and j ($i \neq j$) as:

$$q_{ij} = \begin{cases} 0 & \textit{if } i \textit{ and } j \textit{ differ by more than one nucelotide} \\ \pi_j & \textit{for synonymous transversions} \\ \kappa\pi_j & \textit{for synonymous transitions} \\ \omega\pi_j & \textit{for nonsynonymous transversions} \\ \omega\kappa\pi_j & \textit{for nonsynonymous transitions} \end{cases}$$

Model parameters, including transition bias κ, rate ratio $\omega = d_N/d_S$, and branch lengths $b=\langle b_1,\ldots,b_n\rangle$ can be estimated by ML (**Box 1**). The vector of codon equilibrium frequencies $\pi=\langle\pi_1,\ldots,\pi_{61}\rangle$ is usually estimated from the observed nucleotide frequencies. Given the full set of parameter estimates, the matrix that specifies replacement probabilities is $P(t)=e^{Qb}$ along a branch of length b. The probability model is therefore fully specified by the rate matrix Q and the branch lengths (with the assumed tree topology). Readers are referred to the 'See also' section for a more detailed treatment of Markovian models, rate matrices, and an expanded discussion of models for protein coding sequences. Certain simplifying assumptions are standard in the formulation of those models, and they are thoroughly reviewed in that entry.

Since they are based on the same Markov framework, Bayesian methods share many of the same advantages of ML. However, ML is more widely used, likely because it is less computationally costly. Under ML, the values of ω, κ, b and π_1, $\ldots,\pi_{61}$ that maximize the likelihood function given sequence data are considered the optimal estimates. Nested models can be contrasted to test hypotheses using the likelihood ratio test (LRT) (**Box 2** presents a general introduction). LRTs are routinely used to test the hypothesis that a fraction of sites within a dataset have evolved under positive selection ($\omega>1$). Given a significant LRT, an empirical Bayesian analysis (not to be confused with the full Bayesian approach) is typically used to identify those codon sites within the data most consistent with evolution by positive selection (see **Box 3**). A very large variety of codon models have been developed for identifying sites within an alignment, or branches in the tree, whereby positive selection had likely acted. A comprehensive review of these models is not possible here. Readers are referred to excellent reviews by Anisimova and Kosiol (2009) and Delport *et al.* (2009) for additional information.

The introduction of the first codon models for detecting positive selection ($\omega>1$) was followed by a number of studies claiming that the ML approach is inherently prone to produce false positives or is too liberal in inferring positive selection. Most of these claims were later shown to have overstated the problem (Pond and Frost, 2005; Yang *et al.*, 2009; Zhai *et al.*, 2012). However, there are conditions under which false positives occur at rates slightly higher than the level of significance of the LRT. Anisimova *et al.* (2002), for example, showed that false positive rates generally tend to be elevated when information content is low. Analyses are generally at greater risk of producing false positives when they include too few sequences, are comprised of very similar sequences, or are comprised of sequences that are divergent to the point of saturation.

ML estimates of codon model parameters can be viewed as those that cause the model to 'absorb' as much of the variability in the data as possible. Such estimates are unbiased if the model is a good match for the true evolutionary process. However, if the model is misspecified, then parameters such as ω may be forced to absorb variability in the data produced by processes unrelated to the rate ratio d_N/d_S. In such cases its ML estimate may be statistically biased (e.g., Bao *et al.*, 2008; Bay and Bielawski, 2013). This potential for biased parameter estimates is difficult to diagnose or avoid, but can be mitigated by combining the results of multiple modeling approaches that support the same conclusions.

The applicability of $\omega>1$ as a signature of positive selection is limited to detecting cases that produce a comparatively large number of nonsynonymous substitutions (Yang and Bielawski, 2000), whereas the mainstay of molecular evolution may well involve scenarios where a small number of nonsynonymous mutations are fixed, after which the gene resists further modification. There are several scenarios where repeated nonsynonymous substitutions are likely to occur. These include (1) when there are antagonistic interactions between immune surveillance and immune evasion proteins (e.g., Yang and Swanson, 2002); (2) when there are similar interactions between proteins that promote the competing interests of gametes in diploid organisms (e.g., Swanson *et al.*, 2003); (3) when rapid reorganization of a protein occurs in response to a change in the environment (e.g., Bielawski *et al.*, 2004). Such scenarios may be relatively uncommon, however.

It is therefore incumbent on the investigator to make intelligent use of the existing codon models. There will be many

Box 2 Likelihood Ratio Test

The probability model $f_0(x|\theta_0)$ is nested within another model $f_1(x|\theta_1)$ if they differ only in that the parameter vector θ_0 is the same as θ_1 but with some or all of its components fixed according to a null hypothesis. Continuing with the coin tossing example, we might take the null hypothesis to be that the coin is fair: H_0:$\theta=0.5$. The alternative might be that the coin is not fair: H_a:$\theta\neq0.5$. Given the outcomes of n independent trials, the log-likelihood of $\hat{\theta}$ can be contrasted with the log-likelihood of $\theta=0.5$ by computing the likelihood ratio (LR) statistic:

$$\mathrm{LR} = 2\left(\ell(\hat{\theta}|\{x_1,\ldots,x_n\}) - \ell(0.5|\{x_1,\ldots,x_n\})\right)$$

Statistical theory predicts that the sampling distribution of LR is close to χ^2_1, a χ^2 distribution with one degree of freedom, provided n is not too small. A p-value for LR can therefore be computed from χ^2_1. The null hypothesis would be rejected if the p-value was small, less than 0.05, say.

The LRT is often used to formally test hypotheses about evolution of protein coding sequences by positive selection (e.g., Nielsen and Yang (1998) in Section Codon Analyses), divergence in the distribution of selective constraints between proteins (e.g., Gaston *et al.* (2011) in Section Amino Acid Analyses) and evolution of noncoding DNA by positive selection (e.g., Wong and Nielsen (2004) in Section Analysis of Noncoding Sequences).

Box 3 Bayes' Rule

Suppose 5% of the population is afflicted with a disease so that the probability that a randomly selected person has the disease is $P(D)=0.05$. And suppose there is a test for the disease that is 99% accurate, meaning that the probability that the test is positive given that the person tested has the disease is $P(+|D)=0.99$, and the probability that the test is positive for a person without the disease is $P(-|\overline{D})=0.01$. Then according to the Bayes' rule, the probability that a person who tested positive actually has the disease is:

$$P(D|+)=\frac{P(+|D)P(D)}{P(+|D)P(D)+P(+|\overline{D})P(\overline{D})}$$
$$=\frac{0.99\times 0.05}{0.99\times 0.05+0.01\times 0.95}$$
$$=0.84$$

In this Bayesian analysis, $P(D)$ is the prior probability that a randomly selected person has the disease, and $P(D|+)$ is the posterior probability that arises after the outcome of the test is observed. $P(D)$ was considered in this analysis to be known. If it was not known but estimated from data, then $P(D)$ would be called an empirical prior and the analysis called empirical Bayes.

In the context of Markov models for sequence evolution, parameter values estimated from sequence data typically provide the empirical prior probability that a randomly selected site has evolved under a particular evolutionary regime (e.g., purifying or positive selection). Depending on the type of molecular data, the h^{th} site pattern x_h can be a column of nucleotides, codons, or amino acids within a multisequence alignment. Taking positive selection as an example (Section Codon Analyses), the parameters estimated from codon data provide the empirical prior probability that a randomly selected codon site has undergone positive selection, $P(\omega>1)$. The posterior probability of evolution by positive selection at the h^{th} codon site given the observed site pattern, $P(\omega>1|x_h)$, can then be computed using Bayes' rule. Codon sites are selected for further investigation if they have large posterior probabilities for positive selection. The same approach is used to identify amino acid sites subject to divergent selection intensity (Section Amino Acid Analyses) and noncoding sites evolving by positive selection (Section Analysis of Noncoding Sequences).

scenarios where protein function can evolve adaptively without yielding $\omega>1$, and researchers must avoid overinterpreting the significance of any one measure of sequence evolution, including the ω ratio. To avoid an incomplete picture of adaptive sequence evolution, researchers should seek a comprehensive assessment of natural selection pressure, combining information across different levels (e.g., Nielsen *et al.*, 2005) and from different metrics of functional divergence (e.g., Li *et al.*, 2009).

Amino Acid Analyses

Amino acid data can be used to determine whether the process of molecular evolution is related in some way to divergence of molecular function. While this is not equivalent to distinguishing between different types of selection pressure using a codon model, the results of such analyses do have a selective interpretation. If the 'function' of a gene is defined as any aspect of its phenotype that impacts fitness (structural, biochemical, or developmental), then molecular function can be related to the population genetic parameter for selection intensity. Specifically, neutral theory predicts that amino acid sites will evolve slower under strong selective constraints as compared to sites having weak selective constraints (Kimura, 1985); thus, a change in function that changes the distribution of selective constraints is also expected to yield a change in the distribution of substitution rates across amino acid sites (Gu, 1999). Such analyses have been used to infer the functional and adaptive significance of amino acid substitutions at sites within protein coding genes (e.g., Knudsen *et al.*, 2003; Mertz *et al.*, 2009).

Comparative analysis of amino acid substitution rates typically involves comparisons between two groups of sequences. In this context, methods have been developed to detect one of two broad patterns of sequence variability. The first pattern is simply the case where homologous sites within each group of sequences have different evolutionary rates. Although the moniker 'rate shifting' given to this pattern by Abhiman and Sonnhammer (2005) is accurate, the formal classification is "Type-I functional divergence" (Gu, 1999). The second pattern is characterized by homologous sites that are evolutionarily conserved in both groups but are occupied by amino acids with different physiochemical properties. The moniker "constant but different" (Gribaldo *et al.*, 2003) is appropriate, but the formal classification is "Type-II functional divergence" (Gu, 1999). Any evolutionary event where functional divergence can be plausibly hypothesized (gene duplication event, horizontal gene transfer, cross species viral transmission, adaptive radiation, etc.) can be investigated for Type-I and Type-II divergence. This approach has been most effectively used to investigate functional divergence following gene duplication events (e.g., Gribaldo *et al.*, 2003; Mertz *et al.* 2009; Li *et al.*, 2009).

Various analytical methods have been developed to detect Type-I and Type-II divergence. For convenience we divide them into two broad groups. The first group employs any of a wide variety of site-specific conservation statistics (sequence diversity, information theoretic measures of variability, physiochemical properties, etc.) to identify constrained sites within predefined subgroups (e.g., Capra and Singh, 2008). The methods in this group share a single characteristic in so far as they do not exploit the phylogenetic relationships of the sequences. The second group of methods utilize the phylogenetic information within a dataset through the use of an evolutionary tree and an explicit evolutionary model. These phylogenetically aware approaches substantially improve the inference of site-specific rate differences, as well as the accuracy of predictions about function (e.g., Lichtarge *et al.*, 1996; Pupko and Galtier, 2002; Susko *et al.*, 2002; Gaston *et al.*, 2011). Although information theoretic methods such as those of Lockless and Ranganathan (1999) and Suel *et al.* (2003) have been combined with phylogenetically aware analyses to provide additional insights into the context of functional divergence (see Garvin *et al.*, 2011 for an example), here we focus on the phylogenetcially aware methods alone, as they are better able to distinguish patterns of sequence variation arising

by neutral processes from those that arise from a shift in functional constraints (Gaston *et al.*, 2011).

Typically, a phylogeny is divided a priori into two or more subtrees (usually based on a known evolutionary event(s) such as a gene duplication). Then, the distribution of rates is estimated separately for the subtrees. Methods differ in how rate shifts are measured, how they are tested, and how other parameters of the evolutionary process are modeled (Gu, 1999, 2001; Knudsen and Miyamoto, 2001; Gu and Vander Velden, 2002; Pupko and Galtier, 2002; Susko *et al.*, 2002; Knudsen *et al.*, 2003; Gaston *et al.*, 2011). The most widely used approach of this type was developed by Gu (1999, 2001), and is based on probabilistic modeling of gene sequences as a mixture of two types of sites: (1) those with constant rates throughout the tree, and (2) those with different rates in different subtrees. An LRT is employed to test the hypothesis that the fraction of sites with different rates in subtrees is greater than zero (**Box 2**). Following a significant LRT, empirical Bayes (**Box 3**) is then used to identify sites within a gene for which the rates differ across subtrees. Gaston *et al.* (2011) recently extended this phylogenetic mixture-model framework by employing improved models of amino acid exchangeabilties, such as JTT (Jones *et al.*, 1992), WAG (Whelan and Goldman, 2001), or LG (Le and Gascuel, 2008), and among-site variability in the amino acid usage profiles (Wang *et al.*, 2007). The likelihood-based modeling approaches of Gu (1999, 2006) and Gaston *et al.* (2011) offer a formal statistical framework for detecting both Type-I and Type-II divergence. However, such models assume independence of substitution rates among sites. When functional divergence tends to occur within regions of genes, methods that assume independence will be less powerful. To address this limitation, Huang and Golding (2012) developed a model to account for spatial autocorrelation (at the gene sequence level) among sites evolving under Type-I divergence. More recent work by Huang and Golding (2014a,b) to infer spatial correlation of substitution rates at the level of the protein 3D structure offers a promising direction of future model development.

Often there is either no biological information by which a tree can be split a priori into subtrees or there is some reason to think the available prior information is incomplete. In such cases, inferring the locations of rate shifts is a much more challenging computational task. Dorman's (2007) solution was to apply Bayesian techniques to the phylogenetic mixture model of Gu (2001) for the purpose of permitting Type-I divergence to be modeled as a random variable. Inference about where it occurs in the tree, and the fraction of sites involved, is based on an estimate of the posterior distribution. An alternative approach is to explicitly add a second probabilistic process to model the evolution of 'switching' between different evolutionary rates at a site. This class of models is typically referred to as 'covarion-like,' and readers are referred to Wang *et al.* (2007) for both a description of a general form of this type of model and a review of the key literature. Penn *et al.* (2008) developed a covarion model expressly for the purpose of detecting site-specific functional shifts. Inference under this model employs the likelihood framework, where an LRT (**Box 2**) is used to test for rate shifts and empirical Bayes (**Box 3**) to identify the sites and branches in which rate shifts occurred. Regardless of method, the advantage of not having to partition a tree a priori is offset by the additional difficulty of having to infer both the sites and branches that experienced a rate shift. Assuming that appropriate biological information is available, methods based on a priori specification of subtrees will be more powerful.

Just as methods intended either for polymorphism data or for codon level comparative data have their powers and limitations, so too the amino acid-based methods. The biggest single limitation is that they can't distinguish a rate shift caused by positive selection from a rate shift resulting from a relaxation of functional constraints. Further, the methods are not well suited for detecting adaptive evolution in certain types of genes. Genes that tend to be subject to positive diversifying selection (e.g., abalone sperm lysin) will possess sites evolving under continuously high rates, which does not lead to rate shifts within the data. As with codon models, adaptive evolution in genes where novel function evolved by just one, or a very few, amino acid substitutions (e.g., globins) will go undetected. Lastly, evolutionary processes that strongly violate the underlying assumptions of these methods can elevate their error rates. For example, gene duplication events can cause one copy of a gene to change its genomic location; when this also involves a change in local mutation pressure (e.g., from a GC-rich region to a GC-poor region) a false signal for rate shift can be obtained. Indeed, Bay and Bielawski (2013) demonstrated that statistical tests developed for both codon and amino acid data are subject to increased false positive rates in such scenarios.

Notwithstanding their limitations, these methods have proved useful for understanding the evolution of protein (e.g., Knudsen *et al.*, 2003; Mertz *et al.*, 2009). Under the appropriate conditions they provide a powerful means to formally test for sequence patterns consistent with functional divergence. Given sufficient signal, these methods can even be used to extract information about site-specific changes in the distribution of functional constraints. However, the relationship between sequence divergence and function is highly complex, and no single method can be expected to fully capture all of the signal. Increasingly important contributions will be made by incorporating a variety of comparative methods in the design of functional genomic research.

Analysis of Noncoding Sequences

Eukaryotic genomes contain mostly noncoding DNA, an unknown proportion of which is thought to be instrumental in regulating the interaction of the thousands of proteins encoded within those genomes. Very little is known about the evolutionary dynamics of these regulatory sequences, despite the fact that regulation of gene expression is widely considered to be one of the driving forces of phenotypic evolution (e.g., Wray *et al.*, 2003). The problem is that the functional fraction of genomic noncoding DNA is difficult to identify. This is largely because noncoding DNA are not nearly as constrained as protein coding DNA, which must adhere to the structure of the genetic code. For this reason the functional fraction of genomic noncoding DNA remains poorly characterized.

The most powerful method for identifying putatively functional noncoding DNA is based on the principle of

evolutionary conservation. The basic idea is that new mutations which negatively impact functional noncoding DNA will be rejected by purifying selection leading to lower substitution rates. A variety of approaches have been developed for detecting conserved noncoding sequences (CNSs) according to this principle (Duret and Bucher, 1997; Boffelli *et al.*, 2003; Margulies *et al.*, 2007). While such efforts have uncovered large numbers of CNSs, only some have been shown to contain regulatory sequence elements. Further, the known CNSs are unlikely to comprise all of the functional elements within the noncoding fraction of a genome. Identification and characterization of functional noncoding DNA continues to be an important area of active research.

In order to rule out the possibility that CNSs are merely due to a localized reduction in the mutation rate, their substitution rate should be compared to the rate of evolution at an appropriate reference set of neutral sites. Lower than neutral substitutions rates within CNSs indicates that they are evolutionarily conserved due to the action of purifying natural selection. Several methods have been developed for identifying CNSs based on this criterion (Siepel and Haussler, 2005; Cooper *et al.*, 2005; Asthana *et al.*, 2007; Davydov *et al.*, 2010). Interestingly, when CNSs are identified through comparative sequence analysis, and then their evolutionary dynamics are investigated within populations, a clear signal for selective constraint is often confirmed by the polymorphism data (Drake *et al.*, 2006). The consensus opinion is that a large fraction of CNSs have functional significance, and are not merely mutational 'cold spots.'

Methods analogous to the d_N/d_S ratio for coding sequences have been developed to determine whether some of the evolution among a set of noncoding sequences might have been driven by positive selection. Hahn *et al.* (2004) computed the substitution rate of transcription factor binding sites (d_B) normalized by the rate of intervening non-functional sites (d_I). Under the assumption that d_I is an appropriate neutral reference, positive selection is indicated by a ratio $d_B/d_I > 1$. However, the role of selection on the intervening sites is typically unknown, so it is difficult to demonstrate whether d_I is the appropriate neutral rate. Wong and Nielsen (2004) approached this issue by using the synonymous rate (d_S) of nearby coding sequences as the reference neutral rate. Wong and Nielsen (2004) implemented a full likelihood framework for joint modeling of both coding and noncoding sequences. They introduced a new parameter ζ, which is the rate of noncoding substitution (d_{NC}) normalized by the synonymous rate (d_S). This parameter has a familiar interpretation; $\zeta > 1$ indicates positive selection. Because the model is very flexible, permitting a mixture of sites having different values of ζ, it can be applied to a wide variety of scenarios. The model is fit to a dataset by using ML (**Box 1**), which permits estimation of the fraction of noncoding sites having $\zeta > 1$. The LRT is then used to formally test the hypothesis that the fraction is greater than zero (**Box 2**). Finally, empirical Bayes (**Box 3**) is employed to identify sites within an alignment having $\zeta > 1$. A potential limitation is that the rate of synonymous substitutions is assumed to be homogenous and free from selective effects, whereas there is evidence that evolution of synonymous sites in some genomes is subject to at least weak selection.

Perhaps the most interesting setting for positive selection of noncoding sequences is within particular lineages where there has been exceptional phenotypic evolution. For example, many have speculated that adaptive changes in regulatory sequences played a role in the evolution of human speech production and brain size (Hill and Walsh, 2005). The detection of such a role is very challenging, as inferences must be made about a subset of noncoding DNA (usually small) along just one branch of a phylogenetic tree. One approach is to simply test for an accelerated substitution rate within CNSs along a predefined subset of lineages (Pollard *et al.*, 2006a,b; Prabhakar *et al.*, 2006). The difficulty is that a lineage-specific change in rate can be due to either positive selection or a relaxation of constraint (including a complete loss of function). For this reason, many studies attempt to distinguish the two scenarios by testing for rates in excess of those at neutral sites within the lineage of interest (Pollard *et al.*, 2006a,b; Bird *et al.*, 2007; Kim and Pritchard, 2007; Haygood *et al.*, 2007; Pollard *et al.*, 2010). The study of Haygood *et al.* (2007) is noteworthy, as they extended the modeling framework of Wong and Nielsen (2004) to permit a fraction of sites having $\zeta > 1$ along preselected branches. This allowed them to bring to bear all the advantages of the inferential framework (**Boxes 1–3**) that had been very successfully applied to comparative analyses of coding and amino acid sequences. As mentioned above, a major challenge is determining how to obtain the most appropriate estimate of the neutral rate (e.g., Haygood *et al.*, 2007; Parsch *et al.*, 2010).

Identification of functional noncoding DNA, and interpretation of the role that selection may have played in their evolution, is much more challenging than for coding sequences. First, functional elements within noncoding DNA tend to be smaller than coding sequences, they likely depend on nucleotide states in other (unknown) functional elements, and they lack the clear 'footprint' of functional constraint that protein coding sequences have. Second, it may be that many functional elements have high turnover rates and are undetectable by current methods. Third, the capacity to understand their evolution in response to positive and purifying selection depends on a neutral rate estimated from an appropriate set of reference sites, and such sites can be challenge to identify. Fourth, biased gene conversion can lead to patterns of sequence evolution similar to positive selection (Ratnakumar *et al.*, 2010), making it difficult to tease apart the effects of selective and non-selective evolutionary dynamics (Pollard *et al.*, 2006a,b). Last, studies tend to be restricted to more closely related taxa because the alignment of noncoding DNA is more difficult than coding DNA. Despite these potential limitations, methods for detecting and assessing natural selection in functional noncoding sequences have been informative. For example, two independent studies found an excess of CNSs in the vicinity of protein coding genes related to neuronal development and function (Pollard *et al.*, 2006a,b), and subsequent analyses of the promotor regions of neuronal genes suggested evolution by positive selection (Haygood *et al.*, 2007). Many of the challenges posed by noncoding DNA can be met by combining comparative and population-level analyses (Hahn *et al.*, 2004; Rockman *et al.*, 2005; Drake *et al.*, 2006; Torgerson *et al.*, 2009), and future research will likely involve integrating both levels of analysis at the genome scale.

See also: Molecular Evolution, Models of. Neutral Evolution, Population Genetic Tests of

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Adaptive Mutation Controversy

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Glossary

Adaptive mutation In standard usage, term describes any genetic change that improves fitness. In recent popular usage, the term refers to a hypothetical mechanism said to vary the specificity or increase the intensity of mutagenesis in response to growth limitation. This proposal challenges a widely accepted tenet in evolutionary biology that mutations form as replication or repair errors, independent of need or consequences.

Directed mutation A process proposed to explain results of some selection experiments. A hypothetical mechanism that senses growth arrest, identifies the genomic sites that can restore growth, and preferentially generates mutations at those sites.

F′*lac* The F-plasmid of *Escherichia coli* is capable of transferring copies of itself to recipient cells (conjugation). This circular plasmid can exist free or can insert into the circular bacterial chromosome. Incorrect excision of an inserted plasmid can produce a plasmid derivative (F′) that includes bacterial DNA adjacent to the attachment site. The F′lac_{128} plasmid carries the chromosomal *lac*, *proAB*, and *dinB* genes. The genes encoding the transfer or conjugation functions of F′$_{128}$ are expressed at a high level due to an IS*3* element inserted in the plasmid *finO* gene, which encodes a regulatory function.

Hypermutable state A hypothetical condition proposed to explain the results of some selection experiments. Nongrowing cells are said to induce a mechanism to create mutations genome-wide (adaptive mutation). The mutable state is attributed to a mechanism that evolved to accelerate adaptation.

Defining 'Adaptive Mutation'

Conventionally, this term describes any genetic change that improves reproductive success. Recently the same term has been used to describe a process by which a proposed mechanism might create beneficial mutations in nongrowing cells. Equivalent terms are 'stress-induced mutation,' 'directed mutation,' and 'stationary phase mutagenesis.' It is proposed that this mechanism evolved to accelerate genetic adaptation by increasing the genome-wide mutation rates or by directing mutations preferentially to sites that improve growth. Used in this way, 'adaptive mutation' challenges an accepted tenet of evolutionary biology.

The Classical View of Mutation and Selection

Experimental genetics of bacteria depends on use of positive selection to identify rare cells in astronomically large populations. Selections serve to detect new mutants, assess mutation rates and demonstrate genetic recombination. This field was put on a sound theoretical footing by demonstrations that positive selection could in fact detect mutants without causing their formation. These classic experiments showed that the mutants detected by stringent, often lethal, conditions actually arose during the nonselective pregrowth period before exposure to selection and therefore could not have been formed in response to selection (Cavalli-Sforza and Lederberg, 1956; Lederberg and Lederberg, 1952; Luria and Delbrück, 1943; Newcombe, 1949; Novick and Szilard, 1950).

The 'fluctuation test' devised by Luria and Delbrück showed that mutations form with a constant probability at each cell division. During unselected growth of a culture, cell number increases exponentially and new mutants are added at an exponential rate – more cell divisions, more new mutants (Luria and Delbrück, 1943). Mutants that arise early in the history of a culture appear in a small population and are thus present at a high frequency that remains constant or is enhanced by new mutation events during subsequent growth. Mutant lineages arising at progressively earlier times in the history of the culture are present at exponentially higher frequencies. This is diagramed in **Figure 1**.

When multiple replicate cultures are compared, the variance in their mutant frequencies reflects this exponential growth and the times at which mutations happened to occur. This frequency variance exceeds the mean and deviates from a Poisson distribution. Methods were developed to use the distribution of mutants between parallel cultures to identify a 'Luria–Delbrück distribution' and calculate the mutation rate per cell per division. These methods have recently been reviewed in detail (Foster, 2006; Rosche and Foster, 2000). In fluctuation experiments, cultures are grown in nonselective liquid medium and mutants are detected as rare colonies formed when these cultures were plated on strongly selective solid medium where only these mutants can grow. Observation of a Luria–Delbruck distribution of mutant frequencies between replica cultures (left graph in **Figure 1**) demonstrates that the mutants detected by selection actually form prior to plating and can be used to calculate mutation rate.

With little additional evidence, the conclusion drawn from these beautiful bacterial experiments was broadened and applied to all organisms and conditions regardless of selection stringency. It was accepted that all mutations reflect random errors in DNA replication or repair, regardless of growth rate or phenotypes. Selection thus was inferred to act later to favor or disfavor preexisting mutations, depending on how their phenotypes influence reproductive success. While this conclusion may well be correct, the classical bacterial evidence

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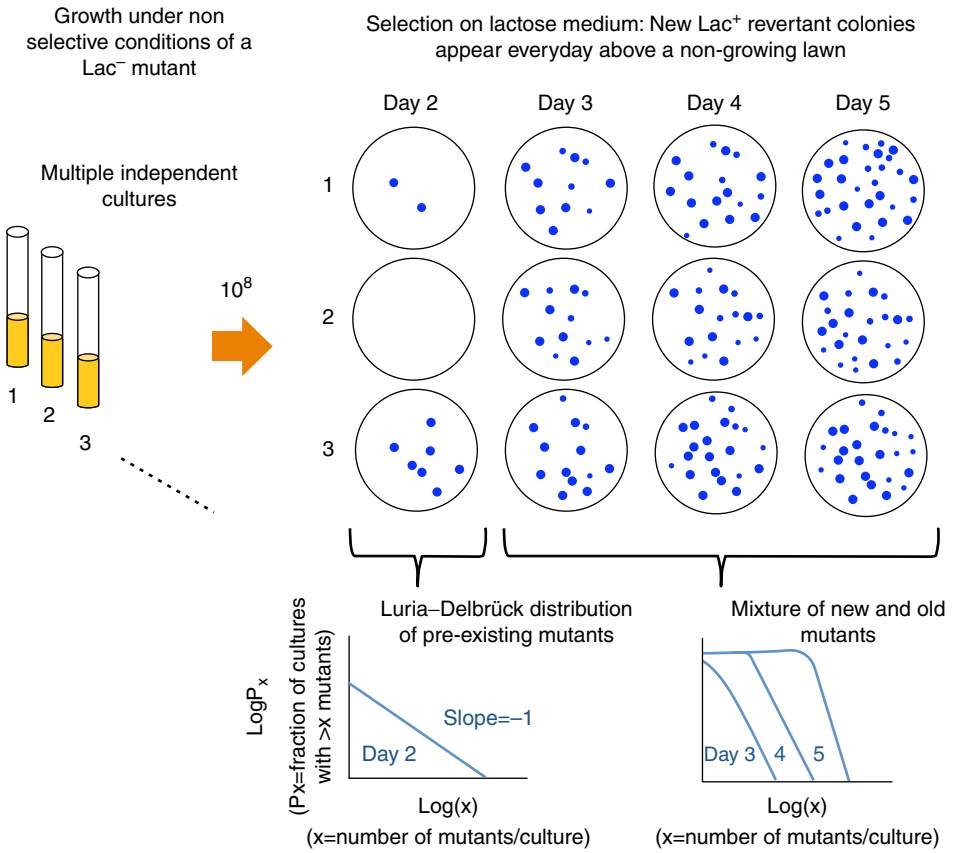

Figure 1 The Luria–Delbrück fluctuation test. Multiple independent cultures are grown nonselectively (left top) and then plated on selective medium to identify mutants that appear over several days (only three cultures are shown). The variance (or fluctuation) in colony number between cultures is used to measure mutation rate (Rosche and Foster, 2000) and also to demonstrate whether the detected mutants arose randomly during nonselective growth prior to plating. Mutants showing a Luria–Delbrück distribution are those that arose during nonselective growth prior to plating and cannot have formed in response to selection. To identify a Luria–Delbrück distribution, the number of mutants (x) in various tubes is plotted as shown at the bottom of the figure above. The horizontal axis displays (log x) for various values of x and the vertical axis shows logP_x, where P_x is the fraction of tubes with a number of mutants equal or greater than x. For a Luria–Delbrück distribution, this plot shows a slope of -1 (as seen in the graph at left). If mutants form only after cells are plated on the selective medium, a Poisson distribution is expected. In the *lac* experiment described below, the few revertants appearing on Day 2, show a Luria–Delbrück distribution and reflect preexisting mutants (Cairns and Foster, 1991). As colony numbers increase with time at a constant rate, the number of mutants on the selection plate shows less variation and deviates from a Luria–Delbrück distribution. (See plots for later days in the graph at lower right). By day 5, the bulk of the mutant colonies seem to have arisen on the plate, consistent with being caused by selection. This conclusion may not be warranted for copy number variants, whose steady-state frequency obscures the Luria–Delbrück distribution (see text).

does not eliminate the possibility that less stringent growth limitation might change the rate or specificity of mutation. This open possibility was addressed by the 'adaptive mutation' challenge described here.

Challenges to the Idea of Random Mutation

In standard bacterial genetic experiments, cell populations are plated on solid selective medium that limits growth. Preexisting mutant cells are detected by the visible colonies they form above the nongrowing lawn of parental bacteria. The procedure reliably detects only preexisting mutants as long as selection is stringent and blocks all growth of nonmutant cells (as was true in the classic experiments). However in practice, this caveat was often forgotten.

In some genetic systems, mutant colonies continue to accumulate for several days after plating, suggesting that selection detects not only preexisting mutants (as did the classic experiments) but also new mutants that arise after exposure to selection. Some authors interpreted this phenomenon as evidence that growth limitation causes the new mutations (Cairns and Foster, 1991; Cairns *et al.*, 1988; Hall, 1988, 1990, 1991; Maenhaut-Michel and Shapiro, 1994; Prival and Cebula, 1992; Pybus *et al.*, 2010; Shapiro, 1984; Sung and Yasbin, 2002; Thomas *et al.*, 1992; Yang *et al.*, 2001).

This conclusion is not warranted when selection conditions are not sufficiently stringent. That is, the new mutants could arise under selection during residual growth of the plated population. In addition, partially revertant cells might arise during pregrowth and initiate the late-appearing colonies under selection. These initially slow-growing clones might

improve (evolve) during colony development. Each act of DNA replication provides an opportunity for mutation and many opportunities are provided during colony growth. This improvement could occur using a constant unenhanced mutation rate.

For most of the exceptional systems used to claim selection-induced mutagenesis, residual growth was not assessed. In some cases, growth and DNA replication proved to be responsible for generating the extra mutants (Gizatullin and Babynin, 1996; Jin *et al.*, 2002; Mittler and Lenski, 1990, 1992; Prival and Cebula, 1996; Quinones-Soto and Roth, 2011). In one system, however, close attention was paid to population dynamics and evidence of associated general mutagenesis. In this system, a *lac* mutant of *Escherichia coli* did not grow on lactose but did give rise to Lac$^+$ revertant colonies on selective plates (Cairns and Foster, 1991; Foster, 1994).

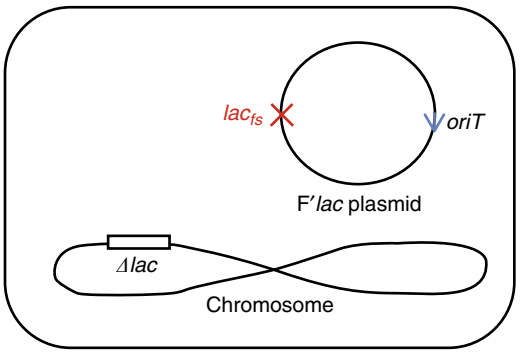

Figure 2 A strain used to seek evidence for selection-induced mutagenesis. The strain FC40 used in the *lac* system carries a leaky (partially functional) mutant *lac* allele on a F′*lac pro* (F′$_{128}$) conjugative plasmid and a deletion of its chromosomal lactose operon. The residual function of the plasmid lactose operon supports slow growth on lactose, which is prevented when cells are plated with a 10-fold excess of scavenger cells. The scavenger cells cannot use lactose but can consume any nutrients revertant than lactose that might contaminate the medium or be excreted by revertant cells on the plate. The tester cells are poised on the brink of growth.

The *lac* System

To explain the high frequency of cancer, which reflects somatic mutations, John Cairns proposed that growth-limited cells – such a mammalian somatic cells – might increase their mutation rate using a mechanism evolved to enhance genetic adaptation (Cairns, 1978, 1998; Cairns *et al.*, 1988). To test the idea of regulated mutagenesis, Cairns and Foster developed a bacterial system in which a cell population is held under selective conditions that prevent growth. Mutants arising in the nongrowing population initiate growth and form colonies that appear and accumulate over several days under selection. The general situation resembles nongrowing somatic cells and derived malignancies.

The parent *E. coli* mutant carries a deletion of its chromosomal lactose operon and a conjugative low-copy F′*lac* plasmid with a *lac* frameshift mutation (see **Figure 2**). A lawn of 10^8 mutant cells is plated on solid lactose medium, where it cannot grow but gives rise to about 100 Lac$^+$ revertant colonies, which accumulate over the course of 5–6 days. The reversion rate of the mutant *lac* allele during nonselective growth is 10^{-8} per cell per division (Foster and Trimarchi, 1994). Under selection, the population produces 100 Lac$^+$ revertant colonies over 6 days. The colonies are of two types. The majority (90%) includes cells that have acquired a compensating frameshift mutation and thereby a fully functional *lac*$^+$ allele. These cells form stable Lac$^+$ colonies when streaked on nonselective medium. The minority type (10%) has an unstable Lac$^+$ phenotype and forms sectored (Lac$^+$/Lac$^-$) colonies when streaked on nonselective rich medium (Andersson *et al.*, 1998). These cells have a tandem *lac* amplification with multiple copies of the leaky mutant *lac* allele (see **Figure 3**).

The course of a reversion experiment is shown in **Figure 4**. The two colony types accumulate on the selection plate with different trajectories. Stable Lac$^+$ colonies accumulate linearly over 5 days, consistent with their arising in a nongrowing population. Unstably Lac$^+$ colonies (10% of total) carry a tandem amplification (10–100 copies) of the original mutant *lac* allele. These colonies accumulate exponentially with time. This suggests that they might develop from a growing

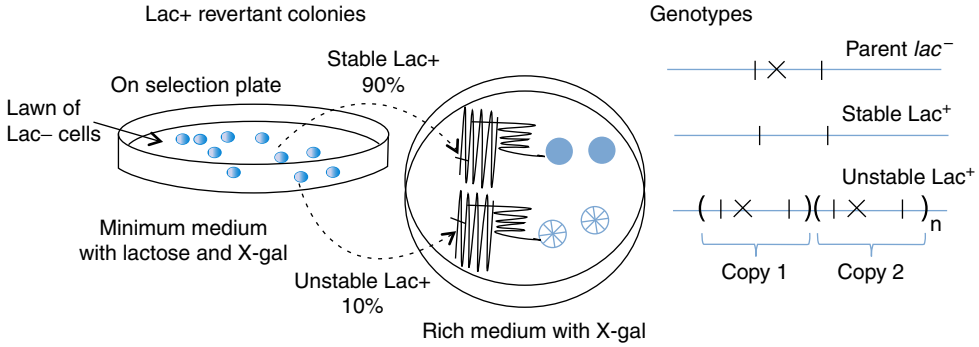

Figure 3 Two types of Lac$^+$ revertant colonies. The majority of the revertant colonies that appear on the selection plate include cells that are stably Lac$^+$ when streaked on nonselective medium with X-Gal, a chromogenic β-galactosidase substrate. These cells have acquired a compensating frameshift mutation and carry a fully functional *lac*$^+$ allele. On day 5, about 10% of revertant colonies are unstably Lac$^+$ and form sectored (blue/white, Lac$^+$/Lac$^-$) on nonselective medium. These cells carry a tandem array of 10–100 (n) copies of the mutant *lac* region and grow under selection by virtue of their multiple partially functional *lac* copies. On nonselective medium, the amplification is frequently lost, leading to a Lac$^-$ (white) colony sector.

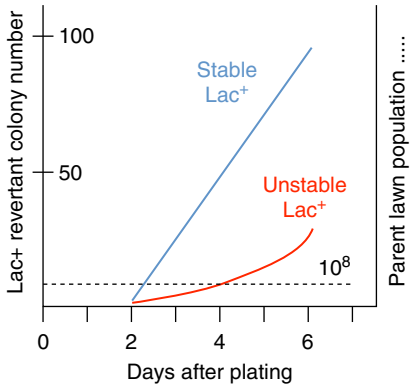

Figure 4 Accumulation of the two revertant types above the nongrowing lawn population. After 10^8 cells are plated on day 0, the lawn shows little or no growth, but revertant colonies accumulate over several days. Revertants are tested by streaking on nonselective medium to test the stability of their Lac^+ phenotype. Colonies of stably Lac^+ revertants accumulate linearly, suggesting formation from a nongrowing cell population. In contrast, unstably Lac^+ colonies accumulate exponentially, suggesting that they form in a growing population. This behavior is explained by a new model described below.

population. The parent lawn population as a whole shows very little growth over the course of the experiment (see **Figure 4**). The question is, 'How does a nongrowing parent population give rise to these two types of Lac^+ revertant colonies?' Some say that growth limitation is mutagenic (perhaps without DNA replication). Others say that selection acts on preexisting partially functional variants allowing them to develop a fully Lac^+ phenotype without mutagenesis (perhaps without cell division).

A clue for solving this puzzle may be the nature of the parent mutant *lac* allele. The *lac* allele carries a leaky frameshift mutation and produces about 2% of the β-galactosidase (LacZ) level found in a revertant. The ability of the strain to grow on lactose using its residual LacZ level is prevented by adding a 10-fold excess of scavenger cells that carry a *lac* deletion mutation. Sufficient scavengers are plated to just barely prevent growth of the tester by consuming carbon sources other than lactose that might contaminate the medium or be excreted by cells on the plate. Thus selection conditions are not stringent – they prevent cell division, but leave cells poised on the brink of growth.

The Adaptive Mutation Paradox

The paradox of this system is that selection appears to cause a 100-fold increase in the number of Lac^+ revertants, but does so with very little evidence of general mutagenesis. Without selection, the parent mutation reverts at 10^{-8} cell per division. Under selection, 10^8 plated cells produce 100 revertant colonies over 6 days. This represents a 100-fold rate increase, if one assumes one division under selection. However, analysis of this system has revealed little evidence of an increase in general mutation rate. That is, the starved lawn population shows no increase in the frequency of unselected mutations on the chromosome (Rosche and Foster, 1999; Slechta *et al.*, 2002b; Torkelson *et al.*, 1997). The revertant Lac^+ colonies show a 10-fold increase in the likelihood of secondary unselected mutation, but that increase is unevenly distributed. About 90% of Lac^+ revertants show no evidence of general mutagenesis, while 10% have experienced a 200-fold increase in mutation rate (Rosche and Foster, 1999). How can the number of Lac^+ revertants increase without exposing the genome at large to mutagenesis?

How Do Lac^+ Mutants Arise under Selection? – Three Models and Their Problems

Directed Mutation

In the initial model, an evolved mechanism senses the physiological situation and directs mutations to genomic sites whose alteration will restore growth (Foster, 1999). Directed mutation would help explain the lack of evidence for genome-wide mutagenesis. The difficulty lies in defining a process that can sense the cause of growth limitation and direct mutations to rare genomic sites that solve the problem. This would seem to require clairvoyance, but there is a precedent in the mammalian immune system, which mutagenizes local genomic regions in response to infection (Teng and Papavasiliou, 2007). Several clever ways to accomplish this in starving bacteria were suggested (Stahl, 1988), but the suggested increase in transcriptional errors or defects in post-replicative mismatch correction system later proved incorrect (Foster and Cairns, 1992; Stahl, 1992). Formation of Lac^+ colonies on selective medium relies on a functional recombinase (RecA) and some DNA replication. The selective amplification model (below) has the effect of directing mutation to valuable sites by amplifying the target gene and selectively maintaining only the revertant copy (Roth *et al.*, 1996).

Selection-Induced Hypermutability

This model proposes that growth limitation induces an evolved mechanism for genome-wide (undirected) mutagenesis in a subset of the growth-limited population. This model was initially suggested by Hall (1990) and supported by Torkelson *et al.* (1997), who found that Lac^+ revertants arising in the Cairns–Foster system were about 20-fold more likely to carry associated, unselected mutations. In this 'hypermutable state' model, mutagenesis of the starved population is not detected because so few starved cells are actually mutagenized. This model, in its simplest form, is not feasible because it requires generation of 100 mutants from a subpopulation 10^5 cells – a rate of 10^{-3} per cell per generation (Roth *et al.*, 2006a). This rate is a 10^5-fold increase over the *lac* reversion rate measured during nonselective growth and is substantially more intense than mutagenesis by any chemical or physical mutagen acting on nongrowing cells (Adelberg *et al.*, 1965; Miller, 1992; Wechsler *et al.*, 1973). The hypermutable state model may explain the modest increase in unselected mutations in revertants, but it cannot alone explain the origin of selected mutants in the *lac* system.

Selective Amplification of the Mutant Gene

This model proposes that mutants with a partially restored LacZ function arise during nonselective growth prior to plating. When placed on selective medium, these cells grow slowly and improve with no increase in mutation rate, leading ultimately to a late-appearing visible colony. It was first suggested that the preexisting cells carry a duplication of the leaky mutant *lac* allele, which supports slow growth on lactose. Growth improves as amplification expands the gene duplication by adding copies successively under selection. Ultimately some copy the *lac* region acquires a mutation that restores a functional *lac*$^+$ allele. The likelihood of such a mutation increases with the number of target sequences – more *lac* copies per cell and more cells in each slowly growing colony. No mutagenesis is required.

The selective gene amplification model has been shown to operate in many biological systems (Andersson and Hughes, 2009; Elliott *et al.*, 2013; Kondrashov, 2012), including adaptation by which poxvirus evades host defenses (Elde *et al.*, 2012), and bacterial acquisition of antibiotic resistance (Nilsson *et al.*, 2006; Paulander *et al.*, 2010; Pranting and Andersson, 2011; Sun *et al.*, 2009). Most notably, this model explains evolution of new genetic functions under continuous selection (Bergthorsson *et al.*, 2007) and has been shown experimentally to underlie evolution of a gene with a novel function within 3000 generations of growth (Näsvall *et al.*, 2012). The model probably explains the *Salmonella* version of the Cairns–Foster system, in which plated cells grow about one division per day (Hendrickson *et al.*, 2002; Kugelberg *et al.*, 2006; Slechta *et al.*, 2002b).

However, despite these successes, the original selective amplification model does not explain the *E. coli* version of system, where plated cells show very little growth. The lack of growth makes it hard to imagine how plated cells with a duplication achieve the amplification and cell number increase needed to explain reversion without mutagenesis.

The Philosophical Problem of Adaptive Mutation – Why Has This Question Been So Hard to Resolve?

Since its first description (Cairns and Foster, 1991; Cairns *et al.*, 1988), this system has been extensively investigated by multiple groups (Foster and Trimarchi, 1994; Galitski and Roth, 1995; Harris *et al.*, 1994; Peters *et al.*, 1996; Powell and Wartell, 2001; Prival and Cebula, 1996; Radicella *et al.*, 1995) from multiple points of view. The accumulated body of data is of high quality and is generally accepted by all participants. However, results have been interpretable in different ways and viewpoints have not converged with time, despite occasional declarations of victory. The subject has been reviewed repeatedly from multiple perspectives (Andersson *et al.*, 2011; Foster, 2004, 2007; Galhardo *et al.*, 2007; Rosenberg, 2001; Roth *et al.*, 2006b). The difficulty in sorting this out may be that many experiments were designed to verify one particular model rather than to decide between models. Resolution of the few data conflicts promises to decide the issue.

The Generally Accepted Body of Evidence

Analysis of the *lac* system has revealed several bits of data that appear central to the mechanism and are not controversial. First, the yield of revertants depends heavily on homologous recombination. Virtually no revertant colonies appear in strains lacking RecA or RecBCD proteins, which are central to recombination initiated at double strand breaks (**Figure 5**; Cairns and Foster, 1991; Harris *et al.*, 1994). However, the opposite effect is seen in strains lacking only the nuclease RecD. In these strains, the number of Lac$^+$ revertants increases in proportion to the increase in F′*lac* plasmid copy number caused by the RecD deficiency (Foster and Rosche, 1999). Second, revertant yield depends heavily on ability of the F′*lac* plasmid to transfer conjugatively to recipient strains (Foster and Trimarchi, 1995; Peters *et al.*, 1996; Radicella *et al.*, 1995). Virtually no revertants are seen when the transfer (*tra*) functions of F′*lac* plasmid are inactive, especially in absence of TraI the endonuclease that initiates transfer replication. Third, the total revertant colony number is reduced about 5-fold in strains that lack the error-prone DNA repair polymerase, DinB (**Figure 5**; Foster, 2000; McKenzie *et al.*, 2001; Slechta *et al.*, 2003). Lack of DinB does not affect unstable revertant number, but reduces the number of stable revertants about 10-fold. Thus without DinB, mutants still arise under selection, but are evenly divided between stable and unstable revertant types.

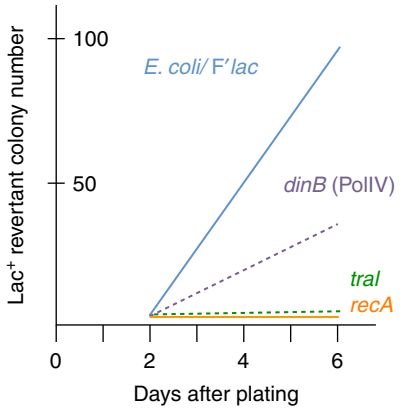

Figure 5 Three requirements for revertant yield in the *lac* system. The revertant yield (blue line) seen in the parent strain is reduced more than 25-fold in strains carrying a *recA* mutation, which blocks homologous recombination (orange line). A similar reduction is caused by a *traI* mutation on the plasmid, which eliminates a single-strand endonuclease needed to initiate conjugational transfer (dotted green line). A functional transfer origin is needed for reversion (Galitski and Roth, 1995; Peters *et al.*, 1996; Radicella *et al.*, 1995), but the role of actual transfer is still unclear. Direct tests show a low frequency of actual plasmid transfer associated with reversion (Foster and Trimarchi, 1995; Maisnier-Patin and Roth, unpublished data). However, in support of mating, Tn*10* is lost during reversion, apparently stimulated by single-strandedness associated with conjugation (Godoy and Fox, 2000). In addition, most Tra proteins are required for reversion, including those needed for mating pair stabilization (Maisnier-Patin and Roth, unpublished data). In the absence of DinB, total revertant yield drops 5-fold (dotted purple line); unstable revertants are unaffected while stable revertants drop 10-fold. Without DinB, residual revertant number is evenly divided between stable and unstable types.

It is interesting that the reversion rate of the same *lac* mutation during nonselective growth is not affected by RecA (Bull *et al.*, 2001) or by the chromosomal DinB allele (Gawel *et al.*, 2008; Kim *et al.*, 2001; Kuban *et al.*, 2004; Strauss *et al.*, 2000; Wolff *et al.*, 2004). The same studies show however a two- to four-fold reduction in mutagenesis on F′*lac* when the *dinB* copy on that plasmid is deleted. The three models outlined above explain the effects of selection in different ways. The key to understanding this system may lie in the conflicting data bits that seem to decide between models.

Resolving Conflicts and Deciding between Models

Do the Cells That Initiate Stable Revertant Colonies Arise Before or After Selection?

Mutagenesis models predict that revertant colonies are initiated on the selection plate by new mutant cells formed in response to the selective environment. In contrast, selection models predict that initiating cells carry a *lac* amplification that forms during nonselective pregrowth. On selective plates, these cells grow slowly and acquire a mutation that generates a *lac*$^+$ allele and allows rapid exponential growth.

The first attempt to decide the 'before or after' question was a classic Luria–Delbrück fluctuation test (Cairns and Foster, 1991). Tests of this type demonstrated long ago that stringent selections detect mutants whose frequency fluctuates from one culture to the next because mutants arise at different times prior to plating (see above). In the *lac* system, the number of revertant colonies formed over several days does not show fluctuation from one parallel culture to the next (Cairns and Foster, 1991). This was taken as evidence that Lac$^+$ mutants were initiated on the selection plate, consistent with either model of 'stress-induced' mutagenesis. Recent results suggest that this conclusion is not warranted for copy number variants whose frequency does not fluctuate.

Unlike point mutants, duplications are not subject to Luria–Delbrück fluctuation. During nonselective growth, cells with duplication or amplification of any gene come to a steady-state frequency maintained by a balance between a high formation rate (typically 10^{-5} per cell per division) on one hand and a higher loss rate (10^{-3} per cell per division) plus fitness cost of the other (Reams *et al.*, 2010). The forces that maintain this steady state obscure fluctuations due to the timing of duplication formation events. If revertants are initiated by copy number variants (as proposed by the selection model), fluctuation tests cannot decide whether they form before or after plating. A different sort of evidence is required.

A new test recently showed that revertant colonies are initiated by preexisting cells with multiple *lac* copies (Sano *et al.*, 2014). These experiments use a *tetA* (tetracycline resistance) gene inserted at various points on the F′*lac* plasmid. The tetracycline analog anhydro-tetracycline (AnTc) induces expression of *tetA*. This induction inhibits growth of cells with multiple copies of *tetA*, but has no effect on cells with a single copy.

The *tetA* gene is located on the same plasmid as *lac* and the two genes are expected to co-amplify. The selection model described above predicts that cells with multiple copies of mutant *lac* allele initiate Lac$^+$ revertant colonies. Pregrowth with AnTc counter-selects rare cells with multiple copies of *tet* (and often *lac*). This reduces then the number of revertant colonies appearing on selective medium. This result was seen regardless of the position of the inserted *tetA* gene on the F′*lac* plasmid, suggesting that the critical cells have multiple copies of the whole F′*lac* plasmid (which includes both *tetA* and *lac*) rather than an internal tandem amplification of the *lac* region. These experiments led to the conclusion that cells with multiple copies of the F′*lac* plasmid initiate stable Lac$^+$ revertant colonies.

Unstable Lac$^+$ revertant colonies are also initiated by cells with multiple copies of F′*lac* (Sano *et al.*, 2014), but the plasmids in these cells appear to carry a tandem amplification of the *lac* region (see **Figures 3** and **4**). Earlier evidence suggested that these unstable revertants are initiated by preexisting cells with a short *lac* duplication (Kugelberg *et al.*, 2006). We suggest that both findings are true – unstable revertants are initiated by cells whose high copy F′*lac* plasmid includes a short *lac* duplication (Kugelberg *et al.*, 2006). In the model below, we will assume that the extra number of *lac* copies supports growth and allows these cells to initiate unstable revertant colonies. Since both stable and unstable revertant colonies are initiated prior to selection, neither revertant type can be induced by selection.

Does the Plated Population Grow Before Mutation?

The lawn population grows very little over the course of a reversion experiment. This has been interpreted as evidence that mutants arise in nongrowing starved cells. However, it is hard to eliminate the possibility that a subset of lawn population grows or that mutations arise within slowly growing colonies (as suggested by the selective amplification model).

Support for reversion within growing colonies was the presence of rare unstable Lac$^+$ cells within colonies of otherwise stably Lac$^+$ colonies. These cells were taken as evidence for unstable precursors of stably revertant cells (Hendrickson *et al.*, 2002). Recently, we confirmed earlier evidence (Hastings *et al.*, 2004) that the unstable Lac$^+$ phenotype of these rare cells is not heritable – all descendent cells are either Lac$^+$ or Lac$^-$ (I. Roush, S. Maisnier-Patin, and J.R. Roth, Unpublished data). This is inconsistent with tandem amplifications of *lac*, which are heritable and suggests that the rare unstable Lac$^+$ cells have multiple copies of the F′*lac* plasmid – some *lac*$^+$ and some *lac*$^-$. Thus the preponderance of evidence implies that plated cells grow very little under selection before the reversion event. This explains the linear accumulation of stable revertants. The new model below explains the mixed plasmid types seen in rare unstable Lac$^+$ cells within otherwise stable Lac$^+$ colonies.

What Activates the DinB Polymerase – Induction or Amplification?

Strains lacking the error-prone DinB polymerase show 10-fold fewer stable revertants, but the same number of unstable revertants (McKenzie *et al.*, 2001). The DinB (polIV)

enzyme, which belongs to the Y-family of DNA polymerases, can accommodate some bulky lesions and catalyze translesion DNA synthesis of damaged template strands (Fuchs and Fujii, 2013; Sale *et al.*, 2012). When overproduced, DinB makes frequent mistakes including many frameshift mutations during replication of undamaged DNA (Kim *et al.*, 2001). Expression of the *dinB* gene is induced by DNA damage as part of the SOS response (Wagner *et al.*, 1999) and by cessation of growth (RpoS) (Layton and Foster, 2003). Mutagenesis models propose that growth limitation induces *dinB* gene expression and consequently causes mutagenesis of the genome (Layton and Foster, 2003; Lombardo *et al.*, 2004). This does not explain the uneven genomic distribution of mutagenesis (Rosche and Foster, 1999). Selection models suggest that the significant increase in DinB levels occurs because the *dinB* gene happens to be located close to *lac* on the F′ plasmid (16 kb away) (Kofoid *et al.*, 2003). This position allows the two genes to be co-amplified during selection for more copies of the mutant *lac* gene.

Determining the effect of *dinB* gene position should be decisive, since mutagenesis models predict no role of position and selection models expect a critical role. Our lab found that revertant yield depended heavily on presence of a functional *dinB*$^+$ allele on the F′*lac* plasmid and not at all on the state of the chromosomal *dinB* gene (Slechta *et al.*, 2002a). Another lab found the position of *dinB*$^+$ to be irrelevant to revertant yield (McKenzie *et al.*, 2001). We have repeated these experiments and are extending the tests, but all current evidence supports the importance of position (I. Roush, S. Maisnier-Patin, and J.R. Roth, Unpublished data). In the model described below, we assume that this conflict will be resolved in favor of a *dinB* gene position effect.

A Model That Fits with All Available Data

Experimental resolution of the remaining conflicts answered the questions listed above, but unfortunately eliminated all three of the initial models. The available data can all be explained by combining aspects of all three models. This latest model (Sano *et al.*, 2014) proposes that revertant colonies are initiated by cells that arise before selection but do not grow (or grow very little) under selection before acquiring a mutation. The central idea of this model is that natural selection can operate without cell division by acting on the population of gene copies made by internal local over-replication of the mutant *lac* region. This model is described in more detail below.

1. All revertant colonies are initiated by preexisting cells with multiple copies of the F′*lac* plasmid (Sano *et al.*, 2014). A single copy of the leaky *lac* allele provides enough energy for reversion (Stumpf *et al.*, 2007), albeit at a very low rate. The latest model proposes that this energy is enough to support plasmid replication, but not enough to initiate replication of the chromosome from its regulated origin. The extra plasmid copies provide more *lac* targets for mutation and multiple copies of the DinB gene. Most importantly, the plasmid provides a means of replicating each copy of the *lac* region. It is the act of *lac* replication that provides opportunities for mutation. (Multiple replicating *lac* copies were aspects of the selective amplification model.)
2. While the mutant cells that initiate stable revertants can replicate their plasmids under selection, they cannot divide or grow exponentially. Cell doubling resumes only after a *lac*+ allele forms by mutation in one copy of the plasmid. (Growth is blocked, as in mutagenesis models.)
3. Initiator cells repeatedly replicate their multiple F′*lac* plasmids using preexisting replication forks, initiated by the plasmid transfer origin or at intermediates in recombination between plasmid copies. These unregulated forks copy the entire plasmid repeatedly over a period of several days with little or no cell division. (Mutations are made more likely by repeated replication of multiple *lac* copies as in the selection model.)
4. The F′*lac* plasmid includes the *dinB* gene, which encodes an error-prone polymerase. Therefore, cells with multiple plasmid copies have more DinB protein and a higher mutation rate. This mutagenesis affects only the plasmid, since the chromosome is not replicating. Mutagenesis is not caused by an evolved mechanism, but is an artifact resulting from the chance location of *dinB* on the F′*lac* plasmid. (Mutagenesis appears directed to the plasmid.)
5. As soon as a mutation event produces a *lac*$^+$ allele in any of the multiple *lac* copies, energy is supplied and cells resume regular division. Revertant cells divide exponentially under selection to maintain their new *lac*$^+$ allele, while losing any non-revertant plasmid copies. The rare unstable Lac$^+$ cells found in a stable revertant colony have a mixture of *lac*$^+$ and *lac*$^-$ plasmid types that have not yet segregated. Selective plasmid retention has the effect of directing mutation to DNA sites that limit growth. Plasmid copy number and repeated replication increases the probability that a mutation will occur on some F′*lac* copy and only the copy with a *lac*$^+$ allele is selectively retained. Recovery of unselected mutations on the plasmid is not enhanced, because only the selected plasmid copy is retained. (Mutagenesis appears directed to the precise sites that limit growth.)
6. Unstable Lac$^+$ revertant colonies are initiated by preexisting cells with a small tandem duplication of *lac* within each of their multiple plasmid copies. Multiple copies of an F′*lac* with a duplicated *lac* region provide sufficient *lac* copies (and therefore energy) to support cell division on lactose. Cells grow slowly under selection and expand their gene duplication into a long amplified array as their plasmid copy number drops by segregation. Cells within these clones grow exponentially and replicate both their plasmid and chromosome, while selectively holding multiple tandem copies of *lac*. Cells whose amplified *lac* region includes *dinB* show increased mutagenesis of their entire replicating chromosome. (This explains the observed modest level of general (undirected) mutagenesis.)

Explaining Behavior of the *lac* System in Terms of Standard Evolutionary Ideas

Basic evolutionary theory predicts that imposed selection will increase the rate at which new alleles become prominent in a

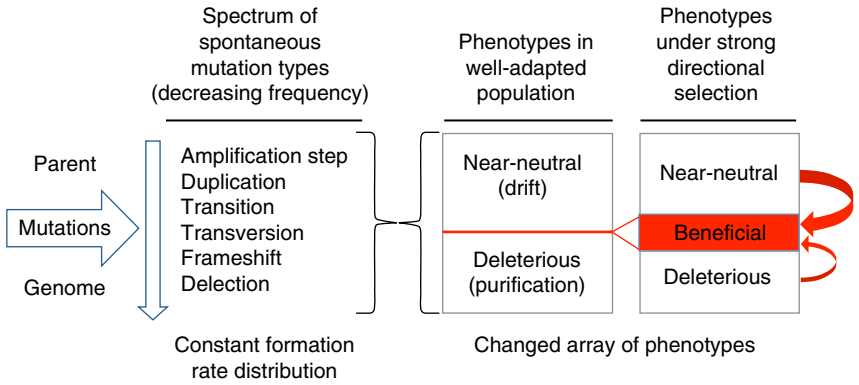

Figure 6 The conventional wisdom and how it can imitate mutagenesis. The primary event is formation of the several mutation types. These arise at a vast array of frequencies and can have phenotypic effects of vastly different magnitude. In *Salmonella*, gain-of-function mutation types arise at rates that vary over a million-fold range. Substitution mutations that cause qualitative changes in a particular protein arise at less than 10^{-8} per cell per generation. Duplications of a gene form at 10^{-5} per cell per generation and further copy number increases at about 10^{-2}. Base substitutions in a particular gene arise at about 10^{-7} per cell per division and most cause a loss-of-function phenotype. The magnitude of their effects range from synonymous and near-neutral conservative missense substitutions that are about third of substitutions to the completely null mutations (nonsense), which, together with frameshift and deletion types make up about 1% of genetic changes. The selective value (or costs) of mutations also varies over a wide range, but beneficial mutations are extremely rare in a well-adapted population. When a population is placed under strong directional selection, a larger fraction of available variability contributes to improved growth. Thus the predominant genotype in a population changes more rapidly under selective, even when the source of variability remains constant.

population, but does this without increasing mutation rates. This is diagramed in Figure 6. Assume that mutations arise as random errors whose frequency and spectrum of types is unaffected by growth limitation. Consider first a well-adapted population growing under optimum conditions. A large fraction of new mutations have phenotypes that are near-neutral (synonymous, silent or conferring a tiny positive or negative effect on growth). These mutations tend to remain in the population as polymorphisms and are subject to random loss by drift. Perhaps a similar fraction of mutations causes a palpable loss-of-function phenotype that impairs growth (deleterious). The latter mutations are continuously removed by purifying selection. Very few new mutations improve growth in a population that is well-adapted to its circumstances.

Consider what happens if one shifts the population to conditions that impair growth but leave open many mutational ways to adapt and improve. A larger fraction of new mutations are beneficial and are brought to high frequency in the population by positive selection. Some of these mutations were previously near-neutral or even deleterious. Due to selection, the preponderant genotypes in the population change progressively. More mutations are being fixed in the genome even though the mutation rate and spectrum of mutation types is unchanged. New mutations form (at an unchanged rate) but rise in frequency and are held in the genome by selection. This looks like mutagenesis, when in fact selection has just detected beneficial alleles in the pool of near-neutral and deleterious types. Selection does not create new mutations but redefines their phenotype. The rate of adding new alleles to the preponderant genome increases due to selection alone. In the 'mutagenesis' models discussed here, these new mutations are attributed to an evolved mutagenic mechanism induced by stress. In the most recent model, polymorphic near-neutral mutant alleles gain a significant phenotype following amplification under selection. Adaptation occurs without mutagenesis.

Evolutionary Insights Provided by the *lac* System

Several principles of broad relevance to evolutionary biology have emerged from study of the *lac* system devised by Cairns. This is true despite the fact that behavior of the system relies on specialized features that are peculiar to the particular strain used. That is, selection is imposed on a *lac* allele that happens to lie near the *dinB* gene, which encodes an error-prone polymerase. Both *lac* and *dinB* genes are carried by a conjugative plasmid with a second replication origin that normally supports transfer from one cell to another. Scavenger cells prevent growth under selection, but poise cells on the brink of growth so even slight improvements initiate growth. Below is a list of generally applicable principles that have emerged from analysis of this peculiar system.

1. Near-neutral beneficial alleles can gain a significant phenotype when amplified under selection. The minimal activity of the mutant *lac* gene in this system supports growth only when present in multiple copies, as seen during formation of unstable revertant colonies.
2. Near-neutral mutations have small phenotypes, such that their frequency is neither strongly increased nor reduced by selection. These mutations are carried as neutral polymorphisms subject only to drift. These polymorphic alleles are available whenever some new selection pressure is imposed. Individuals with multiple copies of a near-neutral allele (and a bigger phenotype) are frequent and a selective response can be rapid.
3. Amplification under selection is rapid and supplies multiple copies of a growth-limiting allele, all of which are

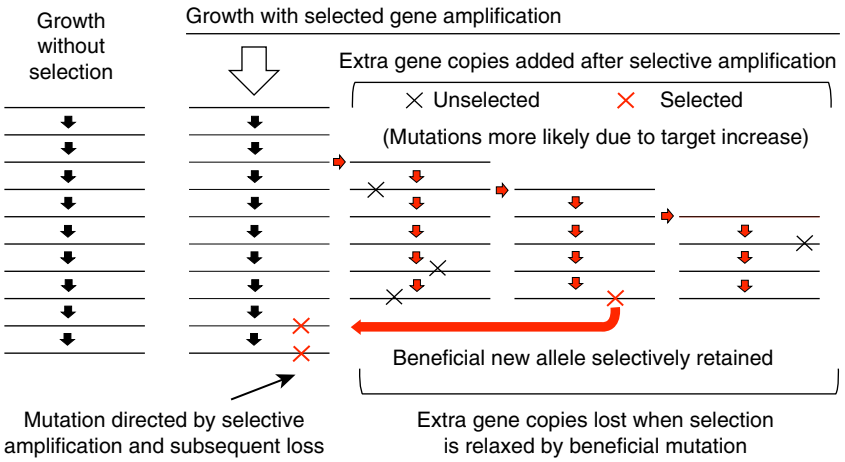

Figure 7 Selective amplification directs new mutations to sites that improve growth. The left column shows a genome region that replicates serially with no selection on copy number. The right column shows the same region that is amplifying under selection for increases in some encoded function. Amplification provides more copies of the entire region and increases the likelihood that a cell carrying this amplification will experience some mutation in this region. If the mutation is beneficial, selection to hold the amplification is relaxed and all copies are lost except the improved version, which is selectively maintained. Any copy with an unselected mutation is lost. The end result is a genome with an improved allele whose probability has been increased without increasing the likelihood of any other mutation in the genome.

subject to mutational improvement. Thus amplification increases the likelihood of sequence improvement by providing more targets (not by mutagenesis). Amplification is reversible such that extra gene copies are lost once selection is relaxed by a beneficial mutation in some copy. This process of selective amplification and later loss has the effect of directing new mutations to beneficial sites.

The apparent direction of mutations to valuable sites by amplification is diagramed in Figure 7. A region including the gene under selection amplifies under selection. This enhances growth and provides more targets for mutation. The extra copies make any mutations in this region more likely, regardless of their effect on phenotype. If a beneficial mutation occurs in some copy, selection for amplification is relaxed and the improved allele is held selectively. This allows all other copies including those with nonselected mutations to segregate and be lost, but retains the copy that has a beneficial mutation. The net effect of this process is to increase the frequency of beneficial mutations in the local region with no effect on nonselected mutations. The chance of retaining an unselected mutation in the amplified region is the same as the likelihood of this mutation arising without amplification. The potential of amplifications to increase apparent mutation rate and to direct mutations to useful site was described some time ago (Roth *et al.*, 1996).

4. Selection can operate in nongrowing cells if a genome region is subjected to local over-replication and beneficial mutations are held selectively. This can be seen in Figure 7, where the extra copies are added by repeated replication even in nongrowing cells. Each act of replication presents an opportunity for mutation. Selection holds only a copy with a beneficial mutation. In essence, the multiple copies of the sub-genomic region serve as a population upon which selection can act in nondividing cells. This process may prove relevant to origins of cancer whose progression is favored by fragile sites in the metazoan chromosome (Albertson, 2006). Repeated breakage and replicative repair of such sites can occur during one generation of a single cell and the lifetime of a single metazoan individual.

See also: Directional Selection and Adaptation. Microbial Experimental Evolution. Mutation, Population Genetic Models of. Natural Selection, Introduction to. Recombination in Bacterial Populations

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Adaptive Radiations: Insights From Evo-Devo

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Glossary

Buccal cavity The whole of the mouth and jaw region and its capacity.

Embryogenesis The early formation of primary structures and patterning that comprise an embryo. Often, most of the developmental events that comprise embryogenesis are shared even among distantly related taxa.

Genetic accommodation The evolution of a developmental system whereby events that are initially induced by environmental cues become an automatic developmental event based on heritable genetic variation. Genetic accommodation can evolve to either increase or decrease the amount of phenotypic plasticity.

Genetic canalization A developmental strategy that is impervious to environmental variation and completely determined by genetic variation.

Gene promoter region An area of the genome adjacent to the sites of gene transcription. These regions are thought to initiate the transcription of genes.

Macroevolution Relatively large evolutionary change that occurs over several million years. Such evolution results in deep splits in evolution creating new genera, families, orders, or kingdoms. This contrasts with microevolution which tends to occur within a single species and can occur rapidly, involving only a few generations.

Multipotent cells Cells that are able to become any number of different tissues through alterations of gene expression, and/or signals from neighboring tissues.

Ontogeny The whole of the developmental process. Development, while often studied at embryonic stages, actually takes place throughout the lifecycle of an organism. Ontogeny, comprised by its often continuous stages, provides a focus for investigation.

Insights into Adaptive Radiations from Evolutionary Developmental Biology

Many early studies in evo-devo focused on investigating large-macroevolutionary patterns and mechanisms that determined phylogenetically deep differences (i.e., between kingdoms and orders). However, evolutionary developmental biology (evo-devo) is now making major contributions to our understanding of adaptive processes down to the level of populations and inter-individual variation (Klingenberg, 2010; Parsons and Alberston, 2013; Nunes *et al.*, 2013). Specifically, evo-devo is providing a mechanistic understanding for how continuous adaptive phenotypic variation arises (and develops), while also providing its own important theoretical contributions for what limits, directs, or enhances adaptive evolution (Pigliucci, 2008; Pigliucci and Muller, 2010). For biologists interested in understanding the evolution of adaptations and more recently their development, there are numerous study systems recognized as 'adaptive radiations.' The hallmark of such systems is that several closely related species exist that arise from a recent single common ancestor, or a few closely related ancestors (Schluter, 2000). These species possess differences in anatomy and behavior that relate to ecological specializations (**Figure 1**). The often rapid nature of evolutionary change in these systems makes comparisons between member species very tractable due to homology in anatomy and similarity in genetic backgrounds (Loh *et al.*, 2008). The process through which adaptive radiations evolve is assumed to be initiated by changes in the environment, or access by a lineage to a new environment through geodispersal events, or perhaps the innovation of a novel trait (Schluter, 2000). These conditions can make new ecological niches available allowing the lineage to persist without competition from other species. Supporting this many adaptive radiations occur across isolated islands or lakes that will be discussed below. Therefore, adaptive phenotypes are often thought to be driven initially by intraspecific competition that drives populations to phenotypically adapt and diverge into species through natural selection.

While the above ecological conditions are commonly thought to drive adaptive divergence, from an evo-devo perspective it is recognized that new environments will also impact developmental processes in novel ways. So far, much of the empirical focus for adaptive radiations has typically come from fields outside of evo-devo. From the late 1980s to the present, evolutionary ecology has repeatedly demonstrated a role for natural selection in the formation of adaptive radiations (Endler, 1986; Schluter, 2000; Kingsolver *et al.*, 2001). Similarly, from the 1990s to present, population genetics (and more recently population genomics) has demonstrated changes in gene flow that coincide with adaptive divergence, while also examining the role of geography in the formation of species (Avise, 1994; Hartl and Clark, 1997; Hartl, 2000). However, while evolutionary ecology has identified adaptive phenotypic variation, and population genetics has identified allele frequency changes in adaptive divergence, there is a significant knowledge gap in evolutionary biology regarding how adaptive variation arises and its mechanisms. In other words, connections between the genotype and phenotype are not yet well characterized in systems displaying adaptive radiations. Given that natural selection only operates directly on phenotypic variation (Mayr, 1997) this is surprising because it is highly relevant to understanding how adaptive phenotypes form, and why specific allele frequencies might change during adaptation. Similarly, the developmental processes that influence both the efficiency and the outcome of

Figure 1 Examples of the phenotypic diversity that figures prominently in cases of adaptive radiation. Darwin's finches (left panels) and African cichlids (right panels) craniofacial anatomy has been the focus of several studies investigating the mechanisms of their development. Finch photographs were generously provided by Jeff Podos. Cichlid images are courtesy of Ad Konings, copyright Ad Konings.

selective processes remain largely unexplored. This is an important area for evo-devo to fill because selection and adaptation can occur over the course of development, and in different developmental environments. Currently most studies of adaptation tend to focus upon performance at a single point in ontogeny (usually in adult phenotypes) in a single environment.

Fortunately, the movement of evo-devo into finer scale evolutionary processes is well underway and seemingly picking up momentum as a wider range of taxa become the focus of study (Nunes *et al.*, 2013). It is now becoming more common for evo-devo approaches and thinking to be applied to cases of microevolution and adaptive radiations to understand how adaptive phenotypes arise (Parsons *et al.*, 2010, 2011; Snell-Rood *et al.*, 2010; Swanson and Snell-Rood, 2014). Further, evo-devo does not simply deal with the outcome of selection (i.e., survival of the fittest), rather evo-devo considers the origins of variation through developmental change

(i.e., arrival of the fittest). This is a key contribution from evo-devo because natural selection cannot occur without phenotypic variation, or be evolutionarily effective without genetic variation. Therefore the study of how variation is formed through development is fundamental for understanding how adaptations evolve – arguably even more fundamental than natural selection (West-Eberhard, 2003). Thus, evo-devo promises and continues to fill current gaps in knowledge by linking the genotype to *adaptive* phenotypic variation through the study of changes in development.

Early Links Between the Study of Adaptive Divergence and Developmental Biology

How did evo-devo enter the realm of adaptive radiations? This involves a long history that continues to unfold. The study of adaptation itself from an ecological perspective did not become rigorous until the 1980s following critiques of the 'adaptationist paradigm' and the publication of landmark volumes that catalyzed approaches for the study of selection at the population level (Gould and Lewontin, 1979; Endler, 1986). At roughly the same time there was a small but fervently renewed interest in the idea that developmental interactions with the environment could contribute significantly to adaptive phenotype variation through phenotypic plasticity – the ability of a genotype to produce a range of phenotypes dependent upon environmental cues (Via and Lande, 1985; West-Eberhard, 1989). Long relegated as 'nuisance' variable or considered developmental 'noise' plasticity was considered unimportant for evolutionary change, and rather was seen as an inconvenience for experimenters especially within the realm of quantifying the heritable contribution to phenotypes.

However, as theory developed it was suggested that plasticity could provide 'alternative' phenotypes that could develop to produce adaptive variation (West-Eberhard, 1989). Further, older theories that hadn't been investigated empirically for decades such as the Baldwin effect – whereby plasticity is favoured because it allows a population to persist in a novel environment through rapid phenotypic change, and genetic assimilation – where an initially plastic response becomes incorporated as part of normal phenotypic development and no longer requires environmental induction – were resurrected by the growing realization that adaptive evolutionary processes were not exclusive to a straightforward change in genetically determined traits. At this time a 'gene-centered' view of evolution still dominated evolutionary biology, whereby genetic variation and allele frequency change were seen as the panacea for evolutionary change (i.e., environmental effects were irrelevant). This slowed progress and acceptance that plasticity, and ultimately variation from development, could be relevant to evolution. For example, some detractors suggested that plasticity provided a 'baroque hypothesis' for how adaptive divergence occurred, or suggested that a renewed interest in plasticity represented a return to Lamarckism (Pigliucci *et al.*, 2006).

Despite these issues, a growing number of biologists viewed plasticity and ultimately development as a relevant contributor to adaptive evolution and began to perform numerous lab experiments across a wide range of taxa. Perhaps one of the most significant findings from these experiments was that plasticity exhibited a degree of heritable variation (Pigliucci, 2005). This meant that plasticity was like other phenotypic traits in that it could itself evolve. In other words, this empirical research showed that the developmental system could evolve and adapt in such a way that it could broaden or narrow its potential range of phenotypes. This was a significant step for bringing developmental thinking into the realm of adaptive divergence, and how population level changes could occur. Notably such thinking is reflected in conceptual models of adaptive divergence (**Figure 2**). For example, the idea of resource polymorphisms, whereby individuals within a population evolve ecological specialization on different ecological resources (i.e., prey, structural habitats), explicitly incorporates developmental thinking through the evolution of plasticity (Skulason and Smith, 1995; Smith and Skúlason, 1996). Specifically, this model suggested how developmental systems would be expected to promote initial variation for divergence through plasticity, and evolve toward increased ecological specialization (**Figure 3**). In the final stages of the model genotypes that can produce adaptive phenotypes without the need for environmental cues are favoured and plasticity is lost (i.e., genetic assimilation) due to its costs (e.g., sensory costs, potential for errors in variable conditions). Thus, resource polymorphism theory provided an early framework for how adaptive evolution could depend on environmentally induced changes in development.

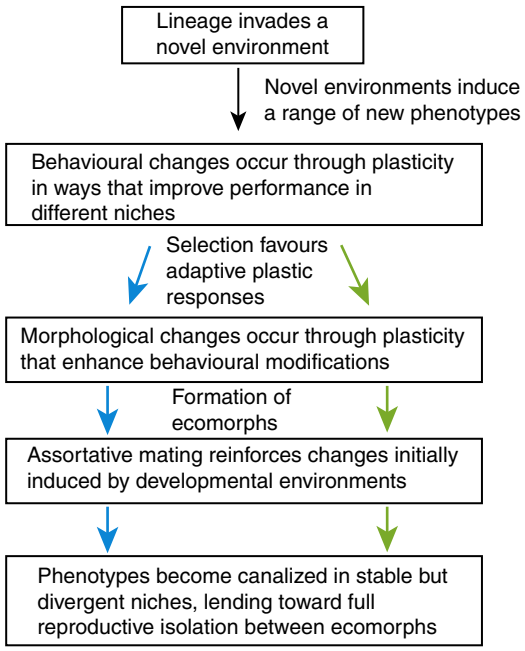

Figure 2 A representative diagram for how adaptive divergence occurs through developmental change within the framework of resource polymorphism theory. Notably, developmental plasticity figures prominently in this model and reflects a process of sympatric divergence. However, it is also recognized that these processes of developmental evolution can occur in allopatry. Modified from Skulason, S., Smith, T.B., 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution 10, 366–370.

Figure 3 Example of ecomorphs from a well-known resource polymorphism of Arctic charr (*Salvelinus alpinus*), in Lake Thingvallavatn, Iceland. Evidence from charr supports the resource polymorphism theory of an evolving magnitude of phenotypic plasticity that coincides with ecological specialization (Parsons *et al.*, 2010, 2011), and some instances of complete reproductive isolation between sympatrically derived charr ecomorphs is documented (Gislason *et al.*, 1999). Within the figure the large benthic (LB), small benthic (SB), piscivorous (PI), and planktivorous (PL) ecomorphs are depicted for head and overall body shape (K. Gunnarsson, and A. Gardarson photo).

More comprehensive challenges to the gene-centered view of evolution were put forth in a series of seminal books through the late 1990s and early 2000s that placed more emphasis on phenotypic development (Schlichting and Pigliucci, 1998; West-Eberhard, 2003). These volumes provide motivation for a great deal of current research as they made the phenomenon of developmental variation much more acceptable as a topic of evolutionary interest. These volumes also helped to reintroduce neglected topics such as how phenotypes may be limited by developmental processes. For example, the idea of phenotypic integration whereby a set of traits are correlated with others became a renewed area of interest (Olsen and Miller, 1958). This is because integration may itself evolve for adaptive reasons, or it can provide limits to adaptive evolution by not allowing a trait to change independently of others (Klingenberg, 2008; Parsons *et al.*, 2012). An extension of phenotypic integration is the idea of modularity whereby subsets of traits can interact tightly, but in a way that is relatively distinct from other traits and modules. The underlying basis of such integration modules can lie in developmental processes, evolutionary history, or functional needs, and now provides an active area of interest in adaptive radiations (Parsons *et al.*, 2011; Claverie and Patek, 2013; Sanger *et al.*, 2012).

While developmental thinking was becoming merged with the study of adaptive variation through the 1990s and early 2000s, pure developmental biology was becoming more directly merged with molecular techniques (Roush and Pennisi, 1997; Dalton, 2000; Goodman and Coughlin, 2000; Hall, 2000). Methodological advances were occurring rapidly with an increasing number of studies beginning to show the expression of genes '*in situ*' where it could be visualized on the embryo, or quantified precisely. This now allowed developmental biology to directly examine the molecular basis of basic phenotypic development such as early embryogenesis (Raff *et al.*, 1999). However, at this time molecular methods in developmental biology were almost entirely limited to 'model' organisms (e.g., zebrafish, mice, xenopus). Through time it became clear that the function of genes during embryogenesis in these widely different organisms was conserved, as was often their nucleotide sequence. This key finding opened the opportunity to take these findings out of the lab and into study systems exhibiting adaptive phenotypic variation (i.e., adaptive radiations). Below, the author provides some examples of where evo-devo has been taking the study of adaptive radiations. Because it is beyond the scope of this article to highlight every relevant system. The author has focused on vertebrate groups because they are among the best characterized in terms of adaptive radiations, and ecology, and currently represent a highly active area of recent investigation.

Fish Adaptive Radiations and the Incorporation of Evolutionary Developmental Biology

Adaptive radiations in fishes, the most species rich vertebrate group, offer an unmatched array of phenotypes that have been investigated from an evo-devo perspective. For example, craniofacial variation is extensive and relates to prey consumption whereby shorter jaws offer a higher mechanical advantage for harder prey, while longer jaws offer a greater volume in the buccal cavity for suction feeding of small waterborne prey (Parsons and Albertson, 2009). Similarly, body shape variation is extensive in fishes with a more elongate fusiform shape conferring an advantage for sustained swimming, while deeper body shapes aid in the navigation of complex structural environments (Webb, 1982, 1983, 1984). Such variation commonly extends across fish adaptive radiations, and is even present within species in the form of 'ecomorphs.' Such ecomorphs are common in fishes, especially those from postglacial environments where competition from other species is lower, allowing for the exploitation of new niches (Taylor, 1999). These attributes whereby ecology can be related to species level phenotypic variation, and even be studied below the species level have made fishes an attractive group for evo-devo studies, especially since many can be readily kept under lab conditions (**Figure 3**).

Fishes can be credited for being a valuable empirical model for helping introduce developmental thinking into the study of adaptive divergence through the study of phenotypic plasticity. For sometime, prior to the emergence of modern evo-devo, fishes have been the focus for studies of morphological phenotypic plasticity (Robinson and Parsons, 2002). Fishes have been shown many times to possess an ability to alter head and body shape in response to diets that mimic the benthic and limnetic prey they often specialize on in nature (Wimberger, 1994; Robinson and Parsons, 2002; Adams and Huntingford, 2004). Such developmental changes correspond to differences in morphology found in nature and ultimately affect the performance of populations (Day and McPhail, 1996; Parsons and Robinson, 2007; Andersson, 2003; Ruehl and DeWitt, 2007). This direct tie between developmental variation, the environment, and adaption suggests broader implications for how plasticity influences evolutionary divergence (**Figure 4**). Indeed, studies have recently shown that

plastic responses themselves can evolve in a number of ways. For example, data from ecomorphs of Arctic charr (*Salvelinus alpinus*) show evidence of genetic accommodation whereby morphological plasticity has been lost with an increasing degree of ecological specialization (Parsons *et al.*, 2010, 2011). Similarly, threespine sticklebacks have been shown to display genetic accommodation in growth rate, whereby recently established populations show a greater degree of plasticity than long established ones. Fishes also demonstrate evidence that plastic responses can determine the initial trajectory of adaptive evolutionary change. For example, the **flexible stem theory** of adaptive divergence posits that the plastic responses of an ancestral population can exhibit the same patterns found between species in a larger adaptive radiation (Wund *et al.*, 2008, 2012). In support of this theory marine threespine sticklebacks, which provide an extant ancestor for contemporary freshwater populations, show a morphological plastic response that matches the patterns of adaptive divergence found between ecomorphs in specialized freshwater populations.

From a molecular perspective, fishes have also contributed to our understanding of the genetic mechanisms underlying adaptive phenotypic development. An advantage of closely related, but adaptively divergent populations of fishes is that different forms can be hybridized to produce offspring that possess a range of phenotypes in the F2 generation. While divergent selection may alter several regions of the genome and cause them to become alternatively fixed for genetic variation between ecomorphs a hybridization event can mix together a number of new combinations. These new combinations allow for relationships between phenotypic variation and genetic variation to be statistically assessed through the methodology known as quantitative trait loci (QTL) mapping. While this method does not typically determine the exact genetic changes related to a phenotype it provides a significant inroad toward this discovery by providing an informed idea of the genomic region where adaptive allelic variation is likely to be present (e.g., Albertson *et al.*, 2003, 2005; Shapiro *et al.*, 2004; Peichel *et al.*, 2001; Parsons *et al.*, 2015; Laporte *et al.*, 2015).

Using the QTL approach, researchers have begun to understand the genetic and developmental basis of a range of phenotypes in fishes. For example, within African cichlids which comprise the largest known vertebrate adaptive radiations, QTL mapping pointed toward the gene bone morphogenetic protein 4 (*BMP4*) as a candidate for adaptive differences in jaw and head shape observed between Malawi cichlid species (Albertson *et al.*, 2005). Indeed, expression of *BMP4* is naturally higher in a species with a more robust jaw morphology, and was shown to alter jaw morphology concordantly in a complementary experiment using zebrafish. A more recent experiment and finer scale sequencing of the QTL region neighboring *BMP4* has now revealed a mutation that is alternatively fixed between species that lies within the gene promoter region (transcriptional co-activator) of the neighboring gene limb bud and heart homolog (*lbh*) (Powder *et al.*, 2014). The function of this mutation was tested using both zebrafish and *Xenopus*, and it was demonstrated that *lbh* mediated the migration of cranial neural crest cells, the cellular source of the craniofacial skeleton (**Figure 5**). The mutation,

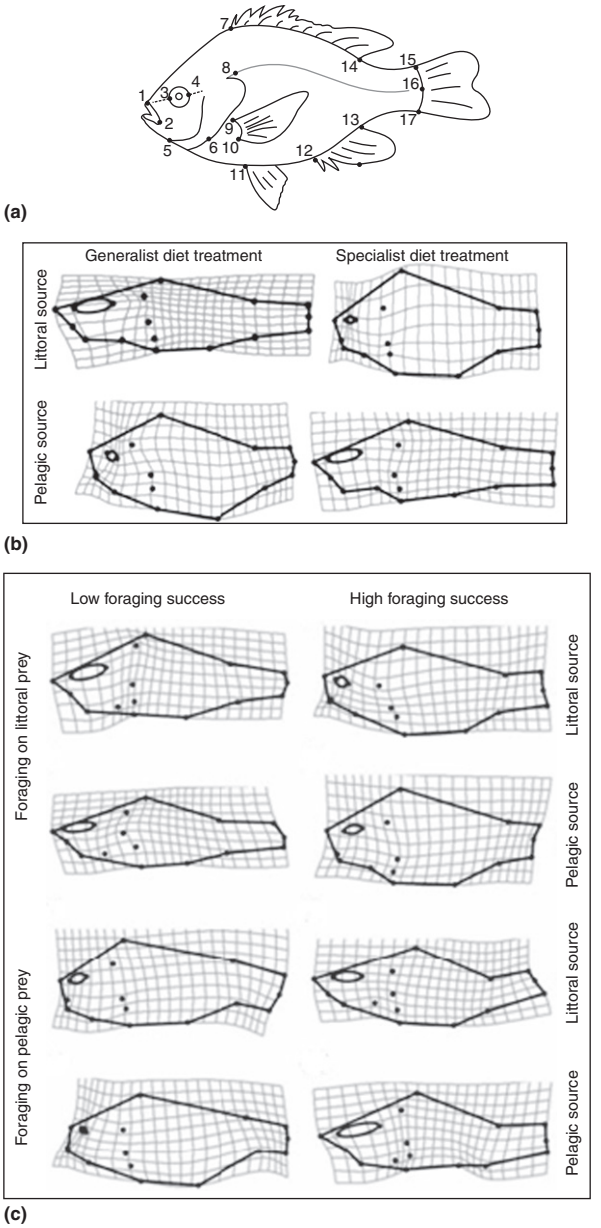

Figure 4 Developmentally plastic responses can mirror adaptive divergence and confer fitness advantages. Here panel (a) shows the shape landmarks collected across the body form of pumpkinseed sunfish (*Lepomis gibbosus*), a species which displays a resource polymorphism between littoral and pelagic habitats. Using measures of geometry to depict shape changes these plastic responses mirror the differences documented between ecomorphs. Panel (b) depicts the plastic responses of sunfish from littoral and pelagic source populations reared under diets that mimic their respective specialist prey, and a generalist diet treatment that alternates weekly between littoral and pelagic prey. Panel (c) depicts the average phenotypes of sunfish from both littoral and pelagic source populations that performed with poor success and high success on littoral (top four grids) and pelagic prey (bottom four grids). Reproduced from Parsons, K.J., Robinson, B.W., 2007. Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favours resource polymorphism. Journal of Evolutionary Biology 20, 673–684.

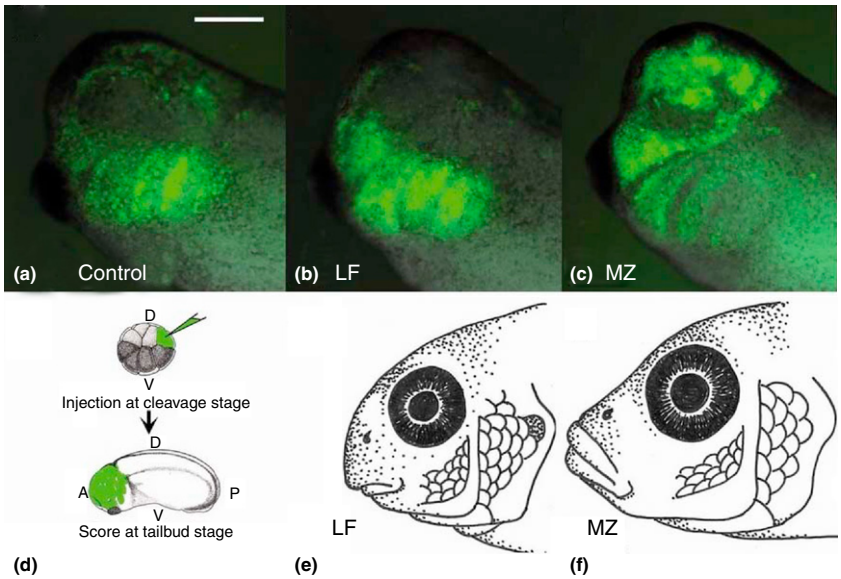

Figure 5 A single amino acid difference in cichlids regulates the migration of neural crest cells, the source cell population for the developing face. Targetted injections of *lbh* transcripts from two species cichlids *Labeotropheus fuelleborni* (LF), and *Maylandia zebra* (MZ) show that they alter cell migration in Xenopus embryos. The top panels depict the differential movement of neural crest cells labeled with green fluorescent protein, including a control (a), LF (b), and MZ (c). The bottom panel depicts the experimental injection of transcripts (d), and the differential facial morphology between species (e,f) that may result from changes in the expression of *lbh*. Images are derived from Powder, K.E., Cousin, H., McLinden, G.P., Craig Albertson, R., 2014. A nonsynonymous mutation in the transcriptional regulator lbh is associated with cichlid craniofacial adaptation and neural crest cell development. Molecular Biology and Evolution 31, 3113–3124.

which comprised of a single amino acid change, resulted in discrete shifts in migration patterns of the multipotent neural crest cells in ways that are consistent with both embryological and adaptive adult craniofacial phenotypes. Among animals, this polymorphism in *lbh* represents a rare example of a coding change that is associated with continuous morphological variation, an important aspect because most adaptive radiations are represented by a continuum of phenotypic variation rather than the presence or absence of traits (Parsons and Alberston, 2013). It is expected that new advances in genomics, and increasingly available resources for fish genomes will continue to enable such precise insights into the developmental genetic underpinnings of adaptive divergence (Brawand *et al.*, 2014; Moczek *et al.*, 2015).

Herpetofauna: Plasticity and Ontogenetic Processes Contribute to Adaptive Divergence

Lizards, amphibians, and snakes exhibit adaptive divergence through a range of phenotypes that correspond with ecological conditions. For example, the anolis lizards form the most species rich genus of amniote vertebrates, and those found across the Greater Antilles provide one of the best studied examples of adaptive radiations. Here anolis occur across isolated islands and have diverged to occupy separate ecological niches. These niches mostly involve the location of foraging within the vegetation with some ecomorphs occupying the crown of trees, the trunk, or underlying shrubs (Losos, 2007). Differential niche use is accompanied by morphological changes mainly related to the diameter of the surfaces they most frequently encounter, with 'twig' ecomorphs having short limbs, while trunk ecomorphs have long limbs. Interspecific comparisons indicate that longer legs confer an advantage for increased running speed on broad substrates, whereas shorter limbs provide greater maneuverability on narrow surfaces.

The anolis system has been the recent subject of evo-devo research. Notably, experimental evidence shows that phenotypic plasticity exists for hind-limb length in *Anolis sagrei* and *Anolis carolinensis*. These plastic responses mirror the pattern of species differences whereby hatchlings exposed only to broad substrates developed relatively longer hind limbs for their body size compared to hatchlings exposed only to narrow substrates (Losos *et al.*, 2000; Kolbe and Losos, 2005). Genetic variation for plasticity is present between species suggesting that plasticity has evolved with and contributed to their adaptive divergence (perhaps in line with the flexible stem hypothesis).

Indeed, other herpetofauna show evidence that developmental flexibility has evolved in concordance with adaptation. For example, comparisons from several populations of tiger snakes (*Notechis scutatus*) isolated (or introduced) on islands from period of less than 30 to more than 9000 years where selection favoured increased head size (adaptive for ingesting large prey) show changes in magnitudes of plasticity (Aubret and Shine, 2009). Specifically, a larger head size is achieved by plasticity in 'young' populations and by genetic canalization in 'older' populations. So far, this system provides rare empirical evidence of genetic assimilation in natural populations, where an adaptive trait has shifted from having a plastic basis to be canalized as part of genetically determined development.

Other developmental processes have been found to influence adaptive radiations in herpetofauna, with recent focus on

ontogenetic processes. For example, allometry, which refers to how a trait changes with increasing size has been viewed as a possible progenitor of variation, but more often a constraint on evolution because of its dependence on size (Klingenberg, 2010). Data from anolis show support for both views in that sexual dimorphisms in cranial shape converge across species and are usually but not always dependant on the same allometric strategy. Specifically, dimorphisms where males have much more elongate faces relative to females are usually the result of the emergence of dimorphism early in ontogeny that gradually increases. However, a novel strategy has also evolved in one clade (*Anolis carolinensis*) whereby dimorphism appears rapidly in the late stages of ontogeny as sexual maturity arises (Sanger *et al.*, 2013). Further research shows that this novel strategy is underlain by sex-specific regulation of the estrogen pathway, in contrast to the androgen or insulin growth factor pathways normally considered to be the primary regulators of sexual dimorphisms within vertebrates (Sanger *et al.*, 2014).

Similarly, allometry plays a role within an adaptive radiation of salamanders. The ontogeny of foot morphology has been investigated using species of European plethodontid cave salamanders (Adams and Nistri, 2010). Foot morphology in this group of salamanders relates to their ecology with the degree of webbing between the toes showing relationships with the ability to cling to substrates. For these salamanders, the ancestral ontogenetic strategy maintains a constant degree of webbing with growth, or little morphological change over ontogeny (i.e., isometric growth). However, at least two evolutionary changes in allometry exist among lineages: one lineage has evolved an allometric growth pattern, where foot webbing increases with size, and a species in this lineage also shows a reversion to isometry. This switch to allometric growth could possibly underlie an adaptation for climbing. Notably, the ontogeny of different species which starts from different juvenile foot morphologies converges toward a shared adult morphology with extensive webbing suggesting an adaptive explanation, and potentially functional optimum (Adams and Nistri, 2010). These and overall findings so far from adaptive radiations of herpetofauna show that they hold great promise for our understanding of adaptive radiations from an evo-devo perspective.

Avifauna: Adaptive Bill Diversity through Developmental Change

Birds are especially noted for variation in bill morphology such as that found in Hawaiian honeycreepers (*Carduelinae*) and arguably the best known example of an adaptive radiation through Darwin's finches (*Geospiza*). *Geospiza* comprise a group of 14 closely related song birds on Galapagos Islands and Cocos Island and have been the focus of several studies focused on the development of bill morphology (Parsons and Albertson, 2009; **Figure 1**). Bills vary among species in ways that are maximally effective for exploiting particular foods, including seeds, insects, and cactus flowers. Broad and deep bills are suited for crushing seeds (small, medium, and large ground finches), whereas long pointed beaks are suited for eating insects or reaching into cactus flowers and fruits. These specializations are apparent during early embryonic development and are clearly influenced by underlying genetic variation. Specifically, the gene transcripts of bone morphogenetic protein 4 (*Bmp4*) and calmodulin (*CaM*) figure prominently in our understanding of finch diversity with deep and broad beak morphology being strongly correlated with *Bmp4* expression (Abzhanov *et al.*, 2004, 2006). Experiments reveal that overexpression of *Bmp4* in chicken embryos causes morphological changes approaching the bill morphology of the large ground finch *Geospiza magnirostris*. Surprisingly, other growth factors such as *fibroblast growth factor* (*Fgf8*) and *sonic hedgehog* (*Shh*), which are expressed in the overlying beak ectoderm and are necessary for patterning of the mesenchyme and cartilage growth (3), do not differ in expression among finch embryos, although recent investigation from other species of birds at earlier stages of development suggests that Wnt signaling, a developmental pathway involved in directing the migration of cells and bone growth in the craniofacial region may actually precede variation in BMP4 (Powder *et al.*, 2010, 2012). Similarly, calmodulin (*CaM1*), a molecule involved in mediating calcium (Ca^{2+}) signaling, is expressed at higher levels in the long and pointed beaks of cactus finches relative to more robust bill types. Also, experiments in which an activated form of the *CaM kinase* (*CaMKII*) was artifically upregulated in the frontonasal prominence of chicken embryos, causes an elongation of the upper beak, similar to cactus finches. Interestingly, this did not affect other beak axes (i.e., width and depth), indicating the potential independence of the pathways controlling beak shape.

Variation in bill width and depth that relates to ecology and bite force shows the potential for extremely rapid evolution through similar developmental mechanisms. For example, such adaptive changes occur in populations of the Sonoran house finch (*Carpodacus mexicanus*), and although variation in bill shape is much less obvious than that found across Galapagos *Geopspiza* species (**Figure 1**), the divergence between urban and rural populations similarly occur in relation to food type. Urban populations feed on larger, harder sunflower seeds, which in turn favor a stronger bite force. Expression analysis during embryonic bill development in urban house finches indicates that *Bmp*s are involved in producing intraspecific differences in bill shape. The bill mandibular primordium of urban finches shows inheritance of an earlier and higher activity of *Bmp*s corroborating the view that the processes involved in speciation (i.e., *Geospiza*) are the sum of smaller but multiple microevolutionary processes.

Conclusions

Evo-devo has an established history of contributions that have shaped our understanding of adaptive radiations. As shown above, environmental influences on development (i.e., phenotypic plasticity) in a range of taxa could contribute to the process of adaptation in a variety of ways and perhaps provide much of the variation that is fundamental for natural selection to operate. As yet, it is largely unknown how such plastic phenotypes arise from a mechanistic perspective, specifically the alleles that determine genetic variation in plasticity. Gaining an understanding of the alleles underlying

plastic responses will provide tractable variation that could directly relate to higher levels of divergence providing an explicit link between early and late stages of adaptive divergence (**Figure 2**). Further, evo-devo has enlightened evolutionary biology about how ontogenetic processes can be altered to produce both novel and convergent phenotypes. These findings suggest a much larger role for ontogeny as a source of variation for adaptive evolutionary change than is currently appreciated. Finally, while lab model organisms initially demonstrated conserved functional roles for genes involved in early development, adaptive radiations also show that 'tinkering' of the same genes or pathways in widely divergent taxa (e.g., *BMP4* for cichlid jaw shape, finch bill shape) can cause roughly similar changes in development to produce adaptive phenotypes (Parsons and Albertson, 2009). Combined these findings are derived from a wide variety of approaches, from those that are highly quantitative to purely molecular approaches (Parsons and Alberston, 2013). However, evo-devo continues to form a synthesis where approaches such as these are becoming increasingly integrated. The advent of newer high throughput sequencing technologies has recently democratized genetic data to any organism of interest which will likely aid in this synthesis. As a more integrative evo-devo moves into this post genomic era it is expected to continue providing novel insights into adaptive processes.

See also: Convergent Evolution, Adaptive Radiation, and Species Diversification in Plants. Ecological Speciation and Its Consequences

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Age-Specific Survivorship and Fertility, Estimating

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Glossary

Bias Difference between the expected value of the estimator of a population parameter and its true value.

Precision An estimator is precise if different samples of the same population result in similar estimates.

Robustness An estimator is robust if its properties – bias, precision – are not too sensitive to underlying modeling assumptions.

Introduction

Survivorship is the proportion of individuals within a cohort still alive after a given period of time, and fertility is the number of offspring born per individual or mating pair. Interest in measuring these two traits started over two centuries ago, but it is mostly in the last decades that modern methods have been developed. In fact, one of the difficulties today is to find the appropriate methods for a given context, depending in particular on the questions investigated, the study design and the information available about individuals. But before investigating how we can reliably estimate survival and fertility in a population, how much they vary and what can cause observed changes, we need first to go through some important statistical concepts. Assume we are interested in estimating a given parameter – let's say survivorship of adult females in the year following birth of their calf, called ϕ. To estimate this parameter, we will usually have to rely on (1) a sample of females from the population, and (2) observing which females will be alive at the end of the year. The naïve way of estimating the survival probability is to consider the ratio of the number of females known to be alive 1 year later to the initial number of females. How can we determine if this ratio is a reliable estimator of ϕ? Because this ratio will be affected by many factors – for example, which females happen to be in the sample, which females were observed to be alive – this ratio will usually differ from ϕ and only the statistical properties of the estimator can be used to assess its reliability. The most important properties of the estimator are its expected value or average and its variance (or its inverse, the precision). These properties are usually calculated considering hypothetical repeated samples from the same population. For example, an estimator is said to be unbiased if its average value is equal to its true (but unknown) value. A large part of the work done in statistics aims at finding estimators, which have low bias, and as small a variance as possible. Another aspect is robustness: we prefer methods that are not too sensitive to the assumptions we make, particularly those that are difficult to assess with the available data. Finally, estimates are of limited value without knowledge of their uncertainty, which is often a function of the bias and precision of the estimators. Why are those concepts important for estimating the survivorship ϕ?

First, the parameter ϕ is a construct, a parameter describing a population and not any single individual. Adult females will differ in terms of factors like age, body mass, and genotype. What we are trying to estimate is rarely a type of underlying constant like one often estimates in physics, but instead is a quantity affected by the factors that structure our populations, and therefore may be confounded with environmental and genetic factors. For example, clearly a population made of old females will result in a lower ϕ than a population made of prime-aged ones. Another consequence is in terms of robustness: we will often assume that there is no heterogeneity of survivorship (ϕ is the same for all females in a given age class) to build estimators and we need to know the sensitivity to this assumption. Second, because of this underlying heterogeneity, the way we sample the population is important: if we have only senescent females in the sample, because they are easier to capture, our estimate will be biased (low, on average our estimate of ϕ is lower than the value for the population). Ideally, we should aim for sampling methods that allow us to know the probability that each female is sampled, as in a survey, but this is rarely the case, for the simple reason that we don't know to start with which individuals are in the population (i.e., we don't have a census as in human populations). Third, another source of bias is the way we assess the number of females that survived – females that survived may not be captured or resighted and therefore won't be counted in the number of females known to be alive. This can be because they have left the population – emigrated – or because they escaped the capture process or were hidden from view. Fourth, survival probability is a stochastic process (think, e.g., of encountering a predator), and therefore estimates will vary from year to year, even if environmental conditions are unchanging. This source of variability is often of interest as we frequently want to assess how environmental conditions affect survival probabilities, and separate these effects from sampling and purely random components (demographic stochasticity). The former component of the variability is often called the process variance, as opposed to the sampling variance associated with the observation of a finite sample of individuals. To summarize, when estimating a life history parameter, it is important to consider what the parameter measures (cf. age structure and mean survival probability), how field methods determine the composition of the sample, both the initial sample and which individuals are later observed, and how the parameter may vary over time and space. Statistical models aim at making explicit the distinction among these sources of variability in order to get estimates that have small bias and as small uncertainty as possible given the sample size.

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Estimating Survivorship

Many approaches to the estimation of survivorship require an initial sample with marked individuals, either upon capture (rings, pit-tags, etc.), because they have natural marks such as spots, bands, or scars (tigers, whales), or because they don't move and can be relocated (e.g., many plants). The main reason for having marked individuals is to obtain robust statistical estimates, whereas other approaches, such as using age structure or age at harvest data, are sensitive to assumptions which are often hard to assess. For example, we could use the ratio of the numbers of individuals aged $(x+1)$ years to those aged x years to estimate survivorship from age x to age $(x+1)$, but this ratio reflects survivorship as well as age-specific capture rates, population structure (size of annual cohorts) and growth rate.

A naïve estimator for a survival probability is the ratio of individuals known to be alive to the initial number of individuals, sometimes called the return rate. Two processes will affect this estimator: recapture, i.e., the proportion of marked individuals present in the population recaptured or resighted (Gimenez *et al.*, 2008; Kéry *et al.*, 2005); and emigration, i.e., the proportion of marked individuals which have left the population. For the latter, one usually refers to apparent survival as being survival minus emigration, i.e., apparent survival is the probability to survive and stay in the population (Schaub and Royle, 2014). Much work has been done on correcting for recapture probabilities which are lower than one, and often vary between capture sessions, age groups, or sex, and this requires that we have more than one recapture session. The principle is that individuals which are not seen at one recapture session but are seen later must have survived but have not been captured. This makes it possible to separate the two processes, survival and recapture (with only one recapture session, we have an estimate of the product – i.e., they have survived and been recaptured – not of each process separately).

The principle behind the estimation of survivorship, and incidentally of recapture rates, is relatively simple to understand. One needs to write every capture history – i.e., that an animal was caught on year 1 and 3, but not in any other years, as a function of survivorship every year ($s1$, $s2$, …) and recapture rates every year ($p2$, $p3$, …). Being caught in year 3 but not year 2 means the animal has survived the first 2 years with probability $s1*s2$, and regarding recapture the probability is $(1-p2)*p3$. Of course, there is software to calculate probabilities of each possible life history automatically. The next step is to have a distribution for the number of animals associated with each capture history, and a multinomial distribution is a natural starting point. Finally, one can constrain the survivorship and recapture parameter to be function of environmental parameters or individual covariates, in a similar manner to a generalized linear model (remember also that probabilities are numbers between 0 and 1 and a link function like the logit limits the range of predicted values to this range).

Next one has to consider (1) if the model adequately fits the data, and (2) how to compare models in terms of their predictive ability or for estimating a given parameter. The first step is essential – using a model with a poor fit, for example, because animals respond to capture and have a lower recapture rate the year after, or because there is a large heterogeneity in recapture rates among individuals, may lead to biased estimates. In practice, the usual approach to assessing goodness-of-fit (comparing observed number of capture histories to expected numbers) will not be efficient because there are many capture histories and expected numbers are low. It is more powerful to restrict the test to specific patterns of lack of fit (such as the trap-dependence mentioned above), so that one can also expand the model to get a better fit (Choquet *et al.*, 2009). The second step, model selection and model averaging, is now done by calculating the relative weight of models, such as could be derived from information criteria (e.g., AIC, BIC, or DIC). Although there is still disagreement on which approach is 'best,' one should be reminded that statistical criteria often aim at a specific objective (e.g., minimizing a measure of the difference between model predictions and the unknown truth) and that biological considerations and design should also be part of the choice of the models retained for final inference. For example, when comparing survivorship in populations differing by selection pressures or management actions (Frederiksen *et al.*, 2014), pressures or actions should be part of all models considered, in order to focus on the main objective of the study – estimating differences among pressures/actions.

As an example, Gamelon *et al.* (2014) estimated survival patterns of senescence in wild boar, a species which is unusual among large mammals because females invest early and heavily in reproduction. Using capture-mark-recapture data, they showed that wild boar males and females had similar senescence rates, and that wild boar females did not have faster senescence than other ungulates of a similar size. However, wild boar females differed from other ungulates because they began to senesce at an earlier age. This work shows how advanced methods allow for a detailed understanding of evolutionary patterns, distinguishing between timing and rate of senescence.

Recapture parameters are often nuisance parameters, i.e., we are not interested in estimating them but they are needed to adequately fit models to the data. It is therefore also important to model their variation adequately (e.g., recapture rates could be a function of age, as well as of time). Although a few years ago it was not practical to consider all possible combinations of models for both survivorship and recapture, this is now easier to do and it seems likely to be a better strategy than modeling recapture rates first and then survivorship.

There are many other methods available for estimating survivorship (David *et al.*, 2010; Skalski *et al.*, 2005; Williams *et al.* 2002) – for example, in studies using biologging, individuals are followed continuously and an approximate date of death is available (or when the equipment was removed/stopped functioning). Methods like survival analysis can then be used. Other approaches have been developed for harvested populations, using in particular the age composition of harvested cohorts, but they often are less robust than approaches using uniquely marked individuals. They, however, are sometimes the only ones available (think of many harvested fishes or large mammals), and it is then important to assess sensitivity to assumptions, for example, regarding age-dependent selective harvesting. Finally, individuals may not be easily identified – think of clonal plants – and spatial location or genetic methods may be necessary to define units for analysis (Lauenroth and Adler, 2008).

Estimating Fertility

Estimation of the number of offspring born per individual is a relatively less unified field than estimation of survivorship. One reason is that there are many stages at which the number of offspring could be measured: for example, one could estimate pregnancy rates by capturing a sample of females (or individuals in the case of hermaphrodites) before birth (or hatching, etc.), count number of eggs or seeds produced by each female, or count the proportion of females with young a few weeks after birth. Such measures might be hard to compare since mortality around birth/hatching is often high, as, for example, in many insect, fish, or plant species producing large numbers of eggs/seeds. At the same time, interfering too much with individuals around birth or hatching may affect the parameters we want to estimate, and without direct observations or measurements made on individuals, we may not be able to identify which offspring belong to which females. In the latter case, we might be able to estimate the average fertility in the population, but not its variability among individuals. This might lead to considerable biases if, for example, the sample of females includes individuals with large variation in age, including females which are too young or too old to reproduce.

Two other issues are observability – the probability that a female will be observed depending on having offspring or not – and state uncertainty – one is not always sure that a female has bred or not, is with or without offspring, or that an offspring belongs to one-specific female. As for survivorship, relying on individually marked individuals is, when possible, the best solution. One can use models similar to those developed for survival to relate capture/sighting rate to female status (breeder/nonbreeder, offspring or not), and use, for example, repeated observations within a short time frame of the same individuals to assess uncertainty in state. Such models with state uncertainty are increasingly used and relevant. Desprez *et al.* (2011) used this approach to disentangle the relationship between reproduction and age versus experience in kittiwakes, a long-lived seabird. As all birds are not observed every year, past breeding status, and therefore experience, is not known accurately and needs to be estimated. Whereas age did not influence adult breeding rates, unexperienced birds had a lower breeding probability than experienced birds.

Finally, access to individually marked individuals makes it possible to estimate transition rates among reproductive states – for example, if females reproducing 1 year also reproduce the year after. Models investigating transitions among, for example, reproductive states (e.g., having 0, 1, and 2 offspring) include a survival component, and represent a fruitful way to combine analyses of survivorship and fertility components.

Conclusions

There is a wide diversity of statistical models now available for analyzing variation in survivorship, and these methods relate such variation to individual characteristics or environmental variables (Gimenez *et al.*, 2012). While the development of these models continues unabated – for example, including information about the spatial localization of each captured individuals (Royle *et al.*, 2015) – it is important to bear in mind aspects such as robustness and sampling design. Estimating individual fertility in the wild is often more complex, because there are many stages at which offspring can be counted and offspring cannot easily be associated with individual females without using specific techniques or intensive observations.

See also: Life History: Age and Stage Structure. Life History: Pike. Life History Theory: Basics

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Aging: Why Do We Age?

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What Is Aging?

Adult plants and animals generally appear to function less well as they age. This senescence is manifested most dramatically in traits related to fitness and most profoundly as decreases in survival and reproductive rates (vital rates). Vital rate declines with age, or 'demographic senescence,' is the best documented examples of aging, and these have been shown to be nearly ubiquitous but highly variable among captive and wild populations of plants and animals (Jones *et al.*, 2014; Promislow, 1991; Ricklefs, 1998). Demographic senescence is caused by 'functional senescence,' or the age-related deteriorations in the phenotypes that support survival and reproduction (Burger and Promislow, 2006; Williams, 1999); however, this sort of aging is not always so clearly expressed and easily detectable (Nussey *et al.*, 2009; Rueppell *et al.*, 2007).

Why aging happens is of immense interest to biologists, who have proposed hundreds of proximate mechanisms to explain how intrinsic or extrinsic changes cause physiological deterioration within individuals (Medvedev, 1990). Like any biological feature, aging can be shaped by evolution. In particular, evolutionary biologists wish to understand how natural selection, a force usually associated with the improved fit of organisms to their environment, can be reconciled with the senescent decline of adaptive function. To this end, evolutionary theory has been developed to guide our study into the origins of the diversity of senescent patterns across individuals, populations, and species. In practice, this requires synthesizing what we can learn about the causal relationships between genes, age-specific phenotypes, and fitness. This article provides an introduction and overview of this theory.

The Origins of the Theory

Several verbal arguments have been proposed to explain the evolutionary cause for aging; these are discussed in this section.

Adaptive Aging

In what might be the earliest evolutionary model of aging, August Weismann proposed that selection will favor aging because the culling of the old directly benefits the young, and this is good for the population as a whole (Weissman, 1889). He argued that death-causing mechanisms should evolve to favor aging, such as deciduous trees have evolved to cast off old leaves in the autumn rather than waste resources to enable them to survive through the winter. However, adaptive aging has been largely rejected by most evolutionary biologists for at least three reasons. First, the model assumes that the old are somehow less important to the population than are the young; this would seem to presuppose the existence of aging. As a result, this model is incapable of explaining an evolutionary 'origin' of aging. A second problem is that an elaborate adaptive mechanism ought to be readily disrupted by environmental or genetic challenges, but no mutation or environmental stressor has been shown to remove aging from a normally senescing organism. Finally, some object to the adaptive aging model because it depends upon some degree of group selection, a perspective of natural selection that has changed dramatically over recent decades and has bred much confusion. While it is not appropriate to reject the model out-of-hand for this reason, a group selection explanation does suggest certain limitations to the strength of such selection, such as the reliance of (perhaps unrealistically) high degrees of relatedness between members of the population. Nevertheless, modern and conceptually formalized versions of group selection (kin selection and multilevel selection) can allow for situations where selection can modify the evolution of aging by further relaxing or enhancing selection for late-age survival or reproduction (Bourke, 2007; Ronce and Promislow, 2010). However, these modifying effects should not be interpreted as strong support for the evolution of aging-adaptations per se.

Mutation Accumulation

In the early 1940s, J.B.S Haldane used evolutionary theory, specifically the antagonistic forces of natural selection and recurring mutation, to explain the persistence of the lethal Huntington disease (HD) in the human population of England (Haldane, 1941). Haldane recognized that the HD allele was largely protected from removal by purifying selection because the disease did not manifest until individuals were in their 40s, a point in most people's lives at which any children they might have were already born. Thus, the genes of individuals that made up the next generation had little do with whether their parents eventually succumbed to the lethal effects of HD. Peter Medawar (1952) extended Haldane's explanation of HD to a more general evolutionary perspective of aging by recognizing that the later the deleterious effect of any gene, the weaker the strength of selection to remove the allele from the population becomes. This age-related attenuation of selection articulated by Medawar forms the conceptual foundation for the evolutionary theory of aging.

In keeping with classical population genetic thinking, Medawar imagined that our genomes were showered with new deleterious mutations every generation. However, he believed that many genes had ages-of-effect that did not necessarily persist over the entire life of the individuals. For example, genes that are deleterious to childhood survival may be irrelevant to adult function. Alternatively, there may be genes (such as HD) that do not affect early function but negatively affect individuals late in life. The latter type of mutation will tend to accumulate to a greater extent than the former over many generations, and aging will result. Medawar's idea represents the first 'mutation accumulation' (or 'MA') model. Unlike Weismann's model, MA models view aging as a

maladaptation: aging, on balance, costs fitness, but natural selection is not sufficiently strong to remove it completely. It is important to note that MA assumes that there is some age-specificity of genetic effects upon survival or reproduction. It does not make any assumptions about the timing of gene expression. A developmental gene that contributes to a phenotype that wears out in late age could be correctly be considered an MA gene because the fitness effect is manifested late in life.

Antagonistic Pleiotropy

George C. Williams embraced Medawar's argument for relaxed selection with increased age while proposing another genetic model to facilitate the evolution of aging (Williams, 1957). Rather than imagining that genes have single, and relatively narrow, age-windows of effect, Williams suggested that some genes might be so pleiotropic with respect to age that they influence fitness in both the young and in the old. If some of these genes had fitness effects that were positive early in life and deleterious late in life, then natural selection would tend to favor their spread through the population. Eventually, evolution by natural selection would increase survival or reproduction in the young at the expense of these traits in the old. Williams' model, commonly called the 'antagonistic pleiotropy' model (or simply 'AP'), does not contribute to an adaptive theory of aging, as aging per se is not advantageous with respect to fitness. Instead, it may be more appropriate to view AP as a model for the evolution of aging by constraint because genetic trade-offs force senescent declines with age. 'Disposable soma theory' merits attention as a popular and biologically motivated AP model that identifies the allocation of limited resources to both self-maintenance and reproduction as the fundamental trade-off relevant to the evolution of aging (Kirkwood, 1977, 2002).

The Mutation Accumulation and Antagonistic Pleiotropy Dichotomy

MA and AP are usually considered the only viable evolutionary explanations for the origins and maintenance of aging, and a tremendous amount of work has sought to determine the contribution of each. A fuller discussion of this work is delayed until the end of this article, but it is worth noting here that the evolutionary mechanism of aging may not be reliably dichotomized because it is possible to imagine genes that demonstrate qualities of both MA and AP. For example, consider an allele with a small positive effect upon early reproduction and a large deleterious effect upon midlife survival. One might correctly call this an AP allele because it demonstrates an early/late fitness trade-off. Alternatively, one could reasonably categorize this as an MA gene if the fitness effects of its early-age advantage are overwhelmed by the fitness lost by its suppression of late-life survival, and the allele persists in the population due to recurrent mutation. For this reason, empirical investigations into the genetic foundations for aging often include the caveat that MA and AP are not mutually exclusive mechanisms.

W.D. Hamilton: Foundations for a Formal Theory

In the mid-1960s, the evolutionary theory of aging was a collection of verbal arguments and models, with little in the way of conceptual rigor. MA and AP offered two genetic models to explain how an age-related relaxation of selection can cause aging to evolve, but exact descriptions of this change in selection were lacking. This changed with William Hamilton's contribution in 1966, where he made explicit the mathematical relationships between fitness and vital rates (Hamilton, 1966). In doing so, he defined exactly how aging changes the strength of selection acting to decrease age-specific survival and increase fertility (**Figure 1**). Quantitatively, he showed how selection against mortality at some particular age x is proportional to the product of: (1) the frequency of cumulative survival, or the fraction of individuals that survive from birth to x and (2) the expectation of future reproduction given survival to that age discounted by future population growth (i.e., early reproduction counts for more when populations are growing, but less when they shrink). Selection for age-specific fertility is cumulative survival, which is also discounted by future population growth. Importantly, the necessary parameters in Hamilton's model are age-specific survival and fertility, which are readily estimable from laboratory and natural populations (at least in principle). These quantitative descriptions of selection are collectively known as Hamilton's 'sensitivities,' as they express the sensitivity of population growth to changes in vital rates (Charlesworth, 1994), where population growth rate is taken as a valid definition of fitness when the age-structure of a population is stable over time. Hamilton derived his sensitivities by implicit differentiation; since then they have been re-derived using population projection matrices (Caswell, 1978, 2001) and multiple linear regression (Moorad, 2014). These approaches allow Hamilton's basic principle to be applied to more complex problems in ecological modeling and quantitative genetics, respectively.

Significantly, Hamilton's sensitivities show that selection against mortality and for reproduction cannot increase with age. Specifically, they prove the following:

1. Selection against age-specific mortality is at its maximum at birth, stays constant until the earliest age of reproduction, and then decreases.
2. The strength of mortality selection declines until the last age of reproduction, after which it ceases altogether.
3. Selection for age-specific fertility is maximized at birth (regardless of whether reproduction is biologically possible) and relaxes with increased age.

There are two ways to interpret these findings. The most general takes Hamilton's model to be a logically incontrovertible description of phenotypic selection acting on vital rates. Consequentially, all valid evolutionary models of aging must incorporate Hamilton's sensitivities as a definition of phenotypic selection for vital rates. Of course, phenotypic selection is not evolution; a heritable basis for traits is required for selection to have any evolutionary change of trait value (Fisher, 1930). Consequently, this interpretation views Hamilton's model as a necessary, but incomplete, component of an evolutionary theory of aging. A full theory requires a

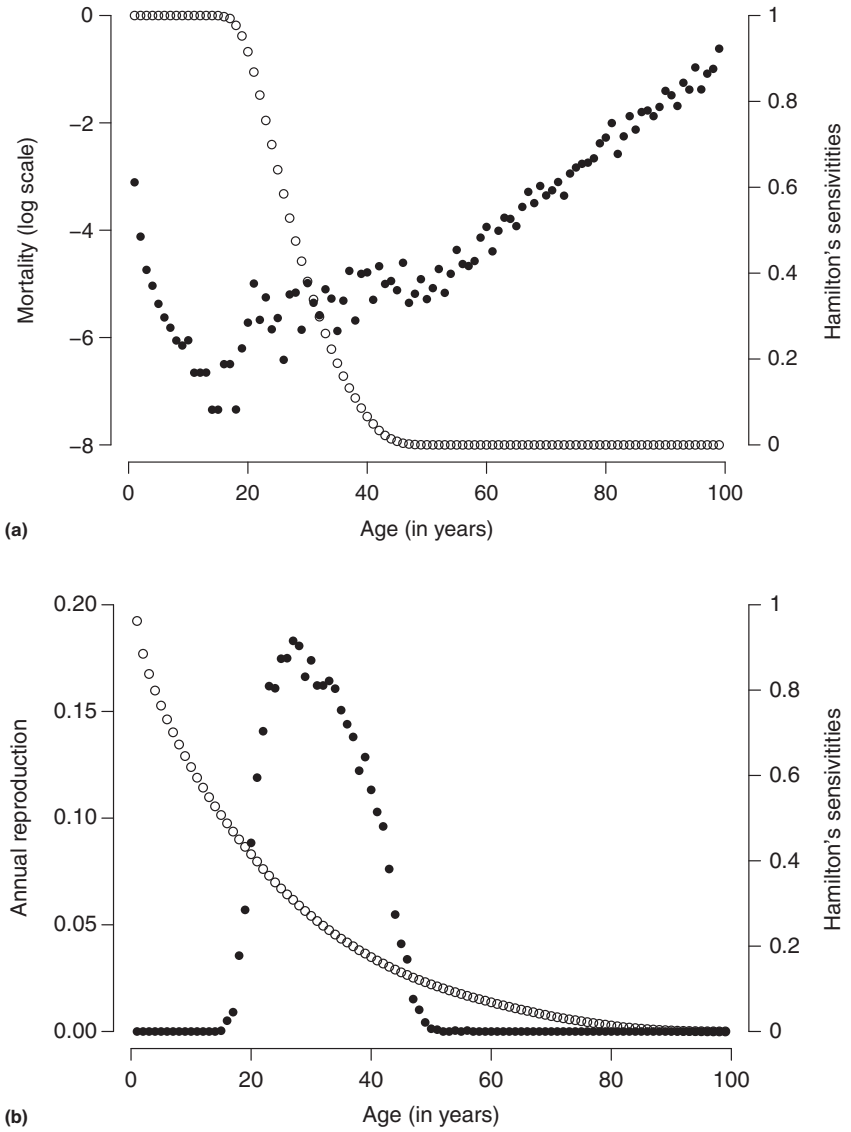

Figure 1 Selection for vital rates in a human population. (a) Age-specific mortality (solid circles) and selection against this mortality (open circles) is illustrated using data from approximately 1600 females born in Utah in 1860. Mortality is log-transformed, and selection against mortality is given by Hamilton's sensitivities calibrated to the generational scale. Note two qualitative departures from the predicted evolutionary endpoints. The first is the elevated juvenile mortality, and the second is continued survival of many females beyond the age of complete cessation of selection. (b) Age-specific fertility (closed circles) and selection for fertility (open circles), also following Hamilton's sensitivities calibrated to the generational scale. Results for both graphs are taken from Moorad, J.A., 2013. A demographic transition altered the strength of selection for fitness and age-specific survival and fertility in a 19th century American population. Evolution 67, 1622–1634.

model of how genes affect vital rates, such as illustrated by MA or AP models.

The second way to interpret Hamilton's sensitivities is to take them to imply directly patterns of aging using a MA model, as Hamilton (1966) himself did. For reasons of simplicity, Hamilton assumed that new mutations had strictly age-specific and additive effects upon mortality (Hamilton, 1966) (i.e., he assumed a linear relationship between mortality and the number of mutations). This simple model permitted him to project his conclusions relating the strength of selection directly to evolutionary endpoints. Hamilton predicted that mortality should be lowest in pre-reproductive years and increase from the age of reproductive onset to the age of reproduction cessation. Beyond this age, the model predicts that nothing can prevent the build-up of deleterious late-age mutation, and mortality will evolve to infinity (a 'wall-of-death'). These qualitative predictions persist over a broad range of more sophisticated population genetic models of vital rates that assume realistic genetic parameters, such as dominance relationships, but still retain Hamilton's assumption of how mutations determine mortality. Significantly, these models can predict the frequently observed log-linear increase in mortality rates in real populations (Charlesworth, 2001). Note that these and many other predictive quantitative evolutionary models of aging often assume many genes of small effect. This practice is adopted in most genetic models of

evolutionary processes to help make them tractable. Applied to aging, this assumption is occasionally confused with a prediction, and the recovery of some longevity genes of large effect has been heralded by some as a challenge to evolutionary theory. However, the fundamental theory is capable of including genes of large effect.

Modifying Hamilton's Model

Clearly, Hamilton's predictions regarding evolutionary endpoints are not always met in real populations. Given the rudimentary nature of his genetic model, which is justified by simplicity rather than any empirical or theoretical insight, this is not too surprising. As a result, his basic model has been refined in various ways to account for departures from theoretical predictions. Observed departures from basic predictions can be grouped into at least three categories. For each, a few examples of appropriate model modifications have been included. By no means is this to be considered an exhaustive catalog of aging models.

Post-Reproductive Survival

Female humans are known to menopause at around 50 years of age. Because reproductive potential ceases at this point, Hamilton's sensitivities indicate that there should be no selection for survival beyond this age. Obviously many women live long beyond this point in clear violation of Hamilton's prediction of a post-reproductive 'wall-of-death.' At least three different evolutionary mechanisms have been proposed to account for post-reproductive survival in human females. First, it has been suggested that late-life survival benefits the females' children (Rogers, 1993) and grandchildren (Lee, 2003). Given that these younger relatives share genes for late-life survival with their older female relatives (one-half of the genes are shared between mothers and offspring and one-quarter between grandmothers and grand-offspring), positive associations are generated between fitness and genes for long-life in younger individuals. These associations are expected to increase the frequency of post-reproductive survival genes over time. A second model recognizes that human males do not menopause, meaning that there is, at least in principle, no age at which selection against male mortality ever disappears completely (Tuljapurkar *et al.*, 2007). If genes that favor late-life male survival also happen to favor female survival, then selection for late-life survival will act indirectly to promote post-reproductive survival in females. However, it is worth noting that this model may be applicable only to humans as men may get more reproduction at later age due to accumulated wealth or social status that may be attractive to some women. Lastly, correlated selection could also encourage the spread of post-reproductive survival genes if positive associations exist between early- and late-age mortality genes (Charlesworth, 2001).

Mortality Deceleration and Plateaus

Hamilton's simple model predicts that mortality should increase ever higher with increased age, but we occasionally observe that mortality rates decelerate late in life; these may even decline sufficiently to create mortality 'plateaus' where mortality risk may actually decline in the very old. Several models attempt to explain these phenomena. The first imagines that all individuals have some amount of 'frailty' which exposes them to some degree of age-independent mortality risk. Cohorts of similarly aged individuals can exhibit 'heterogeneity,' or among-individual variation in frailty. Importantly, correlations between frailty and mortality will cause the cohort's composition to change as it ages, favoring a decrease in mean frailty over time. Even if all individuals' risk of mortality increases linearly (or log-linearly) with age, the biased extinction of high-frailty individuals will cause the population's mean mortality risk to be deflected downwards (Vaupel *et al.*, 1998; Vaupel and Yashin, 1985). Frailty need not imply a genetic basis, but if it does, then variation among individuals for genes with deleterious survival effects at many ages can cause mortality deceleration to evolve (Charlesworth, 2001). Other genetic models can predict mortality decelerations by relaxing Hamilton's assumption that genetic factors act additively on the mortality scale (Baudisch, 2005; Moorad and Promislow, 2011). Still other models point out that if fewer mutations increase mortality late in life compared to early in life, or if late acting mutations have a smaller effect upon mortality, then mortality decelerations can evolve (Moorad and Promislow, 2008). There is some evidence to suggest that these sorts of mutational biases may exist in fruit flies (Pletcher *et al.*, 1998).

Elevated Pre-Reproductive Mortality

Mortality at birth and in early development is often quite high before dropping to a minimum in early adulthood. Hamilton's model, on the other hand, predicts low and constant mortality until the first age of reproduction. As this discrepancy between theory and observations is strikingly general, several explanations have been proposed (Levitis, 2011). Hamilton himself recognized this disagreement between his model and reality and suggested that family-level or kin selection for early or accelerated death in frail individuals could benefit the immediate relatives of the individual (Hamilton, 1966). In this way, he argued, early-death genes would have a fitness advantage over genes that would allow the doomed individual to linger and consume resources that would be put to better use (from the perspective of the genes) if they were consumed by more capable relatives. This idea has been modeled more formally and under a wider range of relationships by Lee (2003), who predicted the widespread evolution of elevated juvenile mortality. Heterogeneity, the demographic phenomenon that might contribute to mortality deceleration, may also cause this pattern if very early mortality removes the most frail individuals from the population before they can be exposed to mortality hazards later in childhood (Vaupel and Yashin, 1985). Another model points out that because the very young are often dependent upon care provided by parents, there are more genetic targets relating to early-age mortality than to mortality later in life to be disrupted by mutation (Moorad and Promislow, 2008). Finally, if the ecological context of the population places the very youngest at a relatively greater risk of mortality, such as by

disease, competition for space or resources, or predation, then high-juvenile mortality may reflect the high frailty of the youngest members of the population, and the phenomenon may simply have no evolutionary explanation.

Tests of the Theory

Extrinsic Mortality

The evolutionary theory of aging couples an age-related decline in selection to various genetic models to predict decreases in mean vital rates with age, and both aspects of the theory are subjected to experimental and descriptive testing. Some researchers have investigated how different degrees and types of environmental risks affect the selection pressures that shape aging rates. However, much of this work is undermined by a misunderstanding in the evolutionary theory that emerged in the early years of its development. Williams (1957) suggested that the strength of selection for survival at some age is proportional to the probability of survival to that age, leading him to predict that selection for late-life survival should be reduced in situations where age-independent (or 'extrinsic') mortality was highest. This prediction is often characterized as a powerful predictive tool for understanding the diversity of aging patterns that have evolved in nature. Williams's intuitive logic proved false, however, as Hamilton's results point out a more complex relationship between cumulative survival and survival selection; we now know that the sort of mortality risk envisioned by Williams should have no effect on selection (Caswell, 2007). In fact, Hamilton's sensitivities show age-related declines in the strength of selection even in immortal populations. Interestingly, some comparative studies appear to support Williams's prediction; these are typically presented as support for the evolutionary theory of aging (e.g., Austad and Fischer, 1991; Shattuck and Williams, 2010). However, predictions made using Hamilton's formal theory recognizes that there are situations where decreased extrinsic mortality can increase or decrease selection for long-life. Indeed, results from natural and laboratory populations can also be interpreted to refute Williams's prediction (e.g., Chen and Maklakov, 2012; Reznick *et al.*, 2004). Neither the theory nor the empirical evidence supports the use of Williams' extrinsic mortality hypothesis for a general test of an evolutionary basis for aging, but there is value in understanding how extrinsic mortality risk affects age classes differently because this kind of mortality has the potential to shape evolution.

Segregating Genetic Variation

Evolution by natural selection requires and consumes additive genetic (heritable) variation. As selection is strongest for traits that determine early survival and reproduction, a straightforward prediction from simple evolutionary models of aging is that there should be less additive genetic variation for early-effect traits compared to traits with late effects (Charlesworth and Hughes, 1996). This prediction is occasionally, but not universally, supported in actual populations. Indeed, even for the same trait within the same species, age-specific mortality in *Drosophila melanogaster*, additive genetic variation has been observed both to increase (Hughes and Charlesworth, 1994) and decrease (Promislow *et al.*, 1996) with age. However, because aging models that include a genetic contribution to heterogeneity can predict nonincreasing additive genetic variance over some ages (Charlesworth, 2001), decreasing additive genetic variance with age does not necessarily provide evidence against the evolution of aging.

Because some MA and AP models make somewhat different assumptions about how genes involved with aging work, age-related changes in nonadditive sources of genetic variation and its derivatives, such as dominance variance, variation among inbred-lines, inbreeding depression, and heterosis, have been suggested as diagnostic of the preeminence of one evolutionary mechanism or the other (Charlesworth and Hughes, 1996; Hughes and Charlesworth, 1994). This suggestion has generated much interest in measuring genetic variation of aging-related traits in laboratory (e.g., Snoke and Promislow, 2003; Tatar *et al.*, 1996) and natural populations (e.g., Charmantier *et al.*, 2006; Nussey *et al.*, 2008). However, it has since been argued that because other AP models can generate the same nonadditive genetic variance predictions as MA models, the diagnostic value of the tests are suspected (Moorad and Promislow, 2009).

Segregating Genetic Covariances

As the AP model is predicated upon genes with early beneficial and late deleterious effects, it is expected that genetic variation at these loci will contribute to negative additive genetic correlations across age-specific traits (such as early reproduction and late survival). These negative correlations can be resolved by artificial selection experiments that find antagonistic responses to selection (e.g., Zwaan *et al.*, 1995) or by direct, quantitative genetic estimates of negative genetic correlations (e.g., Rose and Charlesworth, 1981). These results do seem to offer strong evidence for AP genes, with the caveat that they do not argue against a role for MA (recall that a gene can contribute to aging by being AP and MA). Also, tests for negative genetic correlations might be fairly viewed as conservative, because genes with nonnegative correlated effects (such as some MA and frailty genes) can overwhelm the AP signal by contributing positively to the across age genetic correlations.

De novo Mutational Correlations

Because new mutations are the ultimate source of genetic variation, and genetic variation permits and shapes evolutionary endpoints, it has been suggested that investigating the genetic variation and covariation caused by *de novo* mutation upon vital rates can help us better understand the constraints placed upon the evolution of aging. These studies, so far performed exclusively on *Drosophila* and *Caenorhabditis elegans*, attempt to reduce or remove the editing effects of natural selection through careful breeding designs or the use of balancer chromosomes (e.g., Estes *et al.*, 2005; Gong *et al.*, 2006; Pletcher *et al.*, 1998), with the goal of allowing new mutations to accumulate. These mutation accumulation studies (not to be confused with the MA model of aging) interpret the difference in genetic variances and covariances between

mutation accumulation and control lines as reflecting the contributions of new mutations. These studies show no support for AP-type aging genes and ample support for MA genes. Some aging theory does predict that new AP-type mutations should emerge extremely rarely, perhaps too infrequently to be detected experimentally, but they may still influence the evolution of aging when they do arise (Moorad and Hall, 2009).

Conclusions

The evolutionary theory of aging integrates models of selection and inheritance to explain the diversity of life span and aging patterns across metazoan life. Early aging models identified the age-related attenuation of selection as the evolutionary impetus behind aging, and they provided feasible genetic models for how these age-related differences in selection generate within individual phenotype change. Hamilton's sensitivities formally define the strength of selection acting to increase survival and fertility at different ages, and these provide readily testable first predictions of how aging should evolve. Ecological, social, and genetic additions to the theory provide finer-scaled predictions and a more detailed understanding of how species differ in how they age.

See also: Age-Specific Survivorship and Fertility, Estimating. Life History Trade-offs. Life History, What is?. Life History Patterns

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Amniotes, Diversification of

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Glossary

Amniota A major clade of tetrapods, comprising reptiles, birds, mammals, and related forms. All amniotes have eggs that are characterized by the presence of an amnion, a membrane that encloses the embryo in a fluid-filled chamber.

Clade A group of organisms that comprises the last common ancestor of these organisms and all extant and extinct descendants of that ancestor is called clade.

Cranium The part of the skull that houses the brain and the organs of sense is called cranium.

Diapsida A clade of amniotes characterized by the presence of two openings behind the eye socket on either side of the cranium. Some diapsids no longer retain one or even both of these openings. Present-day diapsids comprise turtles (which have secondarily lost the openings), lizards, snakes, the tuatara, crocodylians, and birds.

Synapsida A clade of amniotes comprising mammals and their extinct close relatives (often misleadingly referred to as 'mammal-like reptiles'). Synapsids are characterized by the presence of a single opening behind the eye socket on either side of the cranium.

Taxon A group of one or more organisms deemed to be a unit within a hierarchical biological classification is called taxon.

Introduction

Reptiles, birds, and mammals and their closest relatives are grouped together as Amniota based on the shared possession of the amniotic (cleidoic) egg (Figure 1). This type of egg is laid on land and differs from that of amphibians in the presence of an outer shell and a set of extraembryonic membranes surrounding the embryo (Packard and Seymour, 1997; Stewart, 1997). The shell is either parchment-like or calcified and protects the egg's contents against desiccation and damage. (With the exception of the platypus and the echidnas, present-day mammals do not lay eggs.) The eggshell is semipermeable, permitting intake of oxygen for respiration and disposal of carbon dioxide. Four membranes surround the embryo. The first is the allantois, which forms a sac attached to the embryo and serves for storage of waste and for respiration. The second is the amnion, which encloses a fluid-filled chamber surrounding and protecting the embryo. The third is the chorion, which envelops the embryo and attached structures and is in close contact with the allantois and with the inside of the shell, facilitating respiration. The fourth is the membrane forming the yolk sac, which provides the developing embryo with nourishment. By contrast, present-day amphibians (and presumably their extinct precursors) typically deposit their eggs in water (Packard and Seymour, 1997). The egg is coated in a jelly-like substance, and the developing embryo receives oxygen and much of its nourishment from the surrounding water. A gilled larva hatches from the egg and continues its development in water. Finally, it undergoes a process of metamorphosis before emerging as an adult capable of life on land. The evolution of the cleidoic egg allowed amniotes to move into drier environments because they were no longer tied to permanent bodies of water for reproduction. No undisputed fossil eggs of amniotes are known from the Paleozoic to date but the fact that present-day reptiles, birds, and mammals all share the amniotic egg suggests that it had already evolved before the reptile-bird and mammal lineages diverged in the Carboniferous. In marsupials and placentals, the eggs develop in the uterus and thus lack a shell; however, the embryo is still surrounded by an amnion (Stewart, 1997).

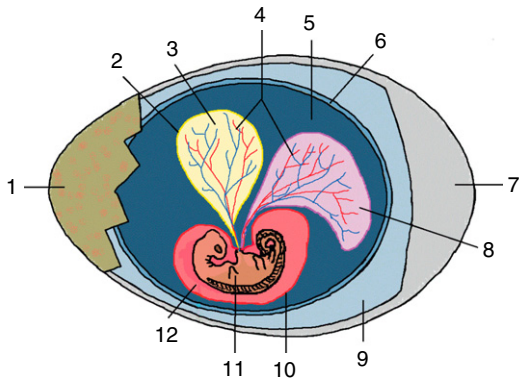

Figure 1 Diagram of the egg of a crocodile to show the basic structure of the amniotic egg. Numbers: (1) eggshell, (2) yolk sac, (3) yolk, (4) blood vessels, (5) coelomic cavity, (6) chorion, (7) air space, (8) allantois, (9) egg white, (10) amniotic sac, (11) embryo, and (12) amniotic cavity. Reproduced from Catsloveme207/Wikipedia.

Amniotic eggs acquire their shell in the oviduct of the female and thus must be fertilized before their encasement. Thus most male amniotes have special organs to deposit sperm inside the female reproductive tract.

Another major difference between amniotes and amphibians is the structure of the skin (Frolich, 1997). The skin of present-day amphibians is relatively thin, weakly keratinized, and rich in glands. By contrast, the skin of reptiles and birds is much less glandular and protected by keratin in the form of scales (Figure 2) or feathers. These features help to reduce water loss through the skin. Although richer in glands than that of reptiles, mammalian skin also contains keratin as well as lipids and is usually covered by hair.

In addition to filling and emptying their relatively small lungs through raising and lowering the floor of their mouth (buccal pumping), present-day amphibians rely extensively on

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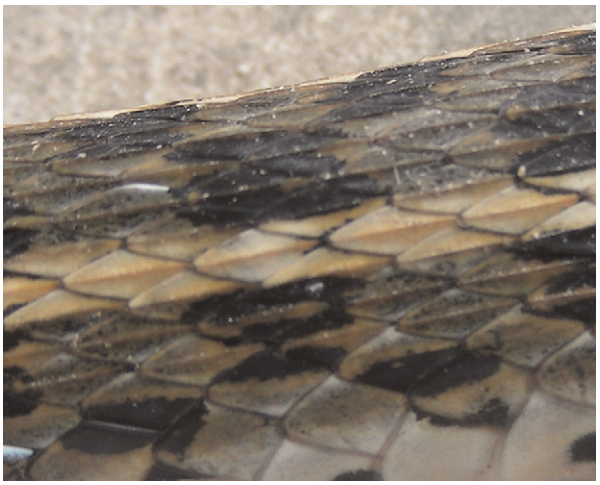

Figure 2 Keeled scales of the buff-striped keelback snake (*Amphiesma stolatum*). The scales protect the skin of reptiles against mechanical damage and help reduce water loss through the skin. Reproduced from AshLin/Wikipedia.

their skin for the intake of oxygen and excretion of carbon dioxide (Frolich, 1997). By contrast, amniotes largely abandoned cutaneous respiration. They fill and empty their larger, more complex lungs primarily by moving their ribs to alter the pressure within the thoracic cavity (Brainerd and Owerkowicz, 2006).

Present-day amniotes comprise six monophyletic groups or clades: mammals (Mammalia), turtles (Testudines), the tuatara (Rhynchocephalia), lizards and snakes (Squamata), crocodylians (Crocodylia), and birds (Aves) (Gauthier *et al.*, 1988). Mammals are characterized by the presence of hair, mammary glands, and many skeletal features. Turtles are distinguished by the possession of a bony shell, which is usually covered by scutes of keratin. Squamates are characterized by the presence of a mobile quadrate bone (along with loss of the lower temporal bar), paired hemipenes, and skin that is composed of superimposed layers of two types of keratin and is shed completely at regular intervals (Vitt and Caldwell, 2013). The tuatara is closely related to squamates but lacks the mobile quadrate bone and elaborate hemipenes. Crocodylians have air spaces in the skull bones surrounding the middle ear, elongate wrist bones, a modified pelvic girdle, and distinctive ankle structure (Nesbitt, 2011). Birds are especially characterized by the presence of wings and other features associated with powered flight, and a greatly reduced tail (Chiappe, 2007). The tuatara and squamates are classified together as Lepidosauria and crocodylians, and birds as Archosauria.

Traditional scenarios of vertebrate evolution held that reptiles separately gave rise to birds and to mammals (Romer, 1956). In recent decades, however, it has been established that the lineage leading to reptiles and their descendants, birds, and the lineage leading to mammals were already distinct by the time both first appear together in the fossil record some 310 million years ago (Gauthier *et al.*, 1988; Clack, 2012).

Amniotes have a vast fossil record worldwide, dating back more than 300 million years. There are over 20 000 known species of present-day reptiles and birds and some 5500 species of extant mammals. Although the Cenozoic is commonly known as the 'Age of Mammals' the combined number of species of reptiles and birds is now almost four times higher than that of mammals.

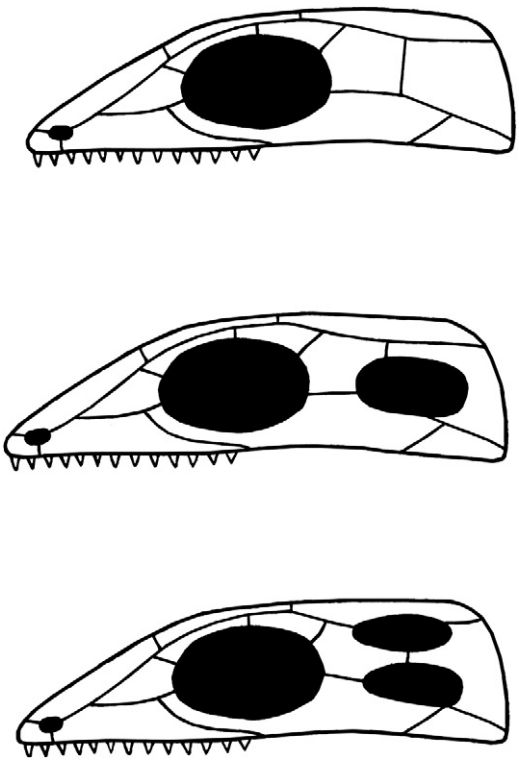

Figure 3 Diagrams of crania of amniote vertebrates. Top: anapsid condition (without temporal openings); center: synapsid condition (with one temporal opening); bottom: diapsid condition (with two temporal openings). Reproduced from H.-D. Sues/Public Domain.

Synapsida

The lineage leading to mammals is characterized by the presence of a single large opening behind the eye socket (orbit) on either side of the cranium (Kemp, 2005). This is known as the synapsid condition (**Figure 3, center**). The opening, called the temporal opening, lightens the skull, and its margins serve for attachment of jaw-closing muscles. Based primarily on this feature, mammals and their precursors are grouped together as Synapsida. Non-mammalian synapsids have traditionally been referred to as 'mammal-like reptiles' but this term has become scientifically meaningless. The earliest synapsids, often referred to as 'pelycosaurs,' are known from the Late Carboniferous and Permian (Kemp, 2005). They included forms with a range of shapes and sizes but superficially lizard-like in appearance. Some were among the first large herbivores whereas others, such as the 'fin-backed' *Dimetrodon* (**Figure 4**), were the first apex predators on land. They retained a sprawling gait, as in other early tetrapods. More derived synapsids, known as Therapsida, comprise a range of increasingly more mammal-like forms that came to dominate global land ecosystems during the latter half of the Permian and much of the Triassic

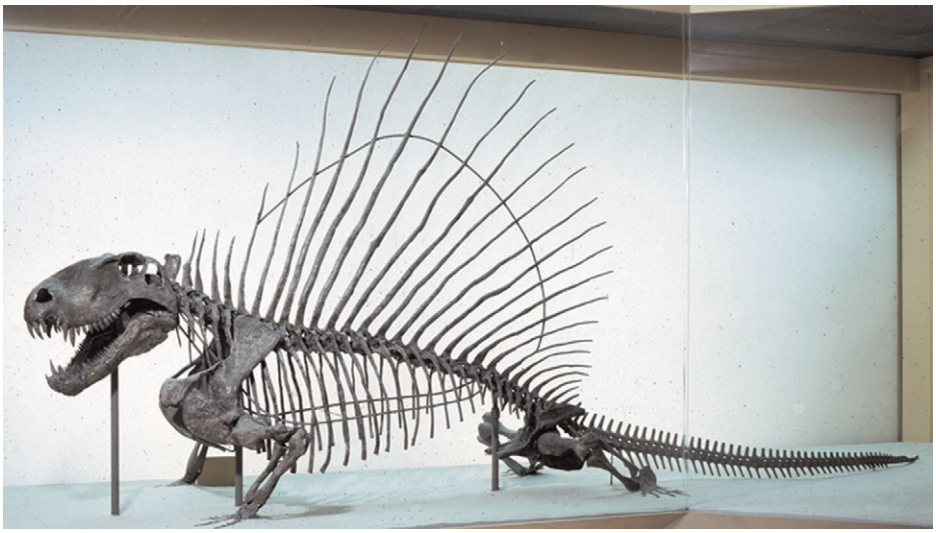

Figure 4 Skeleton of *Dimetrodon grandis*, an Early Permian synapsid. Superficially resembling certain reptiles, *Dimetrodon* is actually closely related to mammals. Reproduced from National Museum of Natural History/Public Domain.

(Kemp, 2005). Therapsids have a much larger temporal opening than 'pelycosaurs.' They also have a relatively larger upper canine, a groove for the ear canal on the squamosal bone, and the angular bone bears a distinct process, which supported the precursor of the eardrum in mammals. The girdle bones of therapsids are less massive and their limb bones are more slender than those of early synapsids, suggesting greater freedom of limb movement and greater ability to lift the body further off the ground. One therapsid group, Cynodontia, includes the immediate antecedents of mammals (Kemp, 2005). Cynodonts have dentitions differentiated into incisors, canines, and postcanine (cheek) teeth, a typically closed secondary bony palate, and a lower jaw in which the tooth-bearing dentary is the principal element whereas the bones behind the dentary became much smaller. The bones behind the dentary subsequently became detached from the lower jaw, attached to the cranium, and formed a chain of three small bones (incus, malleus, and stapes) in the middle ear of mammals. Based on the structure of the dentary bone, the jaw-closing muscles in early cynodonts had already attained a mammal-like differentiation with a superficial masseter and deeper masseter and temporalis muscles. The crowns of cynodont cheek teeth have large cusps and shelves composed of small cusps (cingula), indicating various degrees of oral food processing. The trunk region comprises a thorax with long, mobile ribs at the front and a lumbar region with short ribs further back. The femur (thigh bone) was held closer to the body unlike in 'pelycosaurs,' in which it extended nearly horizontally from the body. Presumably maintenance of a constant high body temperature and higher basic metabolic rates evolved among cynodonts, if not earlier. Development of these physiological attributes was likely accompanied by the development of hair for insulation.

Mammaliaformes, defined as the group comprising Mammalia and their closest extinct relatives, dates back to the Late Triassic (Kemp, 2005). Mammalia, defined as the most recent common ancestor of monotremes, metatherians (marsupials and their closest relatives), and eutherians (placentals and their closest relatives) and all descendants of that ancestor, first appears in the Middle Jurassic. Mammaliaformes is characterized by the possession of a well-developed joint formed by the dentary bone of the lower jaw and the squamosal (temporal) bone of the cranium. (By contrast, the articular bone of the lower jaw and the quadrate bone of the cranium form the jaw joint in other amniotes.) The roots of the cheek teeth are divided, and, in most forms, the upper and lower teeth met in a precise manner (occlusion) to facilitate the chewing of food. Additional characteristic features are present in the structure of the ear region and the braincase, the latter related to a major increase in brain size. Exquisitely preserved fossils from the Early Cretaceous of northeastern China have established that metatherian and eutherian mammals were already distinct groups more than 120 million years ago (Luo *et al.*, 2011). However, the diversification of evolutionary lineages represented by present-day mammals largely postdated the extinction of dinosaurs other than birds some 66 million years ago (Rose, 2006). Today, placentals are the most widely distributed and diverse group of mammals worldwide whereas marsupials are restricted to the Americas and to Australia.

Reptilia

Among reptiles, there are two principal types of skull structure (Rieppel, 1993). In the first type, there are no temporal openings behind the orbit on either side of the cranium (**Figure 3**, top). This is the anapsid condition, which is found only in turtles among present-day amniotes. In the second type, two large openings perforate the side of the cranium behind the orbit (**Figure 3**, bottom). This is the diapsid condition, which is present (often in highly modified form) in most extant and extinct reptiles as well as in birds.

The oldest known reptiles, of Late Carboniferous age, were small and lightly built (Clack, 2012). One group lacks temporal openings but has other skeletal features, such as long

Figure 5 Florida gopher tortoise (*Gopherus polyphemus*). Reproduced from Tomfriedel/Wikipedia.

and slender limbs with slender hands and feet that ally it to diapsid reptiles.

Parareptilia represents an entirely extinct group of early reptiles that were once considered closely related to turtles (Tsuji and Müller, 2009). They ranged in time from the end of the Carboniferous to the end of the Triassic. Some parareptiles have openings behind the eye socket in the cranium but others lack such features. Most have an embayment formed by the squamosal and quadratojugal at the back of the cranium, which probably supported an eardrum. Together with the slender stapes (which conducts sound vibrations from the eardrum to the inner ear), the eardrum formed an impedance-matching ear. This hearing mechanism evolved quite independently from those in other reptiles and in mammals.

All other reptilian groups are united as Eureptilia. Turtles are easily distinguished from other reptiles by their bony shell (**Figure 5**). The shell comprises a dorsal carapace and a ventral plastron, which are linked by a bony 'bridge' on either side (Romer, 1956). Parts of the shoulder girdle and the ribs are integrated into the bony shell. The head, neck, and limbs can be withdrawn into the shell in most present-day turtles. Turtles lack true temporal openings although the back of the cranium is distinctly emarginated in many species (Rieppel, 1993). Thus, most paleontologists assumed that turtles evolved from the earliest known reptiles (Romer, 1956). However, recent discoveries have established that turtles evolved from early diapsid reptiles and some stem-turtles retained two temporal openings on either side of the cranium (Schoch and Sues, 2015).

Aside from turtles, diapsid reptiles comprise archosaurs, lepidosaurs, and their closest extinct relatives. In addition to the two temporal openings, diapsids also share the presence of a large opening in the bony palate, the suborbital fenestra (Rieppel, 1993). Archosaurs and lepidosaurs (which are united as Sauria; Gauthier *et al.*, 1988) share a number of features related to the development of an impedance-matching hearing system with an eardrum (tympanic membrane): the quadrate bone is bowed, the retroarticular process of the lower jaw is long, and the stapes is slender (Rieppel, 1993). These features are still lacking in the oldest known diapsid reptiles. Diapsids also have a more or less L-shaped ('hooked') fifth metatarsal bone, which supports the fifth toe and is thought to assist in extending the foot. The early evolutionary history of diapsid reptiles is still poorly known due to the scarcity of well-preserved fossils from the late Paleozoic. However, from the early Mesozoic onward, diapsids have a rich and varied fossil record (Benton, 2014).

Present-day lepidosaurs include lizards, snakes, and the tuatara (Vitt and Caldwell, 2013). The known fossil record of the tuatara and its relatives (Rhynchocephalia) dates back to the Triassic whereas the oldest fossil lizards are known from the Jurassic and the oldest fossil snakes are known from the Cretaceous. The skulls of lizards have additional joints that allow them to increase the gape of their mouths and manipulate their food more effectively. Lizards generally have slender bodies with long limbs. The two principal anklebones, the astragalus and calcaneum, are fused. Lizards do not grow indefinitely, unlike many other reptiles, and their limb bones have separate ossifications (epiphyses) at their joint ends. Many species reduce or even lose their limbs. Snakes evolved from limbed lizards although their precise relationships remain contentious (Hsiang *et al.*, 2015). They completely lost their forelimbs and shoulder girdle, and the much elongated vertebral column sometimes comprises over 400 vertebrae. The bones of the upper jaws and palate in snakes are loosely joined to each other and to the braincase, and, along with a ligamentous connection between the lower jaws, facilitate the capture and manipulation of prey (Rieppel, 1993). Snakes use constriction or venoms to subdue and kill their prey (**Figures 6–8**).

During the Mesozoic, a number of groups of diapsid reptiles independently adapted to life in the sea (Motani, 2009). They included ichthyosaurs, which are often remarkably fish-like in their body plan, and plesiosaurs, which used four flipper-like limbs for swimming. These groups may be more closely related to lepidosaurs than to archosaurs.

Archosaurs include dinosaurs (including birds), pterosaurs (flying reptiles), crocodylians, and the extinct close relatives of these groups (Nesbitt, 2011). They date back to the Early Triassic. Only birds and crocodylians survived to the present

Figure 6 Tuatara (*Sphenodon punctatus*). The tuatara is the lone present-day survivor of a once diverse group of reptiles closely related to lizards and snakes. Reproduced from Samsara/Wikipedia.

Figure 7 Desert iguana (*Dipsosaurus dorsalis*). Reproduced from Wilson44691/Wikipedia.

day. Birds descended from small predatory dinosaurs and evolved active, flapping flight (Chiappe, 2007). Most researchers now agree that birds should be classified as a subgroup of dinosaurs. Many features commonly associated with birds, such as feathers, the furcula (wishbone), and a specialized wrist joint, were already present in small predatory dinosaurs. A wide range of often exquisitely preserved fossils, especially from the Jurassic and Cretaceous of China, documents a series of intermediate stages in the evolution of the bird skeleton and flight adaptations in great detail (Chiappe, 2007). The major diversification of birds, during which most major modern groups evolved, took place only during the Cenozoic (Mayr, 2014). Starting at the end of the Triassic, dinosaurs became the dominant carnivores and herbivores in continental ecosystems for much of the Mesozoic (Fastovsky and Weishampel, 2012). They included the largest animals that ever lived on land. Pterosaurs are closely related to dinosaurs and evolved active flight long before and independently from birds (Witton, 2013). The wing membrane was attached to the enormously elongated fourth finger. Pterosaurs dominated the air for much of the Mesozoic and included the largest flying animals of all time. Present-day crocodylians are represented only by some 25 species, which are mostly amphibious ambush predators (Grigg and Kirshner, 2015; **Figure 9**). However, crocodylians and their closest relatives were far more diverse during earlier geological periods. Their earliest precursors were land-dwelling pursuit predators.

Figure 8 Western diamondback rattlesnake (*Crotalus atrox*). Reproduced from Gary Stolz, USFW/Public Domain.

Figure 9 American crocodile (*Crocodylus acutus*). Reproduced from Tomás Castelazo/Wikipedia.

Amniotes represent one of the great success stories in the evolution of vertebrates. They attained a global distribution and are found everywhere except in the greatest depths of the ocean.

See also: Amniotes, the Origin of. Birds, Diversification of. Mammalian Diversification

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Amniotes, the Origin of

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Glossary

Amniota Land-living vertebrates (i.e., tetrapods) that have a complex set of embryonic membranes, including the amnion. Also defined as the monophyletic group containing living mammals, reptiles, and birds, their last common ancestor, and all the descendants of this ancestor.
Anapsida Amniotes with no temporal openings in the skull.
Anthracosauia A group of primitive reptiliomorph, albeit still amphibious, tetrapods.
Carboniferous The geologic period that started 359 Mya and ended 299 Mya. Named for the large amounts of coal (carbon) deposited during this period.
Diadectomorpha A group of robust tetrapods of the Carboniferous and Permian that is generally considered to be closely related to Amniota.
Diapsida Amniotes with two temporal openings (dorsal and ventral) in the skull.
Microsauria A group of tetrapods, a part of the Leposondyli, which had an amphibious biology. The microsaurs are usually relatively small and slender compared to other amphibians of the Carboniferous and Permian.
Monophyly The condition that a taxon contains the last common ancestor of all the species included, as well as all the descendants of that last common ancestor.
Reptiliomorpha A group of tetrapods that are more closely related to Amniota than to modern amphibians.
Seymouriamorpha A group of often highly terrestrial reptiliomorphs, with amphibious biology.
Synapsida Amniotes with one ventral temporal opening in the skull. This group is monophyletic, and contains modern mammals.

Introduction

One of the most significant events in the evolutionary history of vertebrates was the origin of Amniota, named for their amnion, which is the innermost of the embryonic membranes found in reptiles, birds, and mammals. This membrane was primitively associated with eggs laid on land (Figure 1), but is also found in all live-bearing amniotes. The amnion is one of the key features that enabled these animals to reproduce and mature in terrestrial habitats, the young generally hatching as miniature replicas of the adults. This is in strong contrast to basal tetrapods with an amphibious way of life, in which the larvae hatch from eggs that are deposited in the water, and only subsequently metamorphose into more-or-less terrestrial adults. The origin of the amniotic egg is linked to the formation of the outer embryonic membrane, the chorion, and an extraembryonic sac called the allantois that grew out of the embryonic hindgut and covered the inner surface of the chorion (Liem *et al.*, 2001). The allantochorion membrane is well supplied with blood vessels and acts as a respiratory organ that allows inward diffusion of oxygen through the permeable shell, as well as the outward passage of carbon dioxide (Strickberger, 1985). The complexity of the amnion and other extra-embryonic membranes which are present in all living squamates, archosaurs, testudines, and mammals, provides strong morphological evidence that amniotes had an ultimate common ancestry not shared by modern amphibians. Thus, both Amniota and Lissamphibia (modern amphibians) appear to be well-defined monophyletic taxa (e.g., Sigurdsen and Green, 2011). Molecular data also supports this basal split between Amniota and Lissamphibia (e.g., Hugall *et al.*, 2007). Sadly, embryonic membranes cannot be found in the fossil record, and fossilized eggs from the period in question are extremely rare (Sander, 2012).

Figure 1 A tortoise hatching from a typical amniote egg. Photo by Mayer Richard.

However, amniotes also generally share several osteological features, notably the transverse flange of the pterygoid in the palate (though this character is often far from clear), solidly ossified vertebrae with large pleurocentra, and a fusion of tarsals to form the astragalus in the ankle. Definitions of Amniota based on such morphological features are commonly used, but an alternative exists in the use of a crown-group

definition, such as that proposed by Gauthier *et al.* (1988). In this definition, Amniota consists of mammals, modern reptiles, and birds, their last common ancestor, and all descendants of this ancestor. Either way, the available evidence shows that the origin and early divergence of amniotes took place in the Upper Carboniferous (Carroll, 1964; Voigt and Ganzelewski, 2010; Clack, 2012). Here, we discuss the existing fossil evidence for the emergence of the amniotes, focusing on the known taxa from around the time of their origin. We also review the phylogenetic position of the pertinent taxa, and problems remaining in our understanding of the early evolution of this key group of tetrapods.

Fossil Evidence

The Earliest Known Amniotes

There is general agreement that true amniotes were present by the early stages of the Westphalian (315 Mya) in the Upper Carboniferous (Carroll, 1964; Voigt and Ganzelewski, 2010; Clack, 2012). The evidence of their existence by the Westphalian A (**Figure 2**) consists of both skeletal material (Carroll, 1964) and preserved trackways (Voigt and Ganzelewski, 2010). At present, the oldest known vertebrates whose skeletal anatomy can be closely compared to that of living

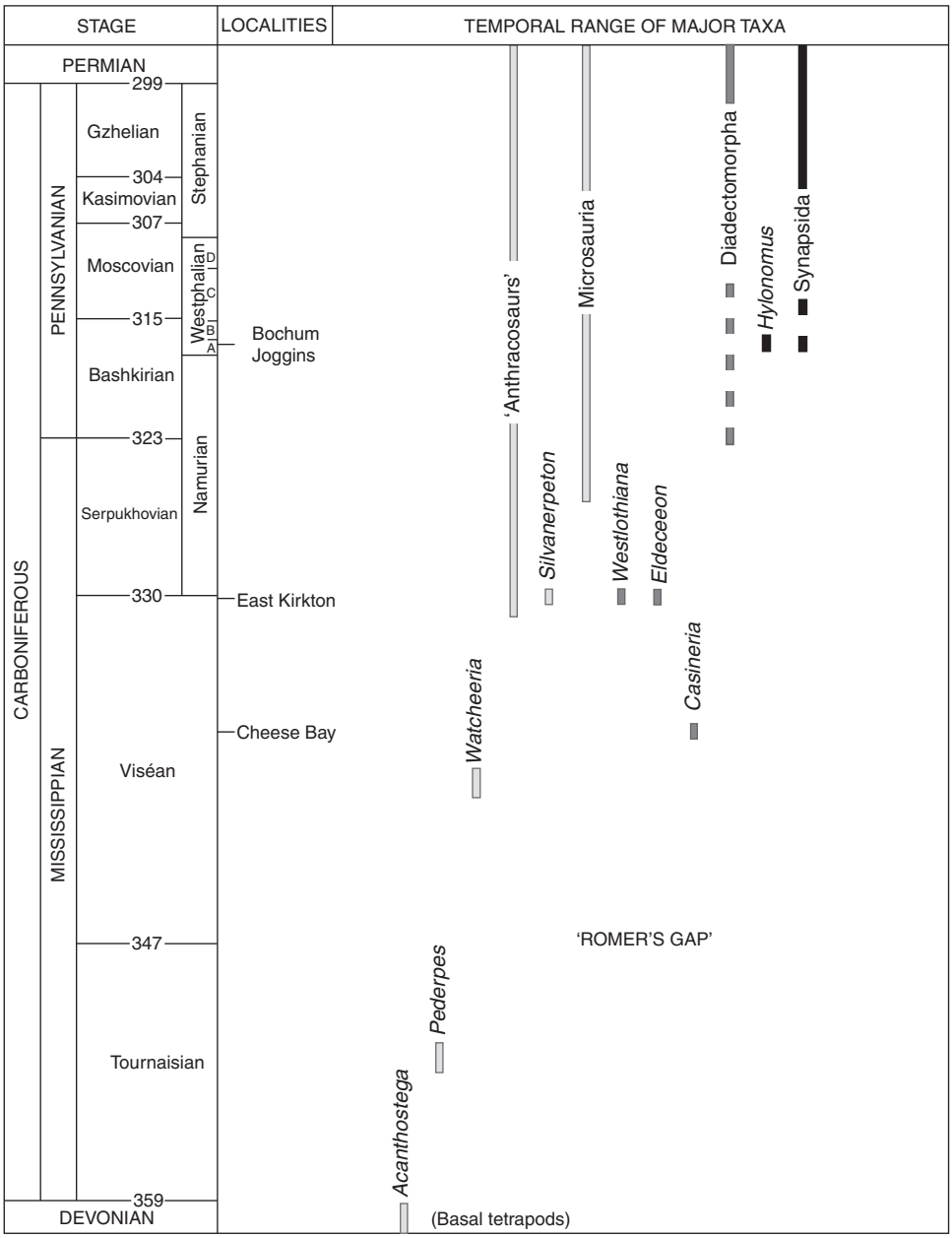

Figure 2 Geological timescale illustrating the time spans of the major groups of terrestrial vertebrates that are associated with the origin of amniotes. The taxa that are generally thought to be more closely related to Amniota are shown in darker shades of gray (taxa considered true amniotes in black). The ages given are in millions of years before present.

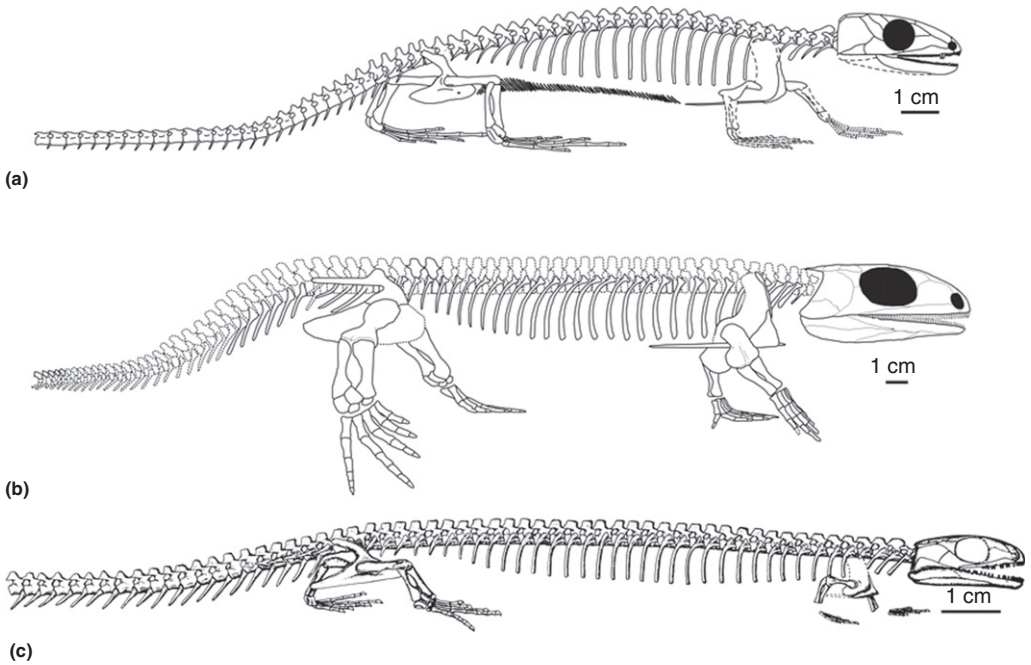

Figure 3 Skeletal reconstructions of three tetrapods from the Carboniferous: (a) The early amniote *Hylonomus lyelli* from Joggins, Nova Scotia. Modified from Carroll, R.L., 1964. The earliest reptiles. Zoological Journal of the Linnean Society 45, 61–83. (b) The basal anthracosaur reptiliomorph *Silvanerpeton miripedes*. Redrawn from Ruta, M., Clack, J.A., 2006. A review of *Silvanerpeton miripedes*, a stem amniote from the Lower Carboniferous of East Kirton, West Lothian, Scotland. Transactions of the Royal Society of Edinburgh: Earth Sciences 97, 31–63. (c) *Westlothiana lizzia*, a reptiliomorph of uncertain phylogenetic position. Modified from Smithson, T.R., Carroll, R.L., Panchen, A.L., Andrews, S.M., 1994. Westlothiana lizziae from the Viséan of East Kirkton, West Lothian, Scotland, and the amniote stem. Transactions of the Royal Society of Edinburgh: Earth Sciences 84, 377–382.

amniotes are specimens of *Hylonomus lyelli* (**Figure 3(a)**) from the Lower Westphalian stage of Joggins, Nova Scotia. This locality (now a United Nations World Heritage Site), is on the shore of the Bay of Fundy, an area that was close to the equator in the Carboniferous (**Figure 5**). The *Hylonomus* specimens, along with many other vertebrates found at Joggins, were preserved within the stumps of upright tree-like lycopodiophytes (plants related to modern clubmosses), of the genus *Sigillaria*. The hollow tree stumps evidently became traps for animals walking on the land surface (Dawson, 1882). The type locality of the Joggins Formation has been dated to the Langsettian (Falcon-Lang *et al.*, 2006) in the Bashkirian (**Figure 2**), more than 315 million years ago (Gradstein *et al.*, 2012). Carroll (1964) established *Hylonomus* as a basal amniote, and this was based on a number of 'reptilian' features of the skeleton (as opposed to stem tetrapod, or 'amphibian' features). These include a transverse flange on the pterygoid, ribs resembling those of modern reptiles, an ankle joint containing an astragalus, as well as phalangeal counts of the manus and pes of 2, 3, 4, 5, 3. Thus, the skeleton of *Hylonomus* had already achieved nearly all of the features associated with modern amniotes.

Apart from *Hylonomus*, Carroll (1964) also suggested that basal synapsids ('pelycosaurs'), the lineage eventually giving rise to mammals, may have been present at Joggins. Although the identification of *Protoclepsydrops* as a synapsid has remained doubtful due to the fragmentary nature of the fossils (see Reisz, 1972), newer studies of tetrapod trackways from the Westphalian A Bochum Formation in Germany support the view that synapsids were indeed present at such an early age (Voigt and Ganzelewski, 2010). The first diapsids, the group containing modern snakes, lizards, crocodiles, and birds, are known from the Upper Carboniferous (Reisz, 1977). Thus, although the evidence is scant, the existing finds suggest that amniotes were already diversifying in the Bashkirian Age (323–315 Mya) of the Carboniferous, perhaps indicating that much of the actual diversity of that period is still unknown to us.

Reptiliomorphs from the Lower Carboniferous

To which group of basal tetrapods are amniotes most closely related? Decades ago, the description of *Hylonomus* and other early amniotes (Carroll, 1964, 1970; Carroll and Baird, 1972; Reisz, 1972, 1977, 1981) significantly extended the known range of amniotes well back in the Upper Carboniferous, but the advanced nature of their skeletons made it difficult to relate them with any other tetrapods known from earlier deposits.

The sister taxon of Amniota within primitive tetrapods remains a problematic topic, as various groups show different amniote-like features to different degrees (for reviews of basal tetrapod groups see Carroll, 2009; Clack, 2012). In general, tetrapods that are more closely related to amniotes than to modern amphibians are known as reptiliomorphs (Clack, 2012). Our knowledge of Early Carboniferous reptiliomorphs is unfortunately limited by the presence of one of the most

notorious gaps in the fossil record; the so-called Romer's Gap (Figure 2). Even though we have recently seen significant finds from these strata (Smithson *et al.*, 2012), the paucity of fossils from this period is still a serious problem to our understanding of the early origin of amniotes. Numerous Carboniferous and Lower Permian tetrapods with amphibious biology have been suggested as possible sister-taxa of ancestral amniotes, and these notably include the anthracosaurs and seymouriamorphs. These problematic groups are significantly more primitive than basal amniotes. However, many of these are known only subsequent to the appearance of clearly recognizable amniotes, and are sometimes specialized in their anatomy and presumed way of life. Still, the possibly paraphyletic anthracosaurs in particular remain a key group in any discussion of the origin of amniotes.

The watcheeriids *Pederpes* and *Whatcheeria* constitute some of the most interesting finds from the early Carboniferous, and when these were first discovered they were tentatively associated with presumed stem amniotes such as anthracosaurs (Lombard and Bolt, 1995). However, recent analyses have left this relationship in serious doubt, and whatcheeriids are now usually regarded as relatively generalized basal tetrapods (Lombard and Bolt, 2006; Ruta and Coates, 2007).

Several sites in Scotland have yielded tetrapods of interest to the study of early amniotes. The Cheese Bay Shrimp Bed of the Gullane Formation near Edinburgh has produced the intriguing potential basal amniote *Casineria kiddi* (Paton *et al.*, 1999). Although fragmentary and lacking most of the skull, *Casineria* shows a very early occurrence of many typically amniote features. The vertebrae are highly ossified with large pleurocentra, and the ribs are long and curved. Not surprisingly, the phylogenetic position of this incompletely known form is uncertain, but it appears to be related to basal amniotes (Paton *et al.*, 1999).

In the mid-1980s, a new fossil-bearing horizon was described at East Kirkton, near Bathgate, Scotland (Wood *et al.*, 1985). The site correlates with the lower part of the Brigantian stage of the Viséan Series in the Lower Carboniferous, estimated at approximately 335 million years ago (Figure 2). Several species collected from this locality have been of special interest in establishing the early evolution of the distinctive characteristics of amniotes. *Silvanerpeton miripedes* (Clack, 1994; Ruta and Clack, 2006) from East Kirkton appears to be a basal representative of the anthracosaurs, and as such shows some features that can be regarded as intermediate between more primitive tetrapods and basal amniotes. It is a robust animal (Figure 3(a)), with well-ossified limb bones. It appears to be adapted to a terrestrial environment, although its phylogenetic position indicates that it might have had an aquatic larval stage (Ruta and Clack, 2006). The skull retains well-developed otic notches of the squamosal bones, and labyrinthine infoldings of the teeth, plesiomorphic features that are typical of basal tetrapods rather than amniotes, although similar otic notches can be found in certain stem amniote groups. *Eldeceeon* also appears to have anthracosaurian affinities (Smithson, 1994). The limbs, especially the rear, were stout and well ossified, indicative of a terrestrial animal with ossified tarsals and a phalangeal formula of 2, 3, 4, 5, 4 (although three terminal elements are missing), which is similar to many basal amniotes. Only the first 14–16 ribs are preserved in the two available specimens, but this is probably due to poor preservation.

Another interesting tetrapod from East Kirkton that may be linked to the ancestry of amniotes is *Westlothiana lizziae* (Smithson *et al.*, 1994). In strong contrast with *Eldeceeon rolfei*, it had a long trunk, with 36 presacral vertebrae, but very short and well-ossified girdles and limbs (Figure 3(c)). The humeri are much shorter than the femora. In addition, the skull shows a combination of traits resembling those of both more primitive tetrapods and amniotes. Most features of the vertebrae closely resemble those of Upper Carboniferous and Lower Permian amniotes, except for the presence of conspicuous ventral lamellae, and the intercentra are conspicuous throughout the trunk region. The greatly elongate trunk and relatively small size resemble many microsaurs, and may perhaps suggest adaptations to a burrowing way of life. *Westlothiana* shows a unique set of features, and its relationships are hard to establish, but it has been suggested to be either a plesiomorphic stem amniote (Paton *et al.*, 1999), or a basal lepospondyl (Ruta *et al.*, 2003).

A noteworthy feature that may link many of these forms to early amniotes is the presence of long, strongly curved, and ventrally pointed ribs. Sigurdsen and Green (2011) argue that such ribs are associated with a clade including reptiliomorphs (with lepospondyls) and amniotes, as opposed to the proposed clade consisting of temnospondyls and modern amphibians.

However, none of these early amniote-like reptiliomorph tetrapods has a definitive astragalus (amniote ankle bone), although some, such as *Eldeceeon*, *Westlothiana*, and *Casineria* appear to have phalangeal counts comparable to that of amniotes. One enigmatic exception is the somewhat later group Diadectomorpha, in which an astragalus sometimes is found (Berman and Henrici, 2003). The diadectomorphs are a group of large and robust reptiliomorphs from the Upper Carboniferous and Lower Permian (Figure 4).

Phylogenetic Studies of Reptiliomorphs and Basal Amniota

Phylogenetic analyses utilizing the theory of cladistics can provide valuable evidence as to potential sister taxa to Amniota within a larger framework of tetrapod evolution. Among the early cladistic studies, Heaton and Reisz (1986) proposed that protorothyrids (including *Hylonomus*, but data mostly based on *Paleothyris*), captorhinids, and Diapsida form a monophyletic clade within Amniota. *Hylonomus* is therefore regarded as the oldest true crown amniote, but hardly representative of the ancestral amniote morphology. It was concluded that the ancestral amniote morphology was not a gracile protorothryrid anatomy, but rather that of slower carnivores, perhaps similar to many synapsid 'pelycosaurs.'

Several authors have found a close relationship between amniotes and diadectomorphs (Gauthier *et al.*, 1988; Laurin and Reisz, 1999; Müller and Reisz, 2006; Ruta and Coates, 2007). Gauthier *et al.* (1988) proposed Diadectomorpha as the immediate sister taxon to Amniota, with *Solenodonsaurus*, Seymouriamorpha, and Anthracosauridae as subsequent sister taxa. Synapsida represents the first group to split off from the rest of the amniotes, and *Paleothyris* is regarded as a stem

Figure 4 Skeletal cast of Limnoscelis, a Permian Diadectomorph. Cast in the Redpath Museum, Montreal.

diapsid. Ruta and Coates (2007) found various diadectomorphs forming subsequently more basal sister taxa (*Diadectes* being closest to *Captorhinus* plus Amniota).

Apart from diadectomorphs, a relatively close relationship between amniotes, *Casineria*, *Westlothiania*, and microsaurs is also well established, albeit the details differ from study to study (Laurin and Reisz, 1999; Paton *et al.*, 1999; Ruta and Clack, 2006). Paton *et al.* (1999) gives an unresolved polytomy at the amniote node. This node includes *Casineria*, *Westlothiania*, and amniotes. The sister taxon to this grouping is microsaurs and, more basally, *Seymouria*. According to Laurin and Reisz (1999) there is no close relationship between seymouriamorphs, embolomeres (anthracosaurs), and Amniota (Lissamphibia being closer to amniotes than these taxa). Diadectomorpha, here regarded as a monophyletic group, is given as the closest sister taxon to Amniota.

Ruta and Clack (2006) focus on 'reptiliomorph' taxa in their analysis, but did not include Diadectomorpha. Here, the sister taxon of Amniota (represented by *Paleothyris*) is a monophyletic clade consisting of *Casineria*, *Westlothiania*, and microsaurs. Seymouriamorphs are a more basal stem amniote group, followed by *Bruktererpeton* and *Gephyrostegus*. Embolomeres and *Silvanerpeton* are the basal-most stem taxa in the clade that includes amniotes, as opposed to the branch leading to temnospondyls.

Focusing on true amniotes, Müller and Reisz (2006) united *Hylonomus*, *Paleothyris*, *Brouffia*, and diapsida in a monophyletic taxon, thus excluding *Hylonomus* from a stem amniote position. This phylogenetic analysis did not include enough non-amniote 'reptiliomorphs' to inform us on the sister-group of amniotes. Synapsida, represented by Caseidae, is given as the basal-most crown amniote clade.

Discussion

Despite more than 100 years of fossil collecting concentrated in North American and Europe, the known fossil record of the Lower Carboniferous remains far less well known than that of the Upper Carboniferous. Broadly speaking, one would expect the fossil record of a given geologic time period to diminish progressively over time as a result of erosion, tectonic processes, continental drift, all of which dissolve, disperse, or cover up carcasses nearly as fast as they accumulate, not to mention normal loss by carnivores and organic decay. As can be seen in the studies by Ruta and Clack (2006), and Carroll (2012), this is a particularly serious problem in tracing the early ancestry of amniotes and other terrestrial vertebrates for which there are extensive gaps within and between major taxa, making it more difficult to establish reliable relationships. It is also notable that the fossil localities that are reasonably well-known from the Carboniferous are from a limited area of the world, paleogeographically speaking. They were actually situated much closer to each other in the Carboniferous than they are today (**Figure 5**), having later been torn apart by continental drift, and it is possible that our relatively small fossil samples may show a skewed picture of the tetrapod faunas of the time. This may add to the great gap in our knowledge of the ancestry of amniotes, as key taxa may have been distributed elsewhere in the world. The fossil preservation seen at Joggins appears to be fairly unusual. Fossilized lycopod trees are known from other horizons subsequent to the Carboniferous, but only four localities, all in Nova Scotia, are known to contain the remains of extinct amphibians or reptiles, and only one other, Sydney, Nova Scotia, contains a diversity of well preserved skeletons.

During the last 50 years, the continued description of tetrapods from the Carboniferous has shed considerable light on the early occurrence of reptile-like tetrapods. However, many problems still remain in the study of the origin of early amniotes. Romer's Gap has been partially filled (**Figure 2**) but the primitive 'reptiliomorph' taxa remain extremely hard to place in relation to the more advanced reptile-like specimens. The difficulties in our studies of early amniote evolution are not unique, and such issues are common to our study of nearly all groups of early terrestrial vertebrates. However, in the case of the origin of amniotes we have the additional obstacle of coping with the paucity of fossils in Romer's Gap, which is still a big part of the problem.

Most authors agree that the sister taxon to crown Amniota is Diadectomorpha (see review by Reisz, 2007), which may or may not be a monophyletic taxon. Diadectomorphs show all the amniote-like characters found in more basal reptiliomorphs, plus some advanced features that parallel true amniotes, such as some taxa within diadectomorpha evolving an astragalus, although the latter is almost certainly

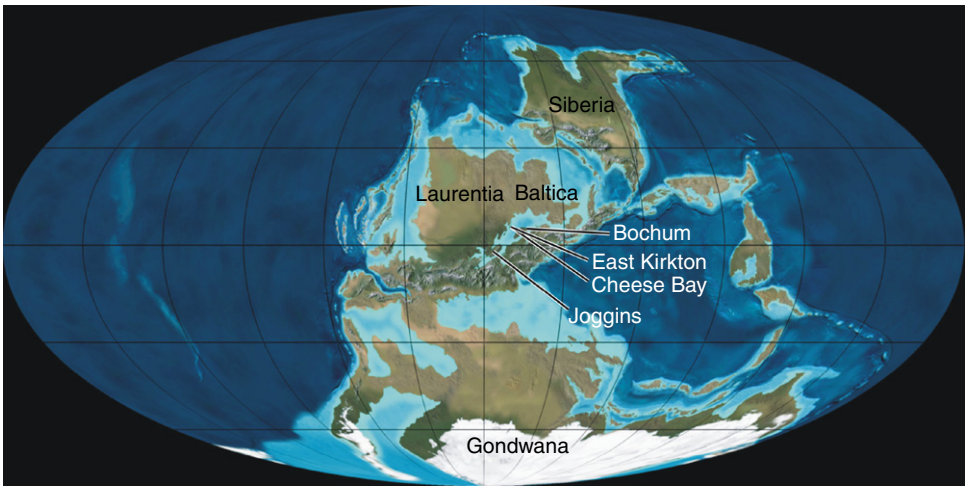

Figure 5 Map of the world in the Upper Carboniferous. Approximate locations of some of the most important reptiliomorph and early amniote localities have been marked. Courtesy Ron Blakey, slightly modified by the authors.

independently derived (see Berman and Henrici, 2003). Microsaurs, seymouriamorphs, and anthracosaurs (monophyletic or not) appear to form subsequent outgroups.

Within Amniota, the traditional classifications divided the major lineages based on the number of temporal openings; the major groups being Anapsida, Synapsida, and Diapsida, with zero, one, and two temporal openings, respectively. This classification has turned out to be problematic, and of these three only Synapsida, containing modern mammals, appears to be unequivocally monophyletic. There is general agreement that the first split within Amniota is likely to have been between Synapsida and the rest of the known groups of amniotes (Gauthier *et al.*, 1988; Müller and Reisz, 2006).

As mentioned above, the earliest known amniotes are represented by *Hylonomus* and some basal synapsid remains (e.g., Carroll, 1964; Voigt and Ganzelewski, 2010). Whereas the position of the synapsids relative to modern animals is clear, the relationships of 'protorothyrids' such as *Hylonomus* have been more contentious. A phylogenetic link between *Hylonomus* and/or *Paleothyris* and Diapsida has been proposed by several authors (Gauthier *et al.*, 1988; Müller and Reisz, 2006). This has been based on the shared presence of slender limbs (represented by two characters in Müller and Reisz, 2006), ventrally keeled vertebral centra, and short postorbital skull length. However, several of these characters are problematic as stem diapsid characteristics, particularly because they also occur in small, gracile synapsids (Romer and Price, 1940). There is a general tendency for smaller animals to have slender long bones. Given its small size, and compared to the basal diapsid *Petrolacosaurus*, the humeri of *Hylonomus* are in fact relatively short and robust (compare Reisz, 1981, Figure 19, and Carroll, 1964, Figure 7). The hind limb proportions are also noticeably more robust than those of *Petrolacosaurus*. Keeled vertebrae and short postorbital skull lengths are common among the early synapsids, and not specific to Diapsida (Romer and Price, 1940). In a preliminary phylogenetic analysis by the present authors, *Hylonomus* belonged to the base of the captorhinid clade (in preparation). Even so, the position of *Hylonomus* is perhaps best regarded as somewhat uncertain, as it retains many basal amniote features, and given the above observations it seems probable that it is more closely linked to both captorhinids and diapsids than to Synapsida. Its anatomy has progressed relatively little beyond that of the most basal amniotes, and a comparison of *Hylonomus* to basal synapsids such a *Varanosaurus* provide a good picture of the anatomy of the last common ancestor of modern amniotes.

Thus, crown amniotes are somewhat better understood than the stem groups. They show several signs of early diversification by the Westphalian A, and at least two main lineages (Synapsida and the species *Hylonomus lyelli*) are present. While *Hylonomus* is possibly linked to the clade containing both captorhinids and diapsids it still shows a morphology that is closely comparable to the expected amniote ancestral state. Interestingly, there is a relatively wide distribution of 'protorothyrid' taxa (including *Hylonomus*) within some phylogenies (e.g., Müller and Reisz, 2006; current authors in preparation). This paraphyletic assemblage share general morphological similarities (albeit symplesiomorphies in some cases), and the fact that such a morphologically homogenous group (reflected in their initial classification as a taxon) might be widely scattered among the earliest amniote lineages could give us an important hint to the basal amniote condition. The ancestor was likely superficially similar to a modern lizard, but with somewhat more stout limb bones and a robust anapsid-type skull, similar to the situation seen in *Hylonomus*.

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See also: Amniotes, Diversification of. Land Vertebrates, the Origin and Evolution of

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Ancestral Reconstruction: Theory and Practice

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Glossary

Dynamic programming It is a computer science method for solving a complex problem by breaking it down into a collection of simpler subproblems. Here, it is used to calculate a result for a whole tree by considering progressively larger parts of this tree, starting from the tree leaves for which the desired result can be easily computed.

Markov process Here, this term indicates a probabilistic process in which there exists a finite set of possible states (e.g., the 4 nucleotides, or the 20 amino acids) and a matrix whose terms are the rates at which a state can change per unit time. This conveniently represents the evolutionary process at the molecular level. Such a process is said to be homogeneous when the matrix is constant through time and through evolutionary lineages.

Phylogenetic tree A tree is an object made of nodes connected by edges such that no closed circuit of edges exists, and there exists a path between any pair of nodes. Tree leaves are those nodes connected to a single other node. In a rooted tree, one node has a special status by being the tree root. A phylogenetic tree represents the evolutionary divergence of biological entities, usually species or genes, the leaves, from their last common ancestor, the tree root. Its nodes figure ancestral entities. Its edges figure evolutionary lineages. In a species tree, biological entities are species. In a gene tree, they are homologous genes, that is, genes sharing a common ancestor.

Introduction

The theoretical possibility of reconstructing ancestral molecules from the sequence of these molecules in extant organisms has been formalized by Pauling and Zuckerkandl (1963), and has become practical and widely applied in the recent years. Various types of traits have been considered for ancestral reconstruction: sequence data, either of the nucleotide or protein types, have been extensively studied. Methods for processing sequence data can often be seen in a more general fashion as methods handling characters with a discrete set of values. Therefore, these methods also allow to consider non-sequence data such as binary characters, e.g., presence/absence. Other sets of methods consider continuous characters (e.g., body mass) and allow the reconstruction of their ancestral values.

Ancestral reconstruction of gene content has also been studied with the objective of understanding the evolutionary history of gene repertoires over time. Methods for discrete characters can be applied in this case, but a model incorporating the processes of gene evolution is more accurate. Indeed, the processes of gene evolution in genomes include duplications and horizontal gene transfer, which are improperly rendered when genes are coded as discrete character data. It has triggered the development of new methods specific for the reconstruction of gene history and hence ancestral genic repertoires. Similarly, the order of genes along chromosomes has been considered as a target for ancestral reconstruction. Because this problem becomes very rapidly computationally intractable, the less ambitious target of reconstructing ancestral adjacencies between genes along chromosomes has been studied.

For very shallow divergences, recent attempts have also been performed to reconstruct full ancestral chromosomal sequences, with both genic and noncoding nucleotides.

This article describes major methods for all these problems, which concern the reconstruction of ancestral traits or sequences along a preestablished phylogenetic tree, and starting from a set of observed trait values (e.g., multiple alignment of extant homologous sequences) (see Figure 1). Notably, the procedures described here are those that have been widely used recently to reconstruct ancestral enzymes in order to study the path followed by a protein along evolutionary time.

Ancestral Character Reconstruction

Inferring the ancestral states of a given trait requires a set of values measured in extant organisms, a binary rooted or unrooted phylogeny relating these organisms and a model assuming a particular process of trait evolution. This model may contain parameters, such as rates of change between states or

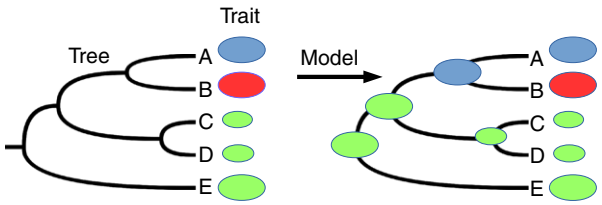

Figure 1 Reconstruction of ancestral characters along a phylogenetic tree.

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the branch lengths of the phylogeny, which are usually estimated from the data in the case of probabilistic reconstructions. Three methodological frameworks are commonly considered to infer ancestral characters: Parsimony (Sankoff, 1975), Maximum Likelihood (ML) (Schluter *et al.*, 1997), and the Bayesian framework (Pagel *et al.*, 2004).

Discrete Characters

Markov models for a finite state space Ω may contain several parameters, depending on the a priori assumptions that are made about transition rates between states (Pagel, 1994; Yang, 2006). When applied on binary or unordered (excepted nucleotides or amino acids, see Section Ancestral Sequence Reconstruction) sets of characters of size k, the models are called M_k. Extensions of M_k are, for example, the Binary and Multi-State Speciation and Extinction models (BiSSE and MuSSE, respectively) developed to simultaneously infer the process of state change with the estimation of speciation and extinction rates along the phylogeny (Maddison *et al.*, 2007; FitzJohn, 2012). M_k model makes the assumption that transition rates are constant across lineages. The Hidden Rates Model (HRM) relaxes this homogeneity hypothesis and intends to capture the variation of transition rates between states across the phylogeny, yielding more accurate estimations of ancestral states (Beaulieu *et al.*, 2013).

Maximum parsimony reconstructions are approximations of solutions when the rates of events are supposed to be low, so that the probability of multiple events on one branch can be considered negligible. In this case, consider a cost d_{ij} of transition between states on any branch, with $i,j \in \Omega$, and ancestral states are chosen in order to minimize the sum of the costs of transitions on all branches. Calculations typically follow a dynamic programming procedure along the phylogeny (Sankoff, 1975). If the state space is small, a cost $C_u(i)$ is computed for every node u of the phylogeny, and every possible state i, starting from the leaves and following a postorder traversal of the tree. For a leaf l, $C_l(i)=0$ if the state i is present at leaf l, and $C_l(i)=\infty$ otherwise. Then, assuming that u_1 and u_2 are the children of u, apply

$$C_u(i) = \min_{j\in\Omega}(C_{u_1}(j) + d_{ij}) + \min_{j\in\Omega}(C_{u_2}(j) + d_{ij})$$

(For u_1, the cost $C_{u_1}(j) + d_{ij}$ is computed for all possible values $j \in \Omega$, and the minimum is retained. Similarly for u_2.)

One can thus obtain the set F_{root} of minimal cost states at the tree root, those for which $C_{\text{root}}(i)$ is minimal. The set F_p of minimal cost states at internal node p, child of node a, is recursively computed, from root to leaves, with:

```
F_p ← ∅ #Initially, F_p is the empty set
#for each minimal cost state assigned to parent node a
for each i∈F_a do
   m ← min_j(d_ij + C_p(j)) #The minimum cost m is computed
#All states j associated with a cost equal to m are added to
the set of possible values
   for each j do
      if d_ij + C_p(j) = m
         F_p ← F_p ∪ {j}
```

The set F_p gives all most parsimonious ancestral states at node p, and the result does not depend on the position of the root. There are two primary issues with the use of parsimony to reconstruct ancestral states. First, parsimony ignores branch lengths, which are indicative of variation of evolutionary rates between lineages. Second, parsimony does not account for variation of rates between the different states of the character, excepted with weighted parsimony, which assign weights to state changes. But, in this case it is always difficult to attribute appropriate weights a priori.

Likelihood calculations follow the same computational framework, with the advantage of probabilistically accounting for the concerns raised above about parsimony. Let $L_u(i)$ be the probability of observing the state i at node u, p_{ij}^l being the probability for state i to turn into j along a branch of length l, computed according to the evolutionary model. Conditional probabilities are computed recursively along the tree from the leaves to the root. For a leaf l, $L_l(i)=1$ if i is the observed value at leaf l, and $L_l(i)=0$ otherwise. For other nodes,

$$L_u(i) = \Big(\sum_{j_1\in\Omega} p_{ij_1}^{l_1} \times L_{u_1}(j_1)\Big)\Big(\sum_{j_2\in\Omega} p_{ij_2}^{l_2} \times L_{u_2}(j_2)\Big)$$

where u_1 and u_2 are the children of node u, and l_k is the length of the branch between u and u_k, which has to be a parameter of the model.

The probabilistic approach considers two types of ancestral character reconstruction, the marginal and the joint reconstructions. The marginal reconstruction gives the probabilities $P(u=i|S)$ of each state i of a given ancestral node u, where S represents the observed values at all leaves of the tree. Using Bayes formula, we obtain:

$$P(u=i|S) = \frac{P(u=i)P(S|u=i)}{P(S)} = \frac{\pi_i P(S|u=i)}{P(S)}$$

where π_i is the equilibrium frequency of character i. Provided it is computed after rooting the tree at its node r, the vector of conditional probabilities at the tree root defined above corresponds to the term $P(S|r=i)$. This procedure is called 'empirical Bayes' by Yang (2006).

The joint reconstruction gives the most probable set of residues across all ancestral nodes, and necessitates a backward traversal of the tree like in the parsimony case (Pupko *et al.*, 2002).

The computations have a quadratic complexity with respect to the state space size k, which makes dynamic programming a weak tool for large or infinite state spaces. If the state space is the integer set, it is possible to see the cost function $C_u(i)$ as an affine function of i and to propagate only the coefficients of the function along the nodes, instead of every value, to reach a complexity that does not depend on the size of the state space (Csuros, 2014), making parsimony calculations feasible. When the state space is large but without a good structure to order it, as with gene orders (Section Gene Order, there can be $n!$ possible gene orders), then the ancestral reconstruction becomes intractable.

Continuous Characters

When the state space Ω is a continuous set of numbers, the parsimony cost function d_{ij} is usually defined as the absolute

value or the square of the difference between i and j. It is not possible to compute the cost $C(i)$ for all values $i \in \Omega$. Instead, the coefficients of a linear or quadratic function of i is computed at each node, following the same dynamic programming principle (for an exhaustive review on parsimony methods see Csuros (2014)). If $d_{ij}=(i-j)^2$, then the parsimony solution is also the ML one under a Brownian motion (BM) with a constant rate, which is the most commonly used Markovian process under continuous characters. BM assumes that the trait value changes as a random walk, with multiple and independent steps drawn from a normal distribution of mean 0 and variance σ^2. The consequence of the independence between steps is that the net change of the trait value along a branch of length t is drawn from a normal distribution of mean 0 and variance $t \times \sigma^2$. With a BM process, the only parameter is the variance σ^2, the trait changes with a constant rate along the phylogeny and drifts neutrally without directionality or evolves toward an optimum that drifts neutrally (Felsenstein, 2004). BM is a special case of the Ornstein–Uhlenbeck (OU) process, which contains an additional attraction parameter toward a central value, increased with the distance from this central value. OU might be of interest when stabilizing selection acts on the trait under consideration (Martins, 1994). Extensions of these models were proposed to account for the heterogeneity in rate of change over time (Blomberg *et al.*, 2003; Harmon *et al.*, 2010; Eastman *et al.*, 2011). For instance, the Early Burst Model (Harmon *et al.*, 2010) allows the rate of the BM process to exponentially change in time.

The estimation of ancestral continuous trait values can be performed in ML (Schluter *et al.*, 1997). Likelihoods of transitions in state between adjacent nodes are easily derived from the BM process formulation, and the joint likelihood of the Brownian rate (σ^2) and all internal states is maximized over the entire tree. Restricted ML (REML) is also often used (Felsenstein, 1985; Paradis *et al.*, 2004). REML does not analyze the raw data directly, but instead realises the ML estimations using the contrasts among the observations. REML has been shown to produce unbiased estimates of variance and covariance parameters. Ancestral values may be estimated in the Bayesian framework (Huelsenbeck *et al.*, 2002; Pagel *et al.*, 2004), allowing to integrate over uncertainty in the tree topology, branch lengths, and rate parameters.

Two alternative methods are commonly considered: the Phylogenetic Independent Contrast (PIC) (Felsenstein, 1985) and the Generalized Least Squares (GLS) methods (Martins and Hansen, 1997). However, they are not usually employed to infer ancestral character values and were rather designed to control the influence of tree topology on the estimation of correlations between evolving traits. PIC assumes a BM-like model to recursively transform the tip values into statistically independent and identically distributed values, called contrasts, over the internal nodes up to the root. Thus, the ancestral values can be estimated with the data of descendant nodes only, contrary to ML estimations. GLS estimates the unknown parameters in a linear regression model. It assumes the model $Y=DX$, where D is a vector of observed values at the tips, X is a matrix describing both the phylogeny unifying the tips along which the trait evolves and the process of trait evolution (usually a BM process). Y is the vector of ancestral trait estimates. In many situations, ML, PIC and GLS produce similar values, especially when a simple BM process is assumed to model the evolution of traits (Martins, 1999).

Ancestral Sequence Reconstruction

Ancestral sequence reconstruction (ASR) from extant molecular sequences (DNA or proteins) consists in computing ancestral residues, given extant residues at the leaves of a phylogenetic tree. As such it requires a multiple alignment of sequences, a phylogenetic tree, and a substitution model. Computations are usually done independently at each site of the aligned sequences.

ASR inherits from all models and methods for ancestral character reconstruction for small state spaces (DNA or amino acids). But models of sequence evolution have their specificities. First, depending on the number of parameters defining the transition rates between states, the M_k model takes different names (e.g., Jukes and Cantor (JC) when all rates are equal or General Time Reversible (GTR) when all rates are different (Yang, 2006)). Second, the variation of evolutionary rates across sequence sites is usually accounted for, modelled by a gamma distribution discretized in K classes of $\frac{1}{K}$ weight. Then, the likelihood equation of Section Ancestral Character Reconstruction becomes:

$$L_u^s(i) = \sum_{c=1}^{K} \frac{1}{K} \left[\left(\sum_{j_1} p_{c,ij_1}^{l_1} \right) L_{u_1}^s(j_1) \left(\sum_{j_2} p_{c,ij_2}^{l_2} \right) L_{u_2}^s(j_2) \right]$$

With sequences, the marginal reconstruction is the most frequently used algorithm to compute ancestral characters. However, an efficient algorithm developed by Pupko *et al.* (2002) can be employed to perform joint reconstruction. It deals with all ancestral nodes together, and the set of sequence residues at all nodes of largest probability is considered to be the best ancestral reconstruction at a given site. While the marginal reconstruction requires a computation time proportional to the number of sequences, the joint reconstruction is exponential in the sequence number when across-site rate variation is modeled with a discretized gamma distribution (Liberles, 2007). Joint reconstruction is therefore difficult for more than a few tens of sequences.

Yang (2006) indicates that joint and marginal reconstructions usually produce consistent results where the most probable joint reconstruction for a site consists of the best marginal reconstruction at each node. Furthermore, when conflicting results arise, neither reconstruction is very reliable.

In practice, the marginal approach of ancestral sequence reconstruction in ML, retaining the most probable residue for each node at each site is most often employed in ancestral protein resurrection experiments. Several software packages implement this procedure: PAML (Yang, 2007), MEGA (Tamura, 2011), DAMBE (Xia, 2013), and bppAncestor, a part of the Bio++ library (Gueguen *et al.*, 2013).

When the goal is the resurrection of ancestral proteins, the residue with largest probability at each node is often retained. In this case, it is interesting to consider the magnitude of this largest probability: values above .9 indicate high confidence in the reconstructed ancestral residue, whereas residues with

moderate probabilities are those where reconstruction is uncertain. This strategy, though, is biased toward the most frequent amino acid at each protein site (Yang, 2006). An alternative strategy that avoids this bias is to generate an ancestral sequence by randomly drawing at each site one residue according to the probability vector computed for this site. The second strategy also allows to generate a small number of putative ancestral sequences in order to measure the sensitivity of inferences to the uncertainty about ancestral sequences.

Several studies have compared ASRs by the parsimony and probabilistic approaches (Yang *et al.*, 1995; Zhang and Nei, 1997). The general outcome is that probabilistic methods are more accurate than the parsimony approach, except when sequence divergences are weak where the two approaches perform similarly. The probabilistic approach has also the advantage of estimating the uncertainty of the reconstruction at each ancestral site. It was also shown that complex probabilistic models which aim at capturing the compositional heterogeneity of the substitution process provide more accurate estimates of ancestral sequences (Groussin *et al.*, 2013). However, the downside of parameter-rich models is that they may require large datasets to accurately estimate all parameters, and may also increase the time and memory requirements of the algorithm.

Models of insertions and deletions on a multiple alignment are necessary to correctly infer the presence or absence of a residue at some site in an ancestral sequence. For this, alignments have to be computed together with phylogenies, so that indels are scored according to an evolutionary model. It is done in parsimony by Loytynoja and Goldman (2008), and statistical models of insertion/deletion are included in alignment algorithms of Diallo *et al.* (2007). In that case the alignment, phylogeny, and presence or absence of ancestral residues are simultaneously given as an input to ASR methods. Diallo *et al.* (2010) present a software for computing indels (and thus, an alignment) given a phylogeny, while Suchard and Redelings (2006) and Lunter *et al.* (2005) propose a Bayesian co-estimation of alignment and phylogeny.

Finally, Groussin *et al.* (2015) highlighted that an erroneous tree negatively impacts ASR accuracy and that the use of species tree-aware gene trees reconstructed with models of duplication, transfer and loss events (see Section Gene Content) strongly increase ASR accuracy. This calls for an additional integration of duplication, transfer and loss in alignment, and phylogeny algorithms.

Gene Content

Several approaches exist for the reconstruction of ancestral gene content onto a species tree. The first one is the analysis of phylogenetic profiles, i.e., a matrix of binary characters coded as presence/absence of genes at the leaves of a given phylogeny of species (see **Figure 2**). A slightly more sophisticated version of phylogenetic profiles considers counts of homologous genes as discrete characters to allow the reconstruction of ancestral copy numbers for the genes under study. But before reconstructing ancestral gene content, it is important to first consider the evolutionary events that may affect the history of genes. For instance, in all organisms, genes can be duplicated or lost. In addition, many organisms have the ability to integrate genes from distantly related donors via lateral gene transfer (LGT). LGT is believed to be frequent in Bacteria and Archaea, and is beginning to be recognized as important in unicellular eukaryotes. It is still considered to be much rarer in multicellular eukaryotes, although several cases have been described. Hence, the application of methods that ignore LGT as a process for the evolution of gene repertoires should be restricted to very particular cases. Dollo parsimony (Farris, 1977), which allows loss of characters, but forbids gains after an initial origination of the gene, has been very popular for genome reconstruction in the absence of LGT. But more realistic approaches attribute asymmetrical costs for gain and loss to account for the possibility of gene transfer with a relatively high cost. The equivalent likelihood methods use a birth-and-death model in which probabilities of gene loss and gain are different, and can be estimated from the dataset (see e.g., Szollosi and Daubin (2012) for review). When the phylogenetic profile represents the number of copies for each gene, and not only their presence/absence, it is possible to estimate the branch-wise rates of duplication, transfer and loss along a phylogeny of species (Csuros, 2010). There is considerable flexibility in the definition of the birth-and-death model for gene evolution, the most simple models being linear, i.e, with rates of gain and loss that are independent of gene family size.

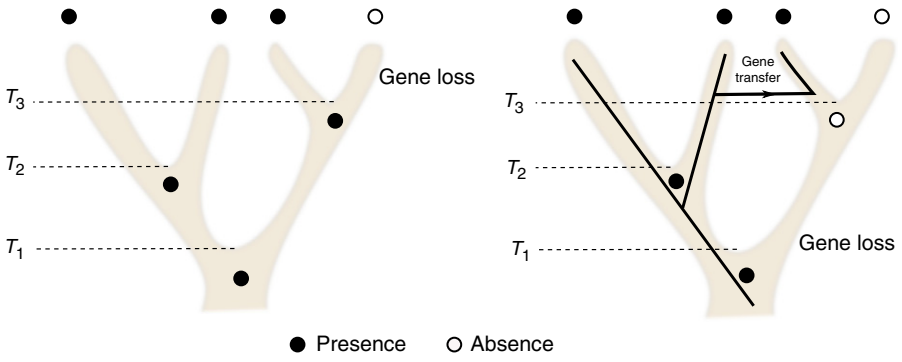

Figure 2 Using phylogenetic profiles or gene phylogenies may reconstruct different ancestral gene contents. The solid lines represent the evolution of the gene within the species tree. Horizontal dotted lines represent the dates of species divergence, restricting lateral gene transfers (LGTs) between species that co-exist in time.

However, the analysis of phylogenetic profiles imperfectly renders the evolution of gene families along the phylogeny of species (see **Figure 2**). Numerous events of duplication and transfer and loss remain invisible to the examination of gene presence/absence or counts. This is the case in particular for gene replacements by LGT. The possibility of reconstructing gene phylogenies based on sequence alignments reveal such hidden evolutionary events. The mapping of a gene family tree onto a species tree, invoking events of duplication, transfer and loss is called reconciliation (Maddison, 1997). A number of algorithms have been proposed, which are more or less complete in the set of events that are modeled (Doyon *et al.*, 2011). An efficient solution to the problem of reconciliation considering duplication and loss is the LCA (for Last Common Ancestor) algorithm (Gorecki and Tiuryn, 2006), which can run in linear time with the number of nodes in the gene tree. The modeling of LGT is more complex. LGT reconciliation has been proposed for the problem of gene replacement specifically. The problem here is to define a minimal number of editing operations to transform the gene tree in the species tree. The Subtree Pruning and Regrafting (SPR) approach reproduces the effect of transfers on phylogeny and has hence been the subject of active research (Nakhleh *et al.*, 2005; Beiko and Hamilton, 2006; Than and Nakhleh, 2008). However, the problem is computationally difficult and only applies to gene families without multicopies. More complete models, that account for duplication, transfer and loss (DTL) and hence apply to the modeling of the evolution of multicopy gene families have been developed, both in a parsimony and probabilistic framework (Doyon *et al.*, 2011; Tofigh, 2009; David and Alm, 2011; Szollosi *et al.*, 2012, 2013; Sjostrand *et al.*, 2014). The problem is still hard, but can become tractable through the addition of biologically relevant restrictions. For instance, an important constraint to include into DTL reconciliation algorithms is the time consistency of gene transfers: LGT can only occur among lineages that are contemporary in the history of species (see **Figure 2**). A promising approach for handling such time constraints is to fully specify the sequence of speciation events in the species tree in which the gene tree is reconciled (Tofigh, 2009), and to allow genes to evolve in lineages that are not explicitly represented in the tree representing the relationships among extant species (Szollosi *et al.*, 2013). A full reconciliation between a species tree and all gene trees yields the best possible reconstruction for the gene repertoire at nodes of the species tree.

Gene Order

Molecular ancestral character reconstruction methods actually began with the organization of genes along chromosomes. Dobzhansky and Sturtevant (1938) constructed the phylogeny and ancestral chromosome conformations of 17 *Drosophila* species, based on the observation of inversions. Already at that time the computational complexity arising from a model of evolution of gene arrangements subjected to inversions was visible (Sturtevant and Tan, 1937): after only a few events, scenarios are difficult to reconstruct even under a parsimony principle. This partly explains that ancestral gene order reconstruction methods seem underage: models are simple but computationally costly, hard to apply to a complex reality and to integrate with other kind of evolutionary signal.

Nevertheless the organization of genes along chromosomes contains valuable information on adaptation and modes of evolution of organisms, which has often been overlooked. And the methodology has slowly come of age.

Dobzhansky–Sturtevant-like methods, modeling chromosomes as permutations subject to inversions, often generalized into double cuts-and-joins to capture more possible events (Yancopoulos *et al.*, 2005), have been used to reconstruct gene orders of some mammalian (Alekseyev and Pevzner, 2009) or angiosperm (Sankoff *et al.*, 2009) ancestors with a parsimony principle. Such techniques are limited to few (typically less than 10) closely related genomes, all with equal gene content, with the notable exception of some including whole genome duplications when ohnologous genes are still present in two copies (Zheng and Sankoff, 2013) or when ohnologous segments can be detected (Gavranovic and Tannier, 2010). A probabilistic model of evolution of permutations by inversions has been implemented in Badger (Larget *et al.*, 2005), and applied to reconstruct animal mitochondria or *Yersinia pestis* ancestral gene orders (Darling *et al.*, 2008). These cannot benefit from the computational facilities of dynamic programming because of the large state space, and use Monte Carlo techniques for space explorations.

A way to bypass the algorithmic complexity and to scale up to hundreds of genomes with unequal gene content is to model the evolution of independent local characters instead of the evolution of a whole genome, just as it is the case for nucleotide sequences and substitutions. This method was also – and is still – applied independently from bioinformatics with cytogenetics data (Svartman *et al.*, 2006). Gene orders may be seen as sequences of adjacencies, which are the links between two consecutive genes (Gallut *et al.*, 2000). Adjacencies can be summed up by a binary character, either two genes are consecutive, or not. The immediate advantage of this view is that it can benefit from the standard methodological arsenal of ancestral character reconstruction on binary characters (see Section Ancestral Character Reconstruction). Yet there are several drawbacks to such an hypothesis of independent evolution of adjacencies. One is that the information connecting the adjacencies, used to estimate inversion distances, is lost. Another is that genomes considered as sets of independently evolving adjacencies are not anymore constrained to be linear arrangement of genes, yet the single cut-or-join model of genome evolution has this linearity constraint (Feijao and Meidanis, 2011; Mikloos *et al.*, 2014). Linearization techniques, often related to Traveling Salesman approaches, have to be applied to sets of reconstructed ancestral adjacencies (Manuch *et al.*, 2012) in order to present bonafide gene orders.

Parsimony approaches (Sankoff or Dollo) on adjacencies have been used, together with linearization procedures to reconstruct ancestors of mammalian (Ma *et al.*, 2006), yeasts (Chauve *et al.*, 2010), monocotyledons (Sankoff *et al.*, 2009), or bacterial (Wang *et al.*, 2006) clades.

Evolution of adjacencies is so simple that it paves the way to integrating gene order and gene content, via gene phylogenies, in a single framework (Sankoff and El-Mabrouk, 2000). Thus, it is now possible to model the evolution of adjacencies along reconciled phylogenies (Ma *et al.*, 2008; Bérard *et al.*, 2012; Louis *et al.*, 2013; Patterson *et al.*, 2013).

Prospective methods have attempted the reconstruction of more ancient animal proto-karyotypes: amniotes (Kohn *et al.*, 2006; Nakatani *et al.*, 2007), bony fishes (Jaillon *et al.*, 2004; Woods *et al.*, 2005; Catchen *et al.*, 2008), vertebrates (Naruse *et al.*, 2004; Kohn *et al.*, 2006; Nakatani *et al.*, 2007), chordates (Putnam *et al.*, 2008), or even eumetazoa (Putnam *et al.*, 2007). However, the accuracy of these ad hoc methodologies has not been studied as thoroughly as for more generic methods, which probably also miss a satisfactory validation process due to the lack of simulators with a diversified enough model of whole genome evolution.

The Complete Reconstruction of Ancestral Genomes

Imagine now a theoretical pipeline that should reconstruct the full sequences of genomes from the past. First give its gene content (Section Gene Content), then order the genes (Section Gene Order), align genic and intergenic regions and reconstruct ancestral sequences (Section Ancestral Sequence Reconstruction).

Although such integration has been attempted and applied to a small fragment of eutherian genomes (Blanchette *et al.*, 2008), or the chromosome of a medieval bacteria (Rajaraman *et al.*, 2013), the problem is still very challenging for several reasons.

One is that there are specific problems to whole genome reconstruction, like borders between genic and intergenic which can be fuzzy: sometimes homologous border sequences are genic in one organism, and intergenic in another, due to the variation of the start and stop positions of genes. Overlapping alignments, possibly with different phylogenetic histories, have to be handled.

Another is that each step highly depends on the others: the construction of phylogenetic trees depends on sequence alignments, while sequence alignment (especially when modeling indels) depends on evolutionary history along a tree (Section Ancestral Sequence Reconstruction); gene order can be informative for gene content (Lafond *et al.*, 2013); every step depends on the accuracy of phylogenetic trees, which in turn are informed by sequence and gene content evolution (Szollosi *et al.*, 2012), and could also benefit from information about the evolution of gene order. The sequencial pipeline adds up the errors of each step without the possibility of any backward correction, while real integrative models are missing. Even small error rates can lead to many errors at the genomic scale.

The validity of all the methods described above is a big issue. True ancestral molecules are unknown and the accuracy of computational estimations can be approached via simulations (but no simulator realistically handles all possible events), via extremely rare cases where an ancient sequence is known (Rajaraman *et al.*, 2013), via expectations about ancestral genomic features (e.g., stability of gene content or linearity of ancestral chromosomes (Boussau *et al.*, 2013)) or via the viability of resurrections (Groussin *et al.*, 2015).

See also: Bayesian Phylogenetic Methods. Maximum Likelihood Phylogenetic Inference. Parsimony Methods in Phylogenetics. Phylogenetic Comparative Method. Phylogenetic Tree

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Angiosperm Phylogeny and Diversification

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Glossary

Apomorphy Derived feature, an evolutionary novelty arising in the immediate ancestor of a clade and characterizing it.
C4 photosynthesis A mechanism of photosynthesis that is advantageous in conditions of drought and high temperatures. C4 photosynthesis spatially separates components of the process allowing for less water loss and increased carbon assimilation.
CAM photosynthesis A mechanism of photosynthesis that is advantageous in conditions of drought and high temperatures. The components of the process are temporally separated, allowing the stomates to be closed during the day to reduce water loss.
Capitulum An inflorescence with a shortened and often expanded axis bearing numerous sessile flowers in a dense cluster.
Clade Any monophyletic group, i.e., a group composed of its ancestor and all of its descendants.
Column A reproductive structure found in orchids in which the stamen is fused to the stigma/style.
Corolla The collective term for all the petals of a flower.
Determinate The apical meristem producing only a limited number of lateral organs, the axis ceasing extension growth.
Ectomycorrhizal A fungal–plant association in which fungal hyphae completely surround the root, but usually do not penetrate its cells.
Endophyte A bacterium or fungus that lives within the tissues of a plant but does not cause disease symptoms.
Endosperm Nutritive tissue, most often triploid, that results from the process of double fertilization that also forms the embryo and that is characteristic of angiosperms.
Epiphyte A plant that grows on another plant for support but that does not harm the plant on which it grows.
Extrafloral nectary Glandular organs that secrete nectar but that are located outside the flower. These generally do not function in pollination but rather attract animals that protect the plant.
Gametophyte Haploid generation of land plants, produces gametes.
Key innovation An apomorphy that facilitates the subsequent radiation/diversification/success of the clade.
Mycorrhiza A mutualistic association between fungi and land plants.
Pollinia Packets of pollen found in orchids and some other species. The packet is carried by a pollinator in its entirety rather than the pollen being dispersed as grains.
Rhizome A horizontal stem, usually underground; a means of clonal vegetative spread.
Stomata Pores in the epidermis, most often of the leaf, through which gas exchange occurs.

Introduction

In order to fully understand angiosperm diversification, we need to have a robust understanding of relationships, dates of divergence, and numbers and distributions of species. We need to know about morphology and other phenotypic characters, and current and past ecological contexts. Each of these elements presents problems. Progress in elucidating relationships has been substantial, much using sequence data from the chloroplast genome; how current ideas of relationships will fare when we use whole genomes or proteomes is an important question. There are very different estimates in the current literature for the dates of many nodes, and molecular and fossil evidence can be at odds (Wilf and Escapa, 2015); one can make estimations of diversification rates within a clade without dates, but many other questions are left dangling. Species numbers have to be interpreted in the context of a phylogeny, not in terms of categorical ranks – a focus on taxonomic ranks or named clades is often not helpful. Distributions of extant taxa can readily be mapped, however, past distributions may be very different from those of the present. We know far less about basic anatomical and morphological variation in angiosperms than we need, but relatively few people are working on detailed morphological studies. Furthermore, as genetic aspects of development are factored in, our understanding of morphological similarities and differences changes. Finally, although quite a lot is known about flowers and fruits and their interactions with pollinators and dispersers, considering the role of ecophysiological changes in the evolution of angiosperms allows us to think about angiosperm – and biosphere – evolution in new ways. However, understanding past ecological contexts and how they affected angiosperm evolution is difficult.

Looking at overall patterns of diversification, it is particular clades within groups like angiosperms, Orchidaceae (orchids), or Poaceae (grasses) that are notably species-rich, so it may be particular features of these clades ('key innovations') in combination with apomorphies of the larger groups that have spurred diversification. Furthermore, extrinsic features like climate or geography can play major roles in diversification (Donoghue and Sanderson, 2015). It is also often suggested that the origin of many features of flowering plants and their effect on diversification are disconnected in time – there is a lag period. This is true of features like genome duplication

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(Tank *et al.*, 2015), where diversification of gene function and reorganization of developmental pathways can be invoked to explain this lag period, and also of more conventional morphological features like extrafloral nectaries (Weber and Agrawal, 2014).

The Background – Some Distinctive Angiosperm Features

The evolution and diversification of flowering plants at one level can be thought of as being spurred by the evolution of the flower, a modified determinate shoot, and the fruit, seeds totally enclosed and protected by tissue developed from the ovary. Interactions between plants and animals (pollination, dispersal, herbivory, and more) have led to the diversification of both. The gametophytic stage of angiosperms is physically much reduced: the male gametophyte consists of two gametes and one other cell, while the female gametophyte usually consists of only eight cells, one of which is the single female gamete. In angiosperms, both male gametes are involved in fertilization (double fertilization), one fertilizing the egg, and the other fusing with (usually) two nuclei of the female gametophyte to form triploid endosperm. Endosperm is unique to angiosperms and is an intermediary in providing nutrients to the developing embryo and often reserves for the mature seed. Angiosperms also have a more efficient transport system consisting of vessel elements, dead cells of wide diameter whose walls are reinforced with the complex polymer lignin in distinctive patterns. Vessel elements are stacked to form elongated tubes for water transport, a process which is facilitated by the perforation of the end walls of the cells which allows formation of an unimpeded water column.

All these features are unique to flowering plants, or are pretty close to being unique. However, practically no feature of flowering plants is unique in the sense that it has evolved once and has never been lost, thus some angiosperms have no vessels and some do not develop endosperm. Similarly, extrafloral nectaries characterize a speciose clade within the legume genus *Senna* and may be involved in its diversification, yet at the same time the loss of those nectaries in *Mimosa*, another legume, also characterizes a species-rich clade (Marazzi and Sanderson, 2010). Furthermore, it is arguable whether or not *Amborella*, the extant species that is the sister to all other angiosperms (**Figure 1**), has vessels, and indeed on a per area basis water transport is as efficient in some conifers as it is in basal angiosperms that lack specialized vessel elements.

The angiosperm life cycle is usually notably shorter than that of extant gymnosperms (Williams, 2012). The pollen tube growth rate in angiosperms is much higher than that of extant gymnosperms, fertilization often occurring within about 24 h of pollination as compared to 7 days or often far more in extant gymnosperms. Angiosperms tend to become mature at a younger age than do gymnosperms (Verdú, 2002).

Among extant seed plants, only angiosperms are herbaceous, and the annual habit is unknown in other vascular plants. Molecular evolution is faster in herbs/annuals (e.g., Smith and Donoghue, 2008). Yang *et al.* (2015) found substitution rates in both synonymous and nonsynonymous sites in protein-coding genes up to three times those in their woody relatives. This correlation may be connected with mutation rate or population size and thence to speciation rate (Gaut *et al.*, 2011). There is a general correlation between rates of molecular evolution (substitution rates) and the rate of speciation (Webster *et al.*, 2003), and the evolution of herbs from trees, or at least a shortened time to maturity, has been linked with a rise in the speciation rate of the former (Verdú, 2002).

Patterns of species richness and diversification rates have been examined at scales from angiosperm-wide to particular clades within families, emphasizing situations where diversification has speeded up or slowed down. Below, the author looks both at species richness and at other aspects of diversification; all species numbers are very approximate!

Angiosperm Diversification

Diversification in Basal Angiosperms and Monocots

The basal angiosperm lineages are species-poor (**Figure 1**). The New Caledonian *Amborella* is monotypic, and neither of the two other earliest diverging lineages Nymphaeales (water lilies) and Austrobaileyales (which includes star anise) has many species. Monocots include some 60 100 species. The adoption by early monocots of wet/marshy habitats might explain their vegetative features including an herbaceous habit, growth via rhizomes (underground horizontal stems), and linear leaves, but this is largely surmise, especially since we know little about the evolution of monocot leaves, the immediate relatives of monocots, etc. Monocots lack taproots, instead a network of 'adventitious' roots develops along the stem (**Figure 2**), making some of these plants such as grasses useful for controlling soil erosion.

The monocot family Orchidaceae (27 800 spp.) is the largest angiosperm family, and hypotheses for why they are so diverse are many (Pridgeon *et al.*, 1999–2014). Orchidaceae usually have very bilaterally symmetric flowers with one functional stamen that is fused with the stigma/style to form a unique structure called the column (**Figure 3**). How orchids are pollinated, the interaction of the pollinator and plant, and how that may underlie the rampant speciation in this family, have long interested biologists, including Charles Darwin. The pollen grains are usually aggregated in packets called pollinia (**Figure 3**) that get attached to pollinators in very specific locations by sticky tissue; the often hundreds of thousands of ovules in the ovary are fertilized by a single visit of the pollinator. Deceit pollination, in which the flower mimics another flower or an insect to attract the pollinator but does not offer any reward, is common – indeed, pollination of perhaps one-third of the whole family occurs during the course of pseudocopulation by amorous male insects. However, rewards of various sorts may be provided, and positive Mullerian mimicry, in which both orchid and the flower it is mimicking offer rewards, also occurs.

Flowers and pollination provide one context for thinking about orchid diversity. However, orchids have also specialized in the epiphytic habit, and features related to this may also explain the diversity of this group (Freudenstein and Chase, 2015). One can argue that all or part of the Epidendroideae, with 21 800 species including most commonly cultivated

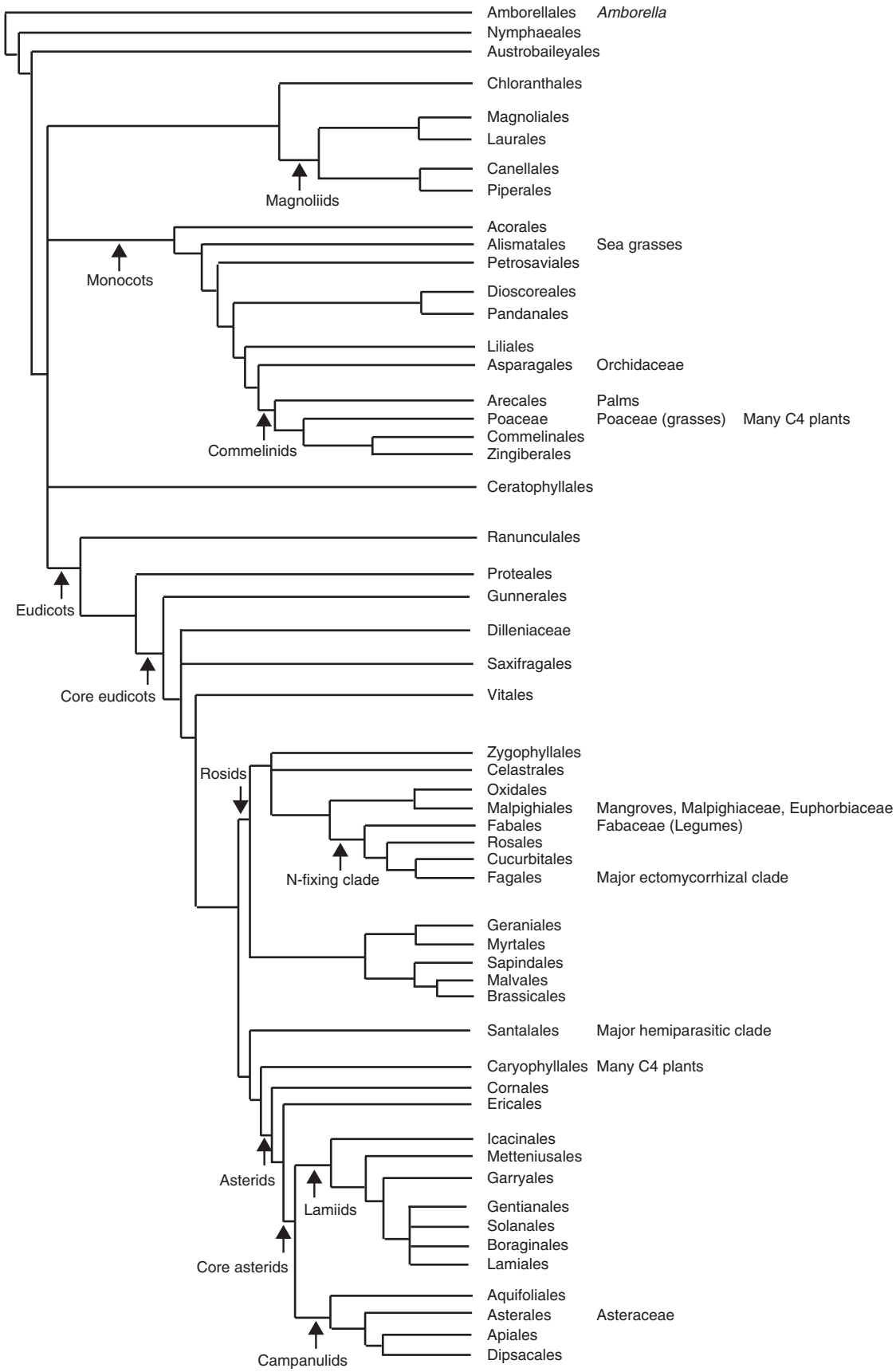

Figure 1 Angiosperm phylogeny, simplified and with additions from Stevens, Angiosperm Phylogeny Website Version 13, accessed 24.viii.2015.

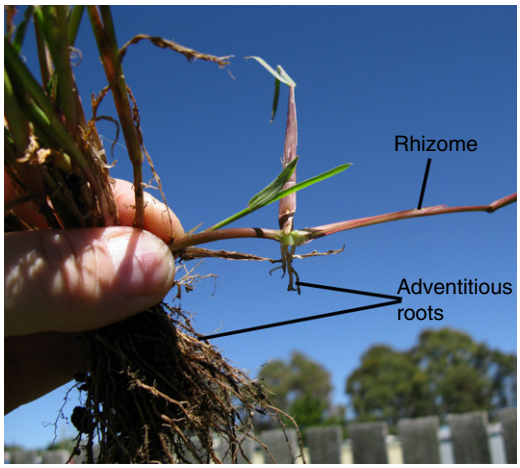

Figure 2 Adventitious roots in the Asian grass *Echinochloa colona*.

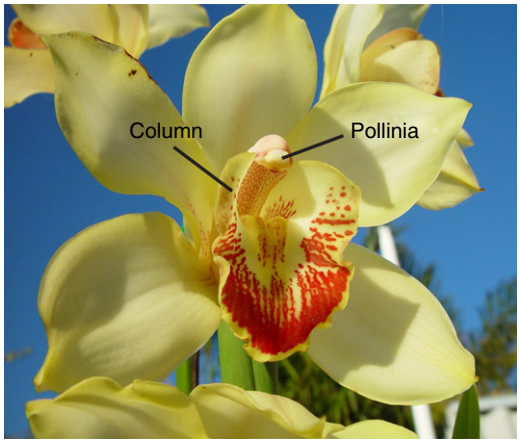

Figure 3 Hybrid *Cymbidium* (Orchidaceae) flower showing column and pollinia.

orchids, are the hyperdiverse clade in contrast to orchids as a whole or to other groups of orchids. Most Epidendroideae are epiphytic. Consistent with life in this aerial environment, with limited access to water, many species employ CAM photosynthesis, they often have exposed fat roots that grasp the substrate and can carry out photosynthesis, and the modified outer surface of the root plays a role in water/nutrient uptake. Such features provide another context for thinking about orchid diversity.

Poaceae (grasses, 11 340 species) are by far the largest wind-pollinated clade in angiosperms, although as with the orchids, it is arguably a single clade within the grasses, called the PACMAD/BOP clade, with 11 310 species, that is most species-rich (Figure 1). This clade includes most familiar grasses such as the economically important cereal grains as well as turf grasses. Even within this group it is particular subclades that are diverse; these subclades have specific features that may have allowed them to diversify: C_4 photosynthesis (PACMAD grasses), a form of woodiness (Bambusoideae, bamboos), association with fungal endophytes, and adaption to cooler conditions allowing the family to move into different climatic zones (Pooideae, including cereal, lawn, and pasture grasses) (Kellogg, 2015). In grasses in particular, fossil and molecular dates are at odds, some molecular estimates being half the age of fossils ascribed to the family.

Diversification in Core Eudicots

One of the distinctive features of nearly all core eudicots, a clade which includes the majority of angiosperm species, is the standardization of floral architecture. Flowers often have 5 sepals, 5 petals, two whorls of 5 stamens, and 3 or 5 fused carpels, with the position of the organs in each whorl alternating with those of the previous whorl (Soltis *et al.*, 2016). It has been suggested that the whorled structure allowed for fusion of floral organs, for instance fusion of the petals into a tube, dish, or bell shape. This allowed for tremendous elaboration of floral morphology, thought to be driven largely by adaptation to pollinators.

One of the largest core eudicot clades is the rosids (roses and much more), with around 85 830 spp. (Figure 1). There are three main groups of rosids that vary in the key features that distinguish them and may have driven their diversification. Malpighiales (16 000 spp.), a heterogeneous group including species such as willows, flax, and violets, represents a disproportionate number of the smaller trees of the lowland tropical rainforest. Diversification in this group is at the subclade level. For instance, Malpighiaceae (1250 spp.) is largely New World, and its members have functionally inverted flowers; oil from glands on the backs of the sepals is the reward for pollinating bees. Malpighiaceae have moved nine times or so to the Old World, but there are few species there, the plant–bee association has broken down, and nectar is often the reward (Davis *et al.*, 2014). Another family in Malpighiales, Euphorbiaceae (*Hevea*, rubber, 6745 spp.), includes one clade of 4860 species characterized by having poisonous latex in the tissues, presumably a defense against herbivory; within this clade the genus *Euphorbia* (poinsettia, crown-of-thorns) alone includes 2420 species. *Euphorbia* species have a great diversity of life forms including succulents superficially similar to New World cacti. Species show both CAM and C_4 photosynthesis (Horn *et al.*, 2014), and the genus is characterized by a unique and remarkable inflorescence in which the flowers are reduced to single stamens or pistils, and these are clustered together and surrounded by showy colorful bracts (Figure 4). Thus the entire inflorescence resembles a single flower, although the adaptive advantage of this is not entirely clear. Thus even within *Euphorbia* high species numbers can be linked with many different factors.

Another large and diverse group within rosids, the 'nitrogen-fixing clade' (31 215 spp.) is characterized by bacterium-mediated nitrogen fixation, of central importance in the global nitrogen cycle and to agriculture (Figure 1). This clade includes legumes, roses, apples, squashes, oaks, walnuts, and much more, and most species do not fix nitrogen. Nonetheless, this bacterial–plant symbiosis appears to have evolved 14 times or more here alone among all angiosperms. In this symbiotic association, bacteria convert atmospheric nitrogen into compounds that are metabolically available to the plants, allowing them to invade and survive in nitrogen-poor soils. The bacteria involved include both gram positive (actinobacteria) and gram

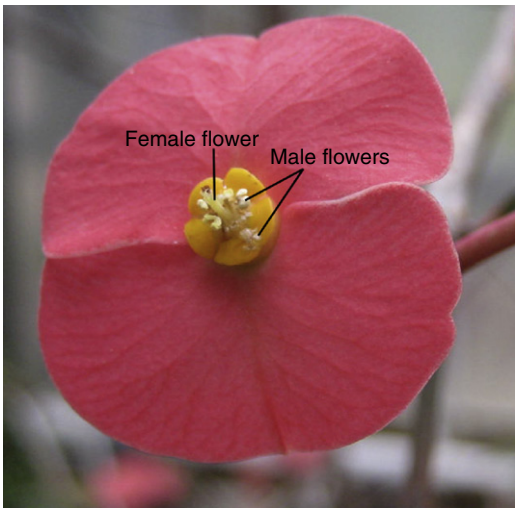

Figure 4 Inflorescence of Gerold's spurge (*Euphorbia geroldii*) showing individual male and female flowers.

negative (alpha and beta proteobacteria). A number of the genes involved in the establishment of these symbioses appear to have been coopted from those involved in mycorrhizal associations, and in legumes, at least, the molecular pathways involved in producing root hairs are also involved in the production of infection threads, structures that facilitate the movement of bacteria deep into the roots where the nodules in which they fix nitrogen develop (Sprent, 2009).

Finally, the core asterids include over 85 000 species (Figure 1). Here the range of floral architecture is further narrowed: petals are fused into a corolla, the stamens are often fused to the corolla, and generally there is only one whorl of 5 or fewer stamens. Like Orchidaceae, basic floral variation is limited, yet overall diversity is very high, the basic floral plan allowing for variation in flower symmetry, organ shape, size, and color; the flowers, when small, may be grouped in inflorescences, the unit of pollinator attraction. One vegetative feature that in some clades is correlated with increased diversification is a transition away from the woody tree habit. In the lamiid (also called euasterid I) clade (mints, nightshades, and many more), the three basal clades are all woody and species poor. Other (largely) woody clades scattered throughout the core asterids are also relatively species-poor. Over 97% of the 23 810 species of Lamiales (including mints) have bilaterally symmetric flowers – and are mostly herbs or small shrubs. Asteraceae (aster family, 23 600 species) predominate within the campanulid (also called euasterid II) clade. They have a characteristic capitulum inflorescence (Figure 5), in which up to hundreds of individual flowers are clustered into a structure that resembles a single flower. Asteroideae is the youngest of 13 Asteraceae subfamilies but includes some 65% of its species. Its members are chemically diverse and have distinctive morphological features, but what spurred their diversification?

Success of a Different Kind

The importance of ecophysiological changes in the tree during angiosperm evolution cannot be overemphasized, since the

Figure 5 Inflorescences of Short's aster (*Aster shortii*) showing individual flowers.

changes have had, and continue to have, major effects on the biosphere. These changes include the evolution of dense leaf venation (above 5 mm mm^{-2}) along with associated changes such as a decrease in stomatal size and increase in stomatal density. Such changes have evolved at least three times in magnoliids, monocots, and eudicots, and were particularly marked in the Late Cretaceous. Their main effects, inseparably linked, are twofold. Photosynthesis can increase, because small dense stomata allow more CO^2 to diffuse in to the leaf, which is well supplied by water from the dense veins (Boyce and Zwieniecki, 2012). At the same time more water is lost through transpiration, and this increases the water content of the atmosphere (the other main component comes from evaporation) and rainfall increases – thus models suggest that venation density may have indirectly facilitated the spread of the diverse lowland tropical rainforest (Boyce and Lee, 2010).

Other ecophysiological changes have occurred in relatively few species, but they have disproportionate effects on the environment (Stevens, 2015). Communities of sea grasses, mangroves, and plants with ectomycorrhizal associations are all involved in massive and surprisingly long-term sequestration of carbon. C_4 plants often dominate in the communities in which they occur, they are often very productive, they cover very large areas of the globe – yet they have come to dominate only within the last 10 million years.

Plant–Pollinator Interactions

The evolution and diversification of flowering plants at one level can be thought of as being spurred by the evolution of the flower and the fruit. Interactions between plants and animals – some kind of coevolution – led to the diversification of both. Pollinator-mediated speciation is thought to be important in evolution, thus for over 150 years the match in corolla and bird bill size and shape has implied coevolution, the bird and flower evolving together. However, looking at what are often thought of specialist pollinators and the plants they pollinate suggests a rather different story.

Some 338 species of hummingbirds pollinate over 4500 species of plants, the plants representing many separate clades in which bird pollination was acquired independently. Hummingbirds began diversifying less than 30 mya, although

the first fossils are from the Oligocene of Europe (Abrahamczyk and Renner, 2015). Two hundred species of orchid bees, also New World, pollinate around 4000 species of plants; the bees evolved around 42 mya. Bumble bees include some 200 species that pollinate around 3000 species of plants. The bees probably diversified within the last 47 my. The story is similar with fruit-eating and flower-pollinating New World bats, and many other insect, bat, bird, and bee groups from all over the world (Stevens, 2015).

It is unclear whether the evolution of the pollinators preceded, was more or less contemporaneous with, or happened after that of the plants they pollinate – all three possibilities have been suggested. Obviously there has been some coevolution between plant and animals, but in many cases the plant may have evolved to 'fit' the morphological and sensory predilections of the animal – much recent evolution has been in the plant, not in the animal (Guimarães *et al.*, 2011; Schiestl and Dötterl, 2012). Diversification of the two parties may have occurred together in hummingbirds, but members of quite different clades of plants can be pollinated by the one hummingbird, and something other than simple coevolution is likely here, too. And classic examples of apparent coevolution such as fig. and fig wasps and yuccas and yucca moths are being reinterpreted (Yang *et al.*, 2015).

Epilogue

All in all, understanding the forces that drive plant diversification can be very tricky. Importantly, how do we measure diversity and success? By the numbers of species in clades, by their morphological, phylogenetic, or ecological, divergence, or their importance to the community/ecosystem/biosphere as a whole? In much literature – especially the earlier literature – on diversification, species numbers are the measure of diversity/success used (c.f. Givnish, 2015), and what they alone can tell us is limited. However, ecologically interesting traits are being mapped onto phylogenies (e.g., Zanne *et al.*, 2014), while databases of critically evaluated morphological characters currently being assembled will allow classical morphology to be mapped to the tree in new ways. Estimates of carbon sequestered in plants, associated fungi, and the soil will allow us to understand better the effects of small clades of ecologically dominating plants on the environment. We are only now beginning to understand diversification.

See also: C_4 and CAM Photosynthesis in Land Plants, Evolution and Diversification of. Plant–Pollinator Interactions and Flower Diversification

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Animal: What Is an Animal?

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Glossary

Adherens junction A cell junction that occurs at points of cell contact in adjacent cells (most notoriously in epithelial cells). Adherens junctions are composed of defined protein complexes that include cadherin receptors, and cytoplasmic adapter proteins (catenins) that link to the actin cytoskeleton.

Blastula A hollow sphere of cells surrounding a fluid-filled cavity. Many animals produce a blastula stage embryo early in development, prior to gastrulation.

Cambrian The first geologic period of the Paleozoic era, lasting from ~542 to 485 million years ago. Most modern animal lineages have a fossil record that extends to this period.

Cell junction Molecularly and functionally defined protein complexes that connect cells to each other or to the extracellular matrix and have functions ranging from adhesion to barrier formation to regulate ionic and molecular transport between cells.

Clade Synonymous with 'monophyletic group.' Refers to an ancestor and all of its descendents.

Ediacaran The last geologic period of the Neoproterozoic era, lasting from 635 to 542 million years ago. This period immediately preceded the Cambrian period.

Eukaryote Cellular organisms with membrane bound organelles such as the nucleus. This large diverse group includes all life forms except for Bacteria and Archaea.

Extracellular matrix The external environment of cells, typically composed of molecules secreted by the cells for structural support and for cell communication.

Extant Alive today (i.e., not extinct).

Filopodia Cytoplasmic projections from cells that contain actin as the primary cytoskeletal component. Filopodia frequently function in cell migration.

Gastrulation The developmental process whereby embryos undergo morphological changes to create internal and external tissue layers.

Homolog In the context of this article, homolog refers to two genes in different organisms that are derived from a single gene in their common ancestor.

Microvilli Cellular protrusions that often function to increase cellular surface area (e.g., in the gut or kidney) or in mechanosensation (e.g., cochlear hair cells). Microvilli are similar to filopodia except that they occur in groups instead of singly, they usually have a polarized distribution on the cell, and they have very regular (and regulated) lengths.

Monophyletic A group of organisms that are descended from a single common ancestor. Synonymous with 'clade.'

Mutagenesis screen A laboratory technique used to create genetically modified organisms. The purpose of a mutagenesis screen is to identify genes that affect specific traits.

Phylogeny A branching diagram that depicts the evolutionary relationships of organisms to each other.

Taxa The plural form of the word taxon, which refers to a named group of organisms such as a species, or even a more inclusive clade such as 'vertebrates.'

Ultrastructure In the context of this article, ultrastructure is used to refer to subcellular organization of an organism.

The word 'animal' conjures images of macroscopic, sexual organisms that swim, crawl, fly, run, and that eat plants and other animals. However, when one begins to look across the full spectrum of modern animal diversity, it becomes more challenging to develop a comprehensive definition. For example, all animals move, but sponges and placozoans lack muscles and nerves and move instead by cellular migration or ciliary action. Likewise, sexual reproduction is an ancient trait, ancestral to all modern animals, but bdelloid rotifers have secondarily evolved to reproduce only asexually (Flot *et al.*, 2013; Welch and Meselson, 2000). Indeed, one can find examples that challenge our concepts about how animals feed, the basic construction of animal tissues, and arguably even the idea that animals are exclusively multicellular.

The first part of this article will review modern animal diversity and will highlight the features that are commonly reported to be diagnostic of animals. The second part will examine the question of animal diversity by looking back through time at the early fossil record of animals, and by looking forward to some of the more bizarre trajectories that animal evolution may take in the future.

Modern Animals and Their Closest Eukaryotic Relatives

Animals evolved in the ocean, and here the overwhelming majority of modern animal phyla are found exclusively. Based upon our current understanding of animal phylogeny, we recognize seven major monophyletic groups: sponges, ctenophores, placozoans, cnidarians, ecdysozoans, lophotrochozoans, and deuterostomes (**Figure 1**). These groups are really not equivalent in any way; least of all in terms of body plan complexity. For example, sponges are one of the most ancient animal lineages, but they exhibit a high degree of morphological homogeneity; in contrast, the relatively younger deuterostome lineage includes organisms as diverse as

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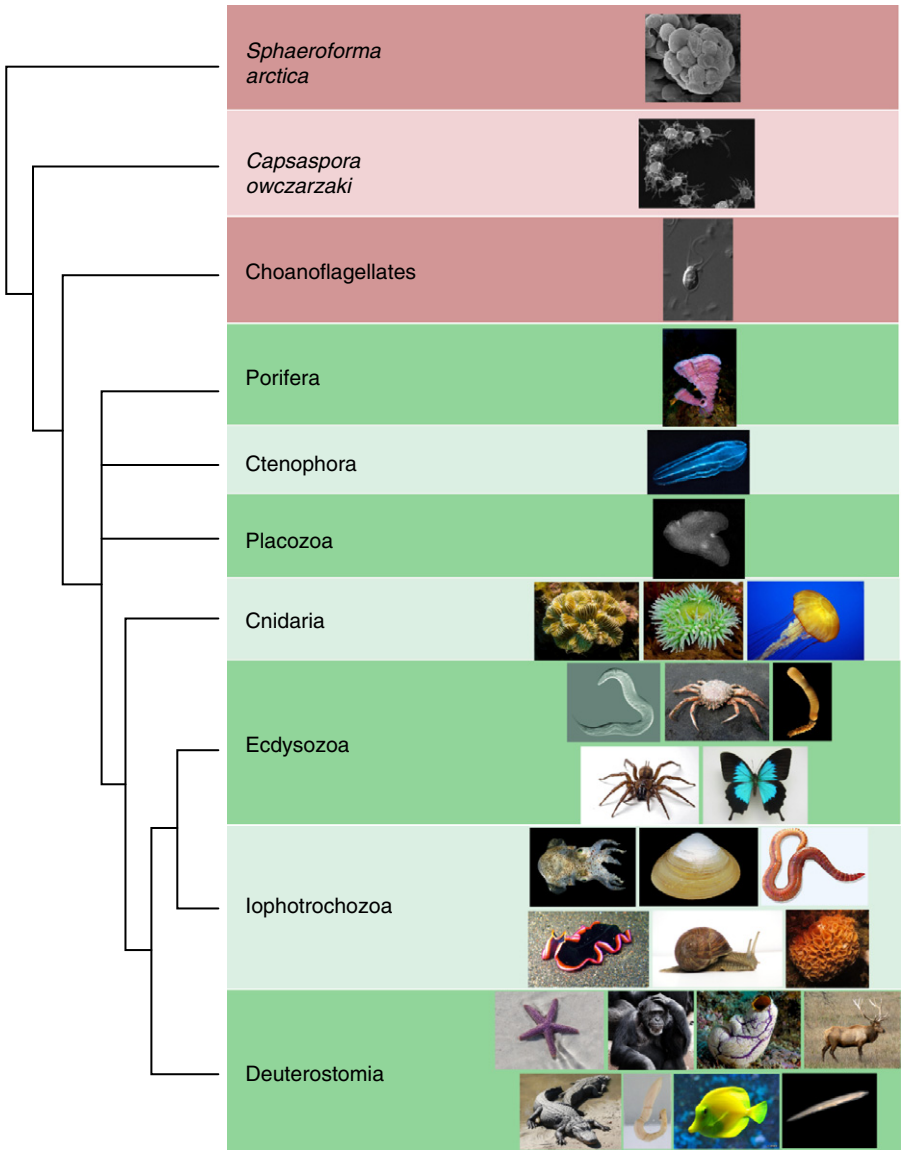

Figure 1 Seven major groups of extant animals and their close relatives. Modern animal diversity (shown in green) is well characterized, but some evolutionary relationships are still uncertain. The largest and most diverse group of modern animals are the bilaterian lineages: ecdysozoa, lophotrochozoa, and deuterostomia. It is from these three lineages that most of our generalizations about animal biology are derived, both because we belong to the deuterostome lineage, but also because the major biomedical research models such as the fruit fly, worm, frog, and zebrafish belong to these lineages. There is considerable controversy about the evolutionary relationships among non-bilaterian animals: the ctenophores, sponges, cnidarians, and placozoans. These lineages diverged from each other and from bilaterians early in animal evolution. Thus, their relative simplicity has long contributed to speculation about the evolutionary sequence by which bilaterian-grade complexity evolved. The evolutionary connection of animals with nonanimals (shown in red) has only recently been discovered. These lineages include choanoflagellates, *Capsaspora owczarzaki*, and ichthysporeans such as *Sphaeroforma arctica*. Research into the biology and genomes of these enigmatic lineages has significantly contributed to our understanding of cellular and genomic evolution in the animal stem lineage.

acorn worms, lancelets, sea squirts, starfish, birds, fish, turtles, and mammals.

Despite major advances in our understanding of animal phylogeny, driven primarily by molecular and genomic data, notable debates continue. For example, there is no community-wide consensus about the relationships among non-bilaterian lineages (**Figure 1**). These are the most ancient animal lineages and it has been proposed that they diverged from each other over a relatively short time period, conditions known to confound current methods of phylogenetic reconstruction (Rokas *et al.*, 2005). Of particular importance is the question of whether ctenophores branch before sponges, or whether sponges branch before ctenophores – a controversy that has emerged from the analysis of large, genome-scale datasets (Dunn *et al.*, 2008; Moroz *et al.*, 2014; Philippe *et al.*, 2009; Pick *et al.*, 2010). Ctenophores have muscles, nerves, and a gut, whereas sponges do not, so resolution of this phylogenetic controversy has implications for how we

interpret their disparate body plans. Did ctenophores evolve these features in parallel with bilaterian organisms, or was the last common ancestor of animals considerably more complex than modern sponges? It may come as a greater surprise that, even within Bilateria, there are a number of taxa that defy confident classification. Examples include acoel flatworms, *Xenoturbellida*, chaetognaths, and Orthonectida (Holland, 2011).

For this discussion of what defines an animal, the most important consideration is that the monophyly of animals is well established. All of the seven major clades identified above (and even the anomalous taxa that so far defy classification) are derived from a single common ancestor, to the exclusion of all other (known) living organisms. Moreover, there is high confidence in the phylogenetic position of the closest eukaryotic relatives of animals. As shown in **Figure 1**, choanoflagellates are the closest relatives of animals, followed by the filasterian *Capsaspora owczarzaki* and icthyosporeans such as *Sphaeroforma arctica* (Ruiz-Trillo *et al.*, 2008). This means that the term animal is phylogenetically well-constrained: even though there are gray areas within the animal tree of life (i.e., about which lineages fit where), there is no ambiguity about which organisms are animals and which are not. As elaborated below, this assertion erodes when the fossil record is taken into account.

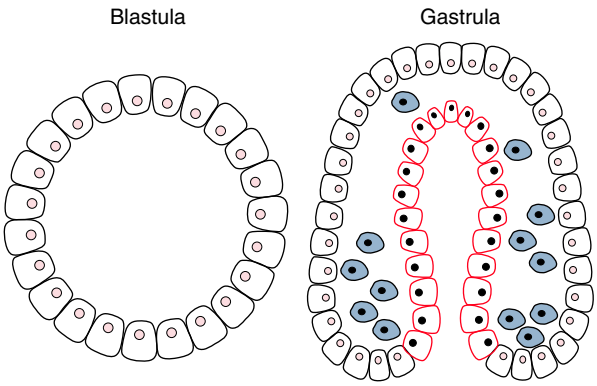

Figure 2 Stereotypical developmental stages of animal embryos. Gastrulation in animals is the transition from a blastula stage embryo (left) to a gastrula stage embryo (right). Whereas the blastula is a hollow ball of cells, the gastrula has an outer layer called the ectoderm, and internal layer called the endoderm (red cells). The cells between these two layers (blue cells) comprise the mesoderm. At this stage, each of these embryonic tissues is fated to give rise to specific adult tissue and organ systems.

What Features Do All Animals Have in Common?

Multicellularity

Perhaps the most fundamental characteristic of modern animals is that they are composed of more than one cell. Moreover, they have differentiated cell types with specialized and integrated functions (e.g., tissues and organs). Whereas multicellularity has evolved many times throughout evolutionary history, the degree of cellular differentiation and integration exhibited by animals is rare. Without delving into a more nuanced discussion of the topic, green plants and possibly fungi, social amoebae, and brown algae also have multicellular states with differentiated tissues, but to a considerably lesser degree than most animals.

So, is there anything unique about animal multicellularity that distinguishes it from other forms of multicellularity across the tree of life? The traditional view is that animals evolved multicellularity independently of other lineages. Part of the evidence for this view comes from distinctive features of animal cell and developmental biology. For example, animals do not have cell walls like fungi and plants, whereas they do have unique features such as specialized cell–cell junctions, adhesion molecules (Abedin and King, 2010), and embryonic features such as a blastula stage that undergoes gastrulation (**Figure 2**). However, the most relevant comparison point for determining whether animals evolved from an immediate unicellular ancestor or an immediate multicellular ancestor is the sister group of modern animals, the choanoflagellates. Multicellular choanoflagellate species (**Figure 3**) are widespread

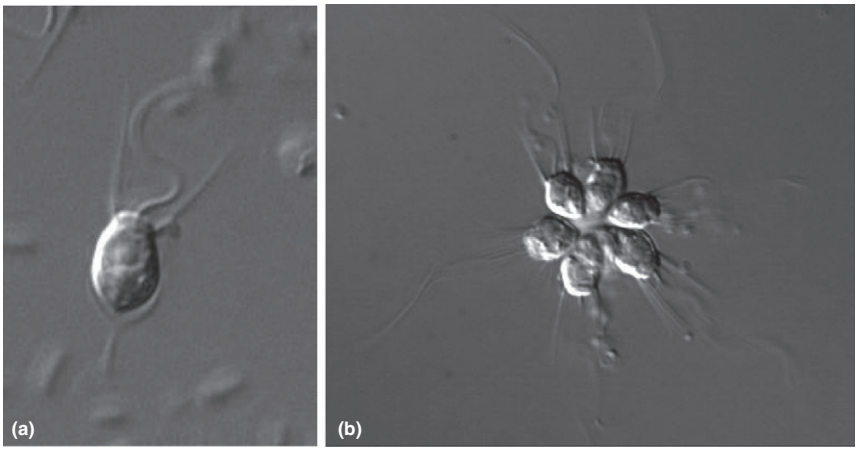

Figure 3 Unicellular and multicellular life history stages in choanoflagellates. Whereas all choanoflagellates have unicellular life history stages (a), some such as *Salpingoeca rosetta* are capable of producing undifferentiated colonies through serial cell division (b). Photo credit: Mark Dayel, Wikimedia commons.

and abundant in nature, but they differ from animals in three important ways: (1) all known species are believed to have free-living unicellular stages; (2) there is no obvious cellular differentiation or division of labor within multicellular colonies; and (3) there are considerable ultrastructural differences between colonies within different choanoflagellate clades, possibly indicating independent origins of multicellular colony formation within choanoflagellates (Nichols *et al.*, 2009). Thus, one possibility is that there may be no evolutionary connection between choanoflagellate multicellularity and animal multicellularity.

A contrasting view comes from recent experimental studies from choanoflagellates. In a study by Fairclough *et al.* (2013), different life history stages of the colony-forming choanoflagellate *Salpingoeca rosetta* were evaluated by gene expression profiling. One conclusion from this study was that genes shared by choanoflagellates and animals were disproportionately expressed in colonial life history stages, and in cells fated to develop into colonies. More recently, Levin *et al.* (2014) used a mutagenesis screen to identify a C-type lectin that is required for the development of a specific multicellular colony morphology (rosettes) in *S. rosetta*. C-type lectins have carbohydrate-binding domains and function in cell adhesion in animal tissues, so this study may indicate a more ancient role for C-type lectins in multicellular evolution.

Finally, evidence for an even more ancient multicellular ancestry of animals may also come from a distant eukaryotic relative of animals, the social ameba, *Dictyostelium discoideum*. On the surface, multicellularity in *D. discoideum* is very different than either choanoflagellates or animals, which both develop clonally through serial cell divisions; *D. discoideum* gives rise to a transient multicellular structure called a 'fruiting body' through aggregation of genetically different individuals (**Figure 4**). Thus, it seems unlikely that there could be any evolutionary or mechanistic connection between these very disparate and phylogenetically disjunct forms of multicellularity. However, a recent study by Dickinson *et al.* (2011) found that candidate homologs of the animal cell adhesion genes α- and β-catenin interact in *D. discoideum*, like they do in animal cells and are required for the organization and polarity of the outer epithelium of the fruiting body in *D. discoideum*. This discovery raises the possibility that transient multicellularity is an ancient feature common to the biology of *Dictyostelium* and animals, and by extension, to intermediate lineages such as choanoflagellates and fungi (Dickinson *et al.*, 2012). However, see Parfrey and Lahr (2013) for a critique of this interpretation.

Figure 4 The fruiting body of *Dictyostelium discoideum*. The social ameba *D. discoideum* is predominantly unicellular in nature. Upon starvation, genetically distinct individuals aggregate to produce a multicellular structure called the 'fruiting body' for the production and dispersal of spores.

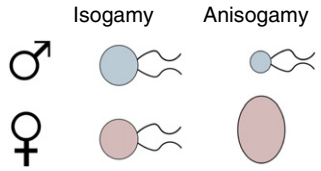

Figure 5 Isogamy vs. anisogamy. Isogamous organisms produce male and female gametes that are indistinguishable in size and shape. In contrast, all sexually reproducing animals are anisogamous and produce large nonmotile eggs and small motile sperm. Indirect evidence suggests that choanoflagellates may also be anisogamous.

Sex and Development

Sexual reproduction is very ancient and vastly predates animal origins, but the details of sexual reproduction in animals distinguish them from their closest relatives, such as choanoflagellates, and also from many other multicellular groups. Specifically, through the process of meiosis, animals typically produce a large haploid egg that is nonmotile, and small haploid sperm that are flagellated (i.e., they are anisogamous (**Figure 5**)). These gametes fuse to produce a diploid zygote that undergoes an orchestrated sequence of cell division, differentiation, and morphogenesis to produce an embryo with internal and external cell layers via the process of gastrulation. How does this compare to sex and development in choanoflagellates?

Sex in choanoflagellates is poorly understood. In fact, sex has only been detected in a single species, the emerging experimental model *S. rosetta*. Specifically, Levin and King (2013) found that the DNA content of isolated nuclei is consistent with a haploid/diploid sexual cycle that could only result from meiosis. Although the ploidy of different cell types and life history stages could not be directly determined in heterogeneous cultures, the existence of considerably distinct cellular size classes (and the observation that these different cell types can fuse) raises the possibility that the anisogamy

Figure 6 The demosponge, *A. queenslandica*, is native to Heron Island, Australia. As the subject of the first sponge genome sequencing, *A. queenslandica* has emerged as an experimental model for studying developmental mechanisms in sponges.

exhibited by animals may have more ancient evolutionary roots.

Comparisons of later steps in development between choanoflagellates and animals yield only one clear similarity: like animals, the development of multicellular structures in *S. rosetta* occurs by serial cell divisions, not by aggregation (Fairclough *et al.*, 2010). However, unlike most animals, cells within colonies are connected by long, thin cytoplasmic bridges rather than the protein complexes that tether animal cells at structures such as adherens junctions (Dayel *et al.*, 2011). Moreover, the process of gastrulation, the morphogenetic event leading to a multilayered embryo (see **Figure 2**), is a hallmark feature of animal development that bears little resemblance to any developmental event in choanoflagellates, or any other eukaryote for that matter.

The (often more varied) patterns of development exhibited by non-bilaterian animals offer a challenge to the idea that gastrulation is a general feature of animal development. In particular, there is currently little consensus over the homology of developmental processes and tissue layers between sponges and other animals. In bilaterian animals, the significance of the layered embryo that results from gastrulation is largely that cells in different regions are destined to different fates. For example, endoderm may give rise to the gut, mesoderm may give rise to muscles, and ectoderm may give rise to the nervous system. Then, how do the developmental programs of sponges, and the resultant adult tissues that arise from development, relate to concepts such as gastrulation and progressively fated tissue differentiation when sponges have neither a gut, muscles, nor a nervous system?

Nagaysu *et al.* (2014) recently used cell tracking methods in the sponge *Amphimedon queenslandica* (**Figure 6**) to examine whether layered tissues in sponge larvae give rise to specific tissue layers in the adult. The results from this study suggest that cells from internal and external larval tissues indiscriminately give rise to both the internal and external tissue types in the adult. Although it is difficult to generalize from the study of a single sponge species, this finding supports the idea that gastrulation is a later evolutionary innovation within animals.

Figure 7 *Riftia pachyptila*. Hydrothermal vent worms of the genus *Riftia* have secondarily lost a functional alimentary canal and instead derive their nutrition from the metabolic activities of symbiotic bacteria.

Feeding

Animals, like the vast majority of living organisms, are heterotrophs: they require organic carbon for growth. So, this is another characteristic that unites animals, but does not distinguish them from nonanimals. Where animals differ from nonanimal heterotrophs is that they are largely micro- or macrophagus, ingesting small or large food particles directly for digestion in a gut. This distinguishes them from osmoheterotrophs like fungi or many bacteria that directly absorb nutrients into their cells through the process of diffusion or active transport across the cell wall. However, even this distinction is not absolute. Animals like tapeworms have secondarily evolved osmoheterotrophy as a means of nutrition. Likewise, hydrothermal vent worms of the genus *Riftia* (**Figure 7**) have evolved to rely on indirect nutrition from symbiotic bacteria and have secondarily lost the need to 'eat' like other animals.

If we look outside of Bilateria, we find more exceptions to the typical modes of animal feeding. For example, placozoans feed in a way that is difficult to classify; they digest food in the space between their ventral surface and the substrate. This resembles digestion in a gut, but occurs outside of the organism. Due to the uncertain phylogenetic position of placozoans, it is unclear if this is an ancestral or derived mode of feeding in animals.

Most sponges feed without a gut altogether. Instead, they have specialized cells that filter bacteria out of the water for direct phagocytosis by individual cells. An exception to this feeding mode are the carnivorous sponges of the family Cladorhizidae (**Figure 8**), which have lost the water canal system and instead capture small animal prey on sticky appendages which undergo morphogenetic changes to surround the immobilized prey with a temporary gut-like structure for extracellular digestion (Vacelet and Duport, 2004).

The ancestral feeding strategy of modern animals remains an open question. It has long been held that the bacterivorous strategy of sponges is ancestral to all modern animals, partly because this feeding strategy is also exhibited by modern choanoflagellates. However, there are two caveats to this view. First, there are diverse multicellular fossils in Ediacaran deposits that are argued to have evolutionary connections to modern animals, either as stem-animals, or as unsuccessful

branches off the animal stem lineage (Figure 9). Many of these organisms are also argued to have bacterial and/or photosynthetic symbionts or to have been osmoheterotrophic (Xiao and Laflamme, 2009).

Another challenge to the view that sponge/choanoflagellate-like bacterivory is ancestral to modern animals is the possibility that modern ctenophores are the earliest evolutionary branch of modern animals rather than sponges (Dunn *et al.*, 2008; Moroz *et al.*, 2014; Ryan *et al.*, 2013). Ctenophores are carnivorous, complete with an alimentary canal much like bilaterian animals and cnidarians. If the proposed phylogenetic placement of ctenophores is correct, then they must have secondarily evolved a gut, or carnivory (or at least macrophagus heterotrophy) is the ancestral feeding mode for modern animals. Indeed, there is compelling fossil evidence that the earliest periods in animal evolution were influenced by interrelated environmental and ecological factors, such as increasing oxygen levels (Knoll and Sperling, 2014) and the evolution of predatory animals (Laflamme *et al.*, 2013). In fact, it has been proposed that the evolution of predation both preceded, and was the primary ecological driver for the diversification of modern animal body plans (Peterson and Butterfield, 2005).

Cells and Tissues

Animals are eukaryotes, and there is little deviation in their basic cellular ultrastructure compared to other eukaryotes. However, a feature common to animal cells that is worth mentioning as a possibly significant evolutionary innovation are the microvilli. Actin-based cellular protrusions are certainly not unique to animals, but a large diversity of animal cells have evolved to use patches of concentrated actin-based microvilli for myriad functions ranging from mechanosensation in vertebrate hair cells and on cnidarian tentacles, to increase surface area for absorption in the gut and kidney, as membrane reservoirs for cellularization of the embryonic epidermis in *Drosophila*, and to lubricate the mucosal epithelium of the vertebrate eye, to name only a few. Thus, it seems that actin-based microvilli represent an ancestral animal innovation that has been the subject of considerable evolutionary tinkering. However, actin-based microvilli are also found in choanoflagellates where they function to capture bacterial prey; as mentioned above, this feature is conserved in sponge choanocytes and possibly reflects the ancestral function for microvilli in the animal stem lineage. Moreover, it has been shown that the microvilli of choanoflagellates are similar in their molecular structure to filopodia (Sebe-Pedros *et al.*, 2013), which are more widespread throughout eukaryotes and are the likely evolutionary precursors to microvilli.

Figure 8 Carnivorous sponge. Demosponges of the family Cladorhizidae have secondarily lost the water canal system used by other sponges for feeding on bacteria. Instead, these sponges have independently evolved carnivory, whereby fine projections capture macroscopic prey which are immobilized and digested extracellularly.

In general, animals exhibit a huge diversity of cell/tissue types (e.g., muscles, nerves, etc.) and common ultrastructural features such as cell junctions (e.g., tight junctions, adherens junctions, etc. (Figure 9)) that are not found in nonanimals. However, the problem remains that it is difficult to generalize due to exceptions such as placozoans and sponges, which each lack some of these features, cnidarians which are argued to have independently evolved striated muscles (Steinmetz *et al.*, 2012), and ctenophores which have been argued to have independently evolved their nervous system (Moroz *et al.*, 2014).

Perhaps the only cell/tissue type that is ubiquitous among animals are epithelial tissues. Epithelia are thought to have been the first animal tissues to evolve (Tyler, 2003). However, there is still no structural or developmental evidence that the tissues of sponges are homologous to epithelia in other animals. They certainly have conserved homologs of the adhesion and polarity markers known from bilaterian epithelia (Fahey and Degnan, 2010; Nichols *et al.*, 2006, 2012), but it has not been experimentally determined how these proteins function in sponges.

A Genomic Perspective

The idea of using molecular characteristics of cells and tissues to distinguish animals from nonanimals is not new. One long-standing example is that an extracellular matrix composed of

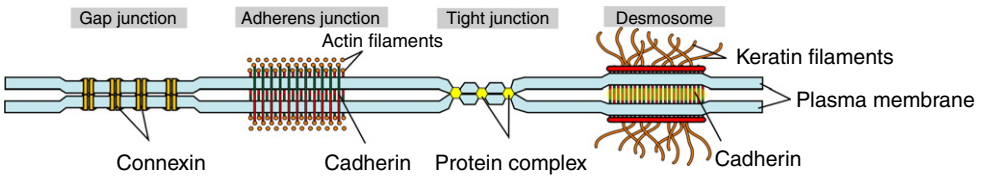

Figure 9 Cell junctions. The cells of animal epithelia have specialized contacts critical for their function. Gap junctions create pores that connect the cytoplasm of neighboring cells and are used for intercellular communication. Adherens junctions and desmosomes tether neighboring cells and mechanically integrate their cytoskeletons. Tight junctions pinch the membranes of neighboring cells together to restrict and transport of materials across tissues via the paracellular space. Each of these junctions is composed of a discrete, defined set of proteins.

collagen has long been considered to be diagnostic of animals (Exposito *et al.*, 2002; Garrone, 1999). However, the dramatic expansion of available genome/transcriptome/proteome scale data from animals and nonanimals in the past 10 years has set the stage for unprecedented molecular comparisons. One such comparison revealed that choanoflagellates have fairly typical eukaryotic mitochondrial genomes, which are larger and contain many genes that are absent in the reduced mitochondrial genomes of animals (Burger *et al.*, 2003). This serves as important corroboration for molecular phylogenetic analyses that support choanoflagellates as sister to animals, rather than being nested within animals (e.g., derived from sponges).

More recent genome/transcriptome sequencing efforts (far too vast to review here) have further revealed that genes required for basic multicellular processes in animals (e.g., developmental patterning, cell adhesion, tissue polarity, etc.) are highly conserved in all modern animals. Even genes that contribute to features such as the neuronal synapse are largely present in all animals, even those such as sponges and placozoans which lack neurons (Burkhardt *et al.*, 2014; Moroz *et al.*, 2014; Sakarya *et al.*, 2007; Srivastava *et al.*, 2008). Thus, there are certainly many genes and protein families that are so far known from all animals, and only from animals, and can therefore be considered to be diagnostic features of animals. However, it is worth noting that there are a number of exciting exceptions to the idea that genes involved in multicellularity are restricted to animals. Examples include the early discoveries that the genome of the unicellular choanoflagellate *Monosiga brevicollis* encodes more receptor tyrosine kinases than the human genome (King and Carroll, 2001; King *et al.*, 2008), and has as many as 23 cadherin-related genes (Abedin and King, 2008). However, these exceptions notwithstanding, the overwhelming pattern to emerge from comparative genomic studies is that all modern animals are united by many genomic features found nowhere else in the tree of life, suggesting that there was considerable genomic innovation in the animal stem lineage.

Extinct Animals and Future Directions of Animal Evolution

It is one thing to define animals based upon the characteristics shared by extant lineages, but things get more difficult when the fossil record is taken into account. The problem is not necessarily with tracing the ancestry of extant lineages; for the most part, most modern animals (at least those that preserve well) can be traced back to the Cambrian. Instead, the problem arises when we ask what life was like prior to the appearance of modern lineages. There has been considerable debate over the identity and interpretation of the so-called 'Ediacaran Biota' that existed just prior to the Cambrian (**Figure 10**). While it is beyond the scope of this article to review the diversity of, and debate about the Ediacaran Biota, it is worth noting that these were among the first multicellular, macroscopic organisms on earth. They existed for more than 60 million years before they suddenly disappeared and were replaced by more modern-like animals at the Cambrian/Pre-Cambrian boundary. One possibility is that members of the Ediacaran Biota represent early animal ancestors. Another possibility is that they represent early branches off the animal stem lineage. Finally, it is possible that they are multicellular eukaryotes with no special relationship to animals at all. Arguments can be made in favor of any one, or combination, of these possibilities. If these were indeed the first animals, then the animals of the past were considerably different from modern animals.

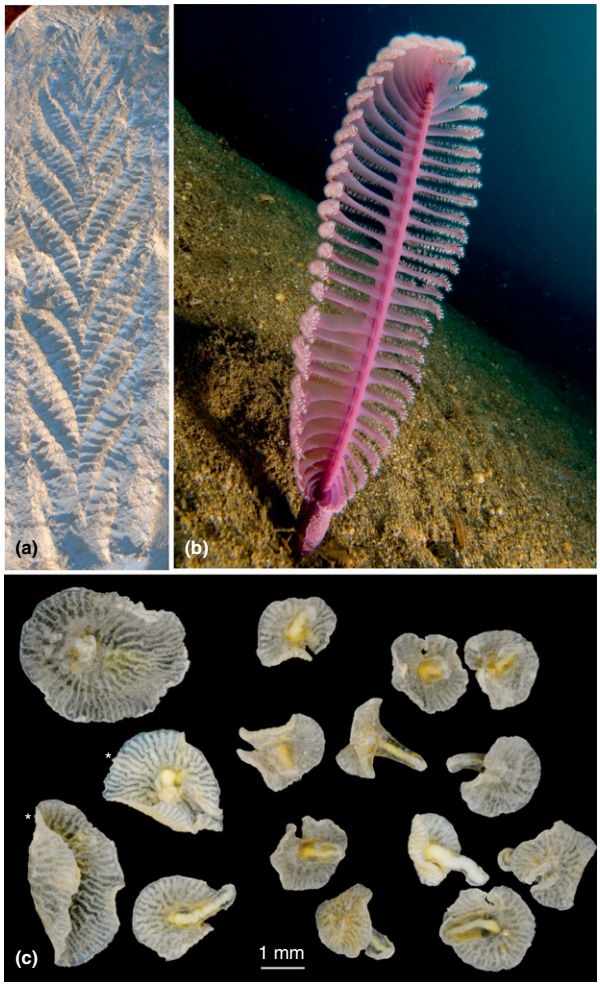

Figure 10 Ediacaran biota. Ediacaran organisms predate the appearance of animals in the fossil record. They may be stem-group animals, or they may represent an entirely independent experiment in multicellularity. Some, such as the members of the genus *Charnia* (a) have a frond-like appearance that resembles modern animals such as sea pens (b). However, the resemblance is believed to be superficial and does not reflect a direct evolutionary connection. Two recently discovered species of the genus *Dendrogramma* (c; Animalia, *insertae sedis*) also have been argued to share features with Ediacaran genera.

It is also worth considering whether any comprehensive definition of animals should be robust to future discoveries of anomalous animals, or even the future evolution of animal lineages away from their modern incarnations. There is still possibly room for the discovery of new, bizarre animals that further challenge our notions of modern animal diversity, albeit slight. One recent example is the recently described deep sea species, *Dendrogramma enigmatica* and *Dendrogramma discoides* (Just *et al.*, 2014; **Figure 10**). These species are intriguing

in that they superficially resemble cnidarians and ctenophores, although they lack diagnostic features of these lineages (cnidocytes and colloblasts). It has been argued that they share similarities with three Ediacaran genera, *Albumares*, *Anfesta*, and *Rugoconites*. At the time of this writing it is unknown if *Dendrogramma* has any evolutionary connection to non-bilaterian animals, much less to Ediacaran genera thought to be extinct for more than 500 million years. Nevertheless, this controversy highlights the potential for future discoveries of cryptic animals (particularly in the deep sea) to challenge our understanding of early animal diversity and evolution.

Finally, an even more controversial, but intriguing, possibility is that the emergence of sexually transmitted cancers may represent speciation events in which new unicellular animal species have evolved from vertebrate ancestors. One example is the canine transmissible venereal tumor (CTVT) (Dingli and Nowak, 2006). The tumor cells themselves are transmitted from one individual to the next during copulation and are not derived from the dog they infect. This tumor cell line has persisted as a self-maintaining, naturally occurring lineage for at least several hundred, and possibly several thousand years (Murgia *et al.*, 2006). Although controversial, CTVT can be argued to represent a novel, unicellular lineage derived from a canine ancestor: in short, a unicellular dog species. This provocative view can certainly be challenged, but it does at least open the door to imagining how future animals could depart dramatically from their modern ancestors. Thus, perhaps the most useful answer to the questions of 'what is an animal?' should not rely upon a checklist of traits, whether morphological, molecular, physiological, genomic, or otherwise. Instead, the only enduring definition may need to be phylogenetic in nature.

Phylogenetic Definition of Animals

In biology, there are seldom absolute answers to even the most basic questions. This is because biology is the result of a dynamic process, evolution, which is contingent upon past events that are often unknown. Moreover, evolution does not stop. Even as you read, evolution continues to 'tinker' with each and every living species, and the future trajectories of these species are entirely unknowable – there are simply too many variables, most subject to chance, to make such predictions. Thus, the challenge to define an animal (or plant, or fungus, etc.) in absolute terms, is fraught from the onset.

The only valid answer to the question, 'What is an animal?,' is that they are all organisms descendent from the last common ancestor of sponges, ctenophores, and mammals. The virtue of this definition of animals is that it includes living and extinct organisms, and it is immune from change. As we discover new lineages that challenge our current concepts of animal diversity, or if in the future animals evolve toward bizarre, unrecognizable forms, this definition holds true.

See also: Cambrian Explosion: A Molecular Paleobiological Overview. Metazoans, Origins of

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Antagonistic Interspecific Coevolution

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Glossary

Allopatric Organisms that do not coexist and that do not have a recent history of coexistence.
Arms Race coevolution Coevolution between pairs of biological antagonists driven by positive directional selection imposed upon each species by the other member of the pair. This type of coevolution can lead to the reciprocal acquisition of novel adaptations in the coevolving partners.
Directional selection Selection on more extreme values of a phenotype (e.g., toxicity and resistance to toxin). Directional selection can either be positive, whereby larger phenotype values have higher fitness, or negative, whereby smaller phenotype values have higher fitness. The former is considered to be the main source of selection underlying Arms Race coevolution.
Environment-allele frequency correlations Analytical approach to detect local adaptation that is based on identifying correlations between environmental variables and allele frequencies while correcting for population structure.
Frequency-dependent selection A form of natural selection driven by a relationship between the population frequency of a given phenotype or genotype and the fitness of this phenotype or genotype. Frequency-dependent selection can be positive, whereby fitness is a positive function of frequency, or negative, whereby fitness is a negative function of frequency. The latter is one of the main mechanisms driving host–parasite coevolution.
Genetic drift Nondeterministic shifts in allele frequencies.
Local adaptation A situation where native individuals possess higher relative fitness than individuals from other populations when measured in the native environment.
Outlier analysis Analytical approach aimed at identifying loci that show significantly distinctive values of population genetic statistics (e.g., Tajima's D, F_{ST}) as a means of, for example, detecting the loci likely to be involved in evolutionary responses to natural selection.
Population structure Nonrandom distribution of alleles across populations, typically detected as a significantly positive F_{ST}.
Red Queen coevolution Coevolutionary dynamics driven by negative frequency-dependent selection favoring rare phenotypes.
Resistance Host ability to reduce the fitness costs of parasitic infection and/or ability of the parasite to infect.
Sympatric Organisms that coexist within the same habitat and have a recent history of coexistence.
Virulence The extent to which a parasite reduces the fitness of its host. Higher fitness reductions translate into higher virulence.

What Is Antagonistic Coevolution?

All organisms face constant attack from biological enemies. Parasites and pathogens (hereafter, 'parasites', except when a particular pathogen species is being discussed) reallocate critical resources from their hosts, release toxins that cause serious host illness, or exploit host bodies for offspring production. Predators and parasitoids (hereafter, 'predators') take a more deadly approach, killing prey and hosts, respectively, to provide food for themselves or for their offspring.

As Darwin recognized in *The Origin of Species* (1859), interactions between species are likely to be a common driving force of evolutionary diversification, speciation, and adaptation. This idea can be taken one step further if we imagine that evolutionary responses in one species generated by selection imposed by a second species will generate new selection on the second species to adapt to the evolutionary change in the first species, a process of reciprocal selection and adaptation that could continue indefinitely. The evolution of reciprocal adaptations as a response to reciprocal selection, so-called 'coevolution' (originally defined by Erlich and Raven, 1964; see also Janzen, 1980), is increasingly recognized as a common and broadly important mechanism driving evolutionary change, adaptation, and diversification in nature (Erlich and Raven, 1964; Woolhouse *et al.*, 2002; Thompson, 2005; Laine, 2009).

The ubiquity of hostile interspecific interactions means that coevolution will often be a product of biological antagonism. Coevolution that is driven by antagonistic interactions, so-called 'antagonistic coevolution,' can be defined as reciprocal adaptation and counter-adaptation of two interacting species for which fitness is negatively correlated. In other words, an adaptation that increases fitness in one species will decrease fitness of the other species (Woolhouse *et al.*, 2002; Brockhurst and Koskella, 2013).

Besides the importance of understanding a common mechanism of evolution in natural populations, antagonistic coevolution is of special significance because of its perhaps unique potential to drive rapid diversification and speciation (Buckling and Rainey, 2002; Thompson, 2005; Paterson *et al.*, 2010; Schulte *et al.*, 2010; Karasov *et al.*, 2014) and the acquisition of novel adaptations (e.g., Hanifin *et al.*, 2008). Antagonistic coevolution is also thought to play a central role in the evolution of disease virulence (Ebert and Hamilton, 1996; Woolhouse *et al.*, 2002; Gómez and Buckling, 2011), mutation rates (Pal *et al.*, 2007), and sexual reproduction (Jaenike, 1978; Hamilton, 1980).

How Does Antagonistic Coevolution Work?

Theoretical and empirical studies of antagonistic coevolution have generally focused on two distinct mechanisms, one driven by negative frequency-dependent selection and the other by positive directional selection (Buckling and Rainey, 2002; Woolhouse *et al.*, 2002; Burdon *et al.*, 2013). These two mechanisms are expected to have fundamentally different influences on important evolutionary phenomena (e.g., the maintenance of genetic variation and sex) (Woolhouse *et al.*, 2002; Neiman and Koskella, 2009; Brown and Tellier, 2011; Hall *et al.*, 2011). Here, we make the distinctions between these two mechanisms clear. We also summarize many of the important distinctions (or lack thereof) between these mechanisms in **Table 1**.

Negative Frequency-Dependent Selection: Red Queen Coevolution

Frequency-dependent selection is defined as a situation where fitness is dependent upon the frequency of a phenotype or genotype in a population. Our focus here is on negative frequency-dependent selection, whereby fitness of a phenotype or genotype increases as its frequency in a population decreases. Negative frequency-dependent selection is believed to be a primary driver of coevolution between biological antagonists (e.g., Hori, 1993; Neiman and Koskella, 2009; Burdon *et al.*, 2013).

The idea that selection favoring rare genotypes or phenotypes might drive antagonistic coevolution began with J. B. S. Haldane (1949). In 1949, biologists were just beginning to discover that many species featured surprisingly high levels of genetic variation for 'resistance' to various diseases – diversity that should quickly be lost if only one or a few phenotypes or genotypes consistently conferred resistance. Haldane (1949) suggested that the maintenance of this diversity could be explained by a situation where parasites disproportionately attack common host types – simply because the parasites have adapted to exploit a common host resource. Here, a mutation that conferred a rare 'biochemical phenotype' (Haldane's words) could confer parasite resistance because parasites have not yet had an opportunity to adapt to this rare phenotype. Because rare hosts are disproportionately uninfected by parasites, they have high fitness relative to the heavily infected common hosts and, as such, do not stay rare for very long.

Table 1 Comparison between the characteristics and signatures of Arms Race and Red Queen coevolution[a]

	Arms Race	*Red Queen*
a. General properties		
Type of selection	Positive directional	Negative frequency-dependent
Fate of selected variation	Selected variants often fix; other variants lost	Cycles
Potential to generate evolutionary innovation	Relatively high	Relatively low
b. Virulence/resistance phenotypes		
Virulence/resistance is inherent property of genotype	Yes	No
Systematic increase in virulence/resistance over time	Yes[b]	No
Universal virulence/resistance	Yes	No
Tradeoff or cost associated with virulence/resistance	Often	Sometimes
c. Molecular signatures		
Local maintenance of polymorphism	No	Yes
Trans-species polymorphism	No	Yes
Linkage disequilibrium associated with selected locus	High relative to mean genome linkage disequilibrium	No clear expectations
F_{ST} of selected locus (in the absence of overall population structure for either member of the coevolving pair)	Low relative to neutral loci	Sometimes low relative to neutral loci
F_{ST} of selected locus (with the presence of overall population structure for either member of the coevolving pair)	High relative to neutral loci	Sometimes low relative to neutral loci
Tajima's D (in the absence of overall population structure for either member of the coevolving pair)	Low relative to neutral loci	High relative to neutral loci
Tajima's D (in the presence of overall population structure for either member of the coevolving pair)	High relative to neutral loci	High relative to neutral loci
Coalescence time of selected locus (in the absence of overall population structure for either member of the coevolving pair)	Short relative to neutral loci	Long relative to neutral loci
Coalescence time of selected locus (with the presence of overall population structure for either member of the coevolving pair)	Long relative to neutral loci	Long relative to neutral loci
d_N/d_S	Elevated relative to neutral loci	Often elevated relative to neutral loci

[a]All cases invoking presence/absence of population structure assume that analyses are across all populations.
[b]Rate of increase of virulence/resistance may slow as costs increase and/or as genetic variation is lost (e.g., Hall *et al.*, 2011).

In parallel, the parasites reduce the fitness of common hosts to the extent that the common hosts are soon made rare, the timing of which will be determined by the degree to which parasite adaptation lags behind the host (Dybdahl and Lively, 1998). The implications are that host rareness could provide a profound fitness advantage that would dissipate as parasites adapted to the formerly unfamiliar host genotype, preventing common host genotypes from fixing and avoiding the loss of rare host genotypes (**Figure 1(a)**). These ideas were further developed by biologists such as Donald Levin (Levin, 1975), Bryan Clarke (Clarke, 1976), John Jaenike (Jaenike, 1978), and William Hamilton (Hamilton, 1980; Hamilton *et al.*, 1990).

Negative frequency-dependent selection is considered by many evolutionary biologists to be a particularly important and interesting form of natural selection because, unlike directional and stabilizing selection, negative frequency-dependent selection favors rare genotypes and can thus maintain high levels of genetic diversity. In contrast to Arms Race models (see below), coevolutionary models like the Red Queen that are based exclusively on negative frequency-dependent

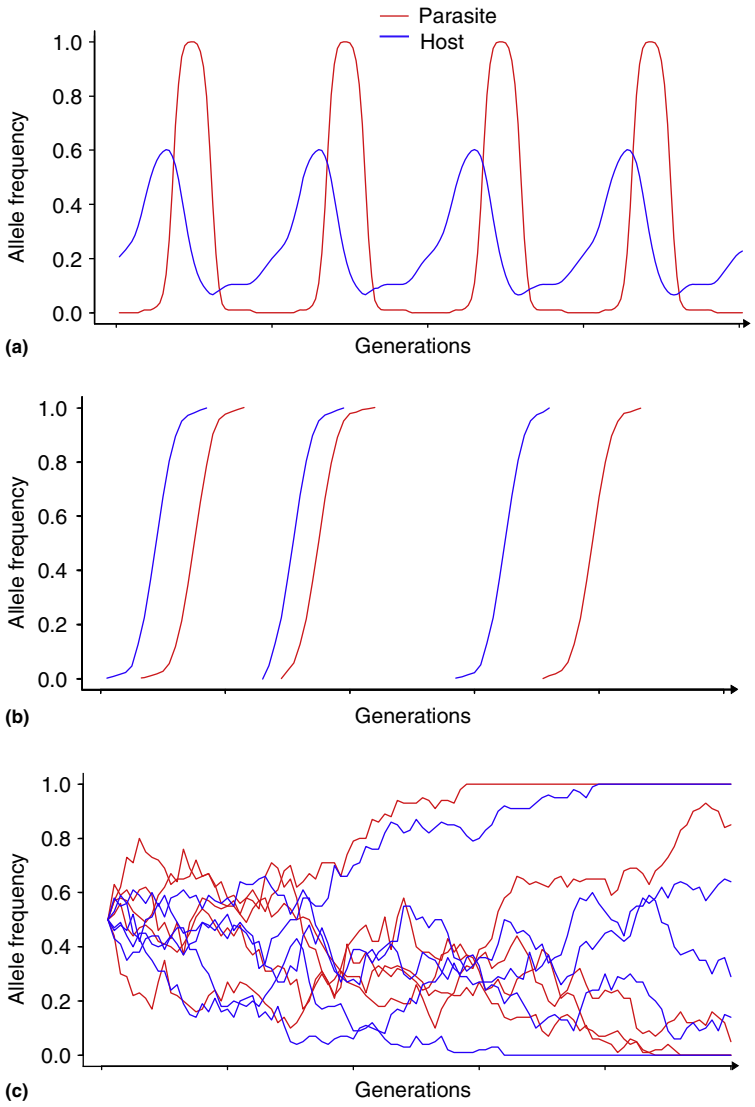

Figure 1 Changes in allele frequencies of loci responsible for coevolutionary dynamics of host (blue lines) and parasite (red lines) under different theoretical models. (a) After Lively (1999), a Red Queen dynamic describes the cyclical, time-lagged increase in the frequency of the parasite allele that infects the common host followed by a decrease of frequency of this parasite allele as the target host allele becomes less common, (b) an Arms Race dynamic wherein the fixation of alleles occur in series in a reciprocal, escalating manner as the coevolutionary process takes place, and (c) shifts in allele frequency resulting from neutral genetic drift, with each line a separate iteration of the same stochastic process. Panels (a) and (b) are adapted from Woolhouse, M.E., Webster, J.P., Domingo, E., Charlesworth, B., Levin, B.R., 2002. Biological and biomedical implications of the coevolution of pathogens and their hosts. Nature Genetics 32, 569–577 and Neiman, M., Koskella, B., 2009. Sex and the Red Queen. In: Schön, I., Martens, K., van Dijk, P. (Eds.), Lost Sex. Amsterdam: Springer, pp. 133–159, with permission from Macmillan Publishers Ltd., © 2002. Panel (c) is the result of the repeated simulation of a population of 50 diploid individuals for 100 generations. Each simulated population was initiated with equal frequencies of two alleles, with individuals randomly selected to reproduce and contribute to the next generation based upon a binomial distribution.

selection rely on standing genetic variation. As a consequence, these negative frequency dependence-based models do not spur the evolution of novel functions (Ebert, 2008).

Positive Directional Selection: Arms Race Coevolution

Under directional selection, relative fitness increases as the value of a trait increases (positive directional selection) or decreases (negative directional selection). Dawkins and Krebs (1979) argued that reciprocal positive directional selection exerted by coevolving hosts and parasites could lead to a situation where hosts continually become more resistant to parasitism while parasites respond by becoming more virulent or evolving new mechanisms of evading host immunity.

Unlike negative frequency-dependent selection, this so-called 'Arms Race coevolution' does not generate a rare advantage per se. Instead, host resistance and parasite 'virulence' are inherent properties of the individual genotype and do not depend on the frequency of the other genotypes. In this scenario, repeated selective sweeps favoring resistant hosts and virulent parasites will lead to the evolution of reciprocal host and parasite adaptations that, once overcome by a counter-adaptation, will not again incur resistance/virulence (**Figure 1(b)**; Woolhouse *et al.*, 2002; Burdon *et al.*, 2013). Another important distinction between Red Queen and Arms Race coevolution is that the latter, in favoring traits that counter adaptations in the biological antagonist, has a high potential for evolutionary innovation (e.g., Kerns *et al.*, 2008; reviewed in Daugherty and Malik, 2012).

A Critical Role for Population Structure

Spatial 'population structure' is both ubiquitous in nature (Slatkin, 1987; Jones and Wang, 2012) and is expected to be one of the major determinants of the presence, extent, and trajectory of coevolution (Gibert *et al.*, 2013). With respect to antagonistic coevolution, the existence of population structure can generate conditions in which there is opposing selection at the individual and group levels and in which the outcomes of coevolution are different than when population structure is absent. For example, theory suggests that with population structure, groups of parasites that are more damaging to their hosts tend to go extinct while groups of parasites that exploit their hosts in a more 'benevolent' way tend to persist (O'Keefe and Antonovics, 2002; Eshelman *et al.*, 2010; Wade *et al.*, 2010). This prediction is in stark contrast to the expectations of antagonistic coevolution between hosts and parasites in the absence of population structure, which is instead expected to culminate in the evolution of relatively high virulence (Eshelman *et al.*, 2010).

A good empirical example of how population structure can affect antagonistic coevolution is provided by Kerr *et al.* (2006) and Eshelman *et al.* (2010), who showed that when coexisting populations of the bacterial host *Escherichia coli* and the viral pathogen T4 coliphage are relatively isolated (migration happens only between neighboring populations or from a randomly selected population within a larger metapopulation), host–pathogen species are more likely to coexist. By contrast, under panmictic or well-mixed conditions (all populations from the full metapopulation are fully mixed and reallocated as individual populations), 'rapacious,' or more virulent, strains of pathogen tended to evolve, eventually resulting in extinction of both host and pathogen.

An additional consequence of spatial structure is an asynchrony in coadaptedness, wherein a subset of a metapopulation that has, for example, been more exposed to an antagonist, has had an opportunity to progress further in the coevolutionary process. In turn, asynchrony in coadaptation can lead to differences in the rates of colonization and/or extinction of host and pathogen due to trade-offs associated with host resistance, wherein more susceptible hosts are likely to colonize new sites (Thrall and Burdon, 1997, 2002; Laine *et al.*, 2011). This particular consequence of population genetic structure is particularly important because by generating variation in host and parasite phenotypes, asynchrony in coadaptation can facilitate the generation and maintenance of genetic and phenotypic diversity (Burdon *et al.*, 2013).

Finally, the presence of population structure and small population size, through the effects of genetic drift, may lead to a scenario in which random changes in allele frequencies in one or both interactants of a coevolving pair can lead to maladaptation (**Figure 1(c)**; Thompson *et al.*, 2002).

How Is Antagonistic Coevolution Detected?

While antagonistic coevolution is likely to be a common feature of natural populations, studying coevolutionary dynamics is not straightforward. Evolutionary biologists have risen to the occasion, developing approaches to study coevolution that either sidestep or directly address these methodological challenges. Here, we present several of the most important established methodologies as well as some newer genomic-based approaches.

The principal method to detect ongoing coevolution of any type is based on controlled experimental exposures of sympatric versus allopatric interactants (e.g., host exposure to sympatric parasites versus host exposure to allopatric parasites). Assuming antagonistic coevolution will result in a process of reciprocal adaptation between sympatric antagonists, direct exposure of allopatric interactants is predicted to result in a different and potentially maladaptive response relative to exposure of sympatric interactants ('phenotypic mismatch'). In the case of predator–prey Arms Race coevolution, phenotypic mismatch may result in an (at least temporary) escape for one of the interactants (Hanifin *et al.*, 2008). Similarly, in the case of Red Queen coevolution, hosts that are resistant to the most frequent sympatric parasite strain may be susceptible to a similarly frequent strain from an allopatric population. Depending upon the genetic basis of resistance/virulence (e.g., gene-for-gene or matching allele models; Luijckx *et al.*, 2013), the degree of asynchrony of coevolutionary processes (which will be driven by variation in population structure), and the spatial scale at which selection takes place (Vale, 2013), some parasite strains may even be incapable of infecting allopatric hosts (Antonovics *et al.*, 2011).

Coevolutionary processes will take place within a spatially structured context (Thompson, 2005), with 'local adaptation'

an expected outcome. As such, many newer methods that have been developed to study local adaptation can also be applied to the study of coevolutionary processes (see also **Table 1**). One such example is 'outlier analysis,' an increasingly common approach focused on the identification of polymorphisms that are enriched within some populations and that can be directly linked to particular biological processes related to local adaptation. While outlier methodology has seen only limited application to detecting coevolution (but see Orsini *et al.*, 2013), a related, more recently derived method, so-called 'environment-allele frequency correlations' (Coop *et al.*, 2010; Günther and Coop, 2013), is emerging as a more effective indirect strategy to determine the genetic basis of adaptation. Fumagalli *et al.* (2011) used this approach to suggest that interactions with parasites have been a principal selective force leading to shifts in allele frequencies amongst human populations experiencing different environments.

It is still unclear whether outlier analysis and environment-allele frequency correlations will provide broadly effective means of identifying the extent and type of coevolution. Because these methods typically rely on molecular summaries gleaned from samples taken from a single time point, they are also limited in their ability to pinpoint the exact genomic regions associated with a given coevolutionary process, which by definition occurs over multiple generations (see Decaestecker *et al.*, 2007, and so-called 'resurrection ecology'). This limitation is particularly clear in the case of Red Queen dynamics: while fluctuating selection will maintain multiple alleles over many generations, in any single generation and any particular population there exists positive selection for individual alleles, each of which may be subject to a different selective regime in different populations. In such cases, distinguishing the type and magnitude of coevolution will be difficult (Tiffin and Moeller, 2006).

Methods based on evaluation of patterns of polymorphism in a (co-)phylogenetic context can present some level of improvement relative or in addition to outlier analysis and environment-allele frequency correlations, providing insight into the presence, extent, and type of coevolution. Under this particular framework, researchers evaluate whether the phylogenetic patterns of the interacting species are concordant (have similar topologies), as expected under coevolution. Because a number of other processes may also generate phylogenetic concordance, the detection of concordance comprises only the first step toward identifying and characterizing coevolution (Althoff *et al.*, 2014). More recently derived comparative phylogenetic tools can provide more information regarding coevolutionary mechanisms and represent a relatively powerful means of evaluating hypotheses relating to the causes of diversification. Specifically, by linking phylogenetic and trait data for a given set of species, one can determine whether coevolutionary dynamics are causal factors in phylogenetic concordance (FitzJohn, 2012; Althoff *et al.*, 2014). Insight into the type of coevolutionary dynamics can come from these approaches as well. For example, Arms Race coevolution is expected to increase diversification rates, as was shown by Smith and Benkman (2007) in the crossbill/lodge pole pine system, while Red Queen dynamics are less likely to provide the sustained source of directional selection that can lead to diversification.

An exciting new approach that could potentially resolve a number of outstanding questions regarding coevolutionary processes and that is just beginning to see wide application is the combined use of experimental coevolution and re-sequencing. Here, experimental populations of species that comprise a potential coevolutionary system are established within a controlled environment. The genomes of each species are sequenced to establish a preexperiment baseline. Next, replicated populations of sympatric and allopatric interactants are exposed to one another for multiple generations in order to allow coevolution to take place. Following this exposure period, the populations of each interactant are re-sequenced in order to identify derived single nucleotide polymorphisms and/or highly diverged genomic regions relative to the initial parental populations (Eshelman *et al.*, 2010; Baldwin-Brown *et al.*, 2014). Comparisons of patterns of molecular evolution in the genomes of the two species before and after experimental manipulation allows evaluation of the magnitude of effect that a particular coevolutionary dynamic has on particular genomic regions, potentially distinguishing (for instance) the role of Arms Race versus Red Queen coevolution. Enrichment of particular alleles across populations relative to the pre-coevolution baseline is a predicted consequence of Red Queen coevolution, where the particular allele increasing in the population of one of the species in the coevolving pair will depend upon the most common allele in the second species. In other words, for Red Queen coevolution, the initial allelic distribution in the coevolving pair will determine the relative increase and/or decrease for the loci responsible for the coevolving dynamic (Thompson, 2005). By contrast, Arms Race coevolution is expected to result in fixation of particular alleles, potentially in series, as each reciprocal adaptation continues to escalate the coevolutionary arms race (**Figure 1(b)**). Confirmation of these predictions will require careful experimental design as well as a detailed understanding of the ecological dynamics of each species.

Recent theoretical work has also suggested that, depending upon the type of coevolutionary process taking place, one or the other interactant may actually be more effective for detecting the type and magnitude of coevolution. For example, Tellier *et al.* (2014) use a simulation approach to find that the genomic signature of Arms Race coevolution can be effectively determined in both interactants through the use of Tajima's D. By contrast, the typical signature of Red Queen, balancing selection, is often undetectable in the host species but apparent in the parasite.

Evidence for Antagonistic Interspecific Coevolution in Nature

Here, we provide brief summaries of two exemplar cases of Red Queen and Arms Race coevolution that have been documented in natural populations. We also discuss some new evidence that both types of coevolution can occur in series or even simultaneously.

Red Queen Coevolution: Snail and Trematode

Potamopyrgus antipodarum, a New Zealand freshwater snail, is often infected by sterilizing trematode parasites

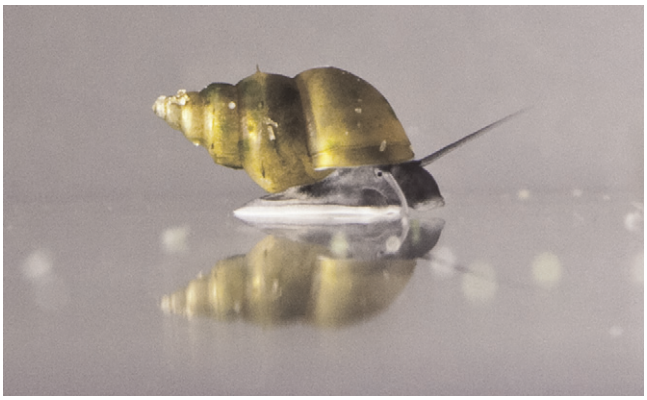

Figure 2 Adult female *Potamopyrgus antipodarum* (Photo Credit: Bart Zijlstra).

(Winterbourn, 1973). The possibility that these trematodes, and particularly *Microphallus* sp., contribute to the maintenance of sexual versus asexual reproduction in *P. antipodarum* (e.g., Lively, 1987; King *et al.*, 2009) has inspired multiple empirical studies aimed at addressing whether the interactions between snail and trematode fit those expected under a scenario where negative frequency-dependent selection driven by disproportionate infection of common host genotypes (i.e., Red Queen coevolution) helps favor sexual reproduction. Proof of Red Queen coevolution has several key requirements, including demonstration of advantages to rare alleles, tight genetic specificity for infection/resistance, and a time lag between increase in frequency of a (formerly) rare host genotype and disproportionately high infection of this now common host genotype (reviewed in Neiman and Koskella, 2009). Direct or indirect empirical support exists for all of the necessary components of Red Queen coevolution in the *P. antipodarum–Microphallus* system (e.g., Dybdahl and Lively, 1998; Dybdahl and Krist, 2004; Lively *et al.*, 2004; Jokela *et al.*, 2009), providing a very convincing example of antagonistic coevolution driven by negative frequency-dependent selection in natural populations (**Figure 2**).

Arms Race Coevolution: Newt and Garter Snake

Newts of the genus *Taricha* are a major prey of multiple species of garter snakes of the genus *Thamnophis*. *Taricha* newts generate a neurotoxin called tetrodotoxin (TTX) that has evolved independently in a number of *Taricha* taxa (Brodie, 2009, 2010). TTX is stored in the skin and acts as a chemical defense to most predators. In turn, the garter snakes have evolved resistance to the TTX produced by the newts, generating one of the best-characterized examples of coevolutionary arms race dynamics (Castoe *et al.*, 2011). The intensity of this arms race varies over the shared range of the snakes and newts. In some locations, the resident newt population can produce a much higher skin concentration of the TTX and the garter snake population is capable of resisting much higher TTX concentrations than in other parts of the range, generating what is sometimes referred to as a geographic mosaic of coevolutionary interactions (Thompson, 2005). Importantly, there is no known instance of a newt reaching concentrations of TTX that the local garter snake cannot overcome. The absence of

Figure 3 *Thamnophis sirtalis* consuming *Taricha granulosa* (Image Credit: Edmund Brodie III).

universal newt toxicity suggests a rare, and probably temporary, Arms Race escape for the garter snakes (Hanifin *et al.*, 2008; McGlothlin *et al.*, 2013). While the exact genetic basis of variation in TTX concentration is unknown, the genetic basis of variation in TTX resistance has been localized to functional amino acid substitutions in the skeletal muscle sodium channel (NA_v 1.4), though other parts of the genome (potentially other sodium channels) may contribute as well (Feldman *et al.*, 2010; McGlothlin *et al.*, 2013). Greater understanding of the genetic mechanisms underlying TTX resistance generated an additional line of evidence that clearly marks the newt–garter snake coevolutionary system as an Arms Race: there are multiple, independently derived alleles responsible for adaptation to increased TTX that now exist at high frequency within garter snake populations. By contrast, under Red Queen coevolution, alleles for generalized resistance to TTX would not exist. Instead, resistance would be connected to whichever particular resistance alleles happened to be recently or presently rare (Feldman *et al.*, 2010, 2012; **Figure 3**).

Simultaneous and Sequential Red Queen and Arms Race Coevolution: *Daphnia*, Bacterium-Microsporidian, and Bacterium-Phage

While we have focused primarily on pairs of distinct interactants, coevolutionary processes might involve more than two

taxa (Noguchi and Arakawa, 2008; Koskella, 2013; Ryder *et al.*, 2014), which themselves may act on the same or different components of individual genomes. Once such example is provided by the coevolutionary interactions between the cladoceran water flea *Daphnia magna* and two of its parasites, the sterilizing bacterium *Pasteuria ramosa* and the microsporidian *Hamiltosporidium tvaerminnensis*. Routtu and Ebert (2015) used a quantitative trait locus (QTL) approach to both uncover distinct genetic architectures for resistance to the two pathogens and describe evidence that the coevolutionary dynamic taking place between *D. magna* and *P. ramosa* is best characterized by Red Queen dynamics, while that of *D. magna* and *H. tvaerminnensis* instead fits the framework of Arms Race coevolution.

There is also evidence that these two distinct types of coevolution can occur sequentially within a pair of antagonists. For example, Hall *et al.* (2011) show that in coevolving populations of the bacterium *Pseudomonas fluorescens* and pathogenic phage SBW25Φ2, initial Arms Race dynamics are succeeded by Red Queen-like fluctuating selection, likely resulting from costs associated with host resistance. While identification of sequential coevolutionary dynamics will often be rendered difficult if not impossible due to the necessity of observation of the initial coevolutionary interactions, a powerful exception may be provided by coevolution in the context of biological invasions (Burdon *et al.*, 2013). Here, coevolutionary associations can arise across relatively recent and short time scales allowing biologists a powerful means of understanding how incipient coevolutionary processes arise. An especially important potential consequence of connections between biological invasions and coevolution is provided by the possibility that naïve interactants, especially a naïve host, may be unable to adapt to a novel parasite. The implications are that the host, and, eventually, the (now hostless) parasite, may go extinct in the new range, with potentially disastrous consequences for native taxa. As such, understanding the sequence of events that take place during the emergence of a new coevolutionary system and how Red Queen and Arms Race dynamics affect the maintenance of biological diversity is of primary importance to conservation efforts (Thompson, 2005).

Outstanding Questions and Future Directions

The increasingly widespread availability of inexpensive ways to generate large amounts of sequence data means that we are on the verge of addressing important questions like the extent to which antagonistic coevolution drives evolution in nature, which mechanisms of antagonistic coevolution are the most common, whether antagonistic coevolution is likely to be a main contributor to the maintenance of sexual reproduction, the genetic/genomic basis of coevolution, and how coevolution is affected by the presence of more than two interacting partners. The applicability of these methods to characterizing the nature of coevolution of hosts with their microbiota and of microbiotal components presents an especially exciting angle for future research. Identifying and discriminating between particular coevolutionary mechanisms remains a challenge. As such, future research efforts should include some focus on the continuing development of analytical methods that can be applied effectively to coevolving systems.

See also: Coevolution, Introduction to. Intraspecific Coevolutionary Arms Races. Predation and Parasitism. Secondary Metabolites, the Role in Plant Diversification of

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Archaeplastida: Diversification of Red Algae and the Green Plant Lineage

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Glossary

Gametophyte The haploid life cycle stage that is the conspicuous stage in most green algae and in bryophytes; called gametophyte because it produces haploid gametes through mitosis.

Glaucophytes They are relatively rare unicellular protists possessing primary plastids with chlorophyll a and have several similarities to free-living cyanobacteria.

Oogamy Sexual reproduction involving a large nonmotile egg and a smaller swimming sperm.

Peptidoglycan Chemical making up the cell wall of cyanobacteria and certain other bacteria.

Phragmoplast A type of cell division found in charophyte algae and land plants, with a distinct arrangement of microtubules and mode of cell-plate formation; this mode of cell division is thought to enable persistent intercelllular cytoplasmic connections called plasmodesmata.

Phycobilisomes Small round cellular structures on the photosynthetic membranes of cyanobacteria and in the plastids of glaucophytes and red algae.

Plasmodesmata Microscopic tube-like connections that extend across the walls between plant cells that maintain cytoplasmic continuity throughout the plant.

Pollen tube The tube that grows from a pollen grain that has landed on the receptive surface of the female portion of a flower; the tube carries the sperm cells to the egg inside the flower without them being exposed to the outside world.

Primary endosymbiosis The acquisition in the ancient past of a prokaryotic cell into a eukaryotic host cell to become an organelle; for Archaeplastids, cyanobacteria were engulfed by a eukaryotic host cell and transformed into a primary plastid.

Secondary endosymbiosis The acquisition of a eukaryotic red or green alga into a host cell to produce a secondary plastid.

Sporophyte The diploid life cycle stage in land plants and certain algae; in vascular plants this is a large, conspicuous stage, whereas in bryophytes it is a less conspicuous stage that grows on the haploid gametophyte; called a sporophyte because it produces haploid spores through meiosis.

Thallus A general term for the body of an alga.

Introduction

The Archaeplastida are a large and diverse group of photosynthetic organisms that include all of the green land plants that we are familiar with, as well as the red and green seaweeds and freshwater algae, plus a small group of rare and relatively obscure freshwater single-celled protists called glaucophytes (Figure 1). These three main groups of Archaeplastida diverged from a common ancestor at least 1.5 billion years ago, or perhaps much earlier (Butterfield *et al.*, 1990).

Evolutionary Origins

The Archaeplastida are the product of an ancient evolutionary partnership of a single-celled eukaryotic protist with a prokaryotic oxygen-producing photosynthetic bacterium (Delwiche, 1999). This well-accepted endosymbiosis hypothesis is that a eukaryote, already possessing a nucleus and mitochondria, engulfed a free-living cyanobacterium, which then became permanently incorporated as a plastid within the protist (Figure 2). (Mitochondria are also believed to have been acquired through an endosymbiotic acquisition of a nonphotosynthetic bacterium.) This merger of a cyanobacterium with a eukaryotic host cell resulted in a green algal-like cell that could harness sunlight to produce sugars through photosynthesis. Cyanobacteria, which had much earlier evolved the innovation of capturing sunlight to manufacture carbon-containing sugars, thus became permanent intracellular boarders, or organelles called plastids, whose sugars fueled the now photosynthetic host protist. This process, known as endosymbiosis, set the stage for the evolution of hundreds of thousands of species of algae and land plants, from single-celled phytoplankton to giant redwoods, that are found in nearly every habitat on Earth.

Although protists, red seaweeds, green algae, and plants are very different in appearance, the evidence of their evolutionary kinship is clear (Figure 3). Their cell structure and physiology exhibit a number of unique similarities, including the light capturing green pigment chlorophyll a in the plastid, inherited from the ancestral cyanobacterium. Two membrane layers surround each plastid, one a membrane from the engulfing host cell and the second inner membrane a vestige of the original cell wall of the cyanobacterium. Additional evidence of their shared ancestry is the arrangement of membranes within their mitochondria into flat layers, or cristae, and the production of starch as the primary storage product of photosynthesis.

In recent decades, studies of DNA in the Archaeplastida have reinforced the hypothesis that these three major lineages are evolutionarily related. DNA exists in three compartments in the cell: the nucleus where most of it is stored, and in separate clusters of organellar DNA inside the mitochondria and plastids, each of which contains a handful of genes that are essential to their function. These compartments are the nuclear genome, mitochondrial genome, and plastid genome, and though separate in the cell they are interdependent in helping the cell function. The relatively small amount of DNA

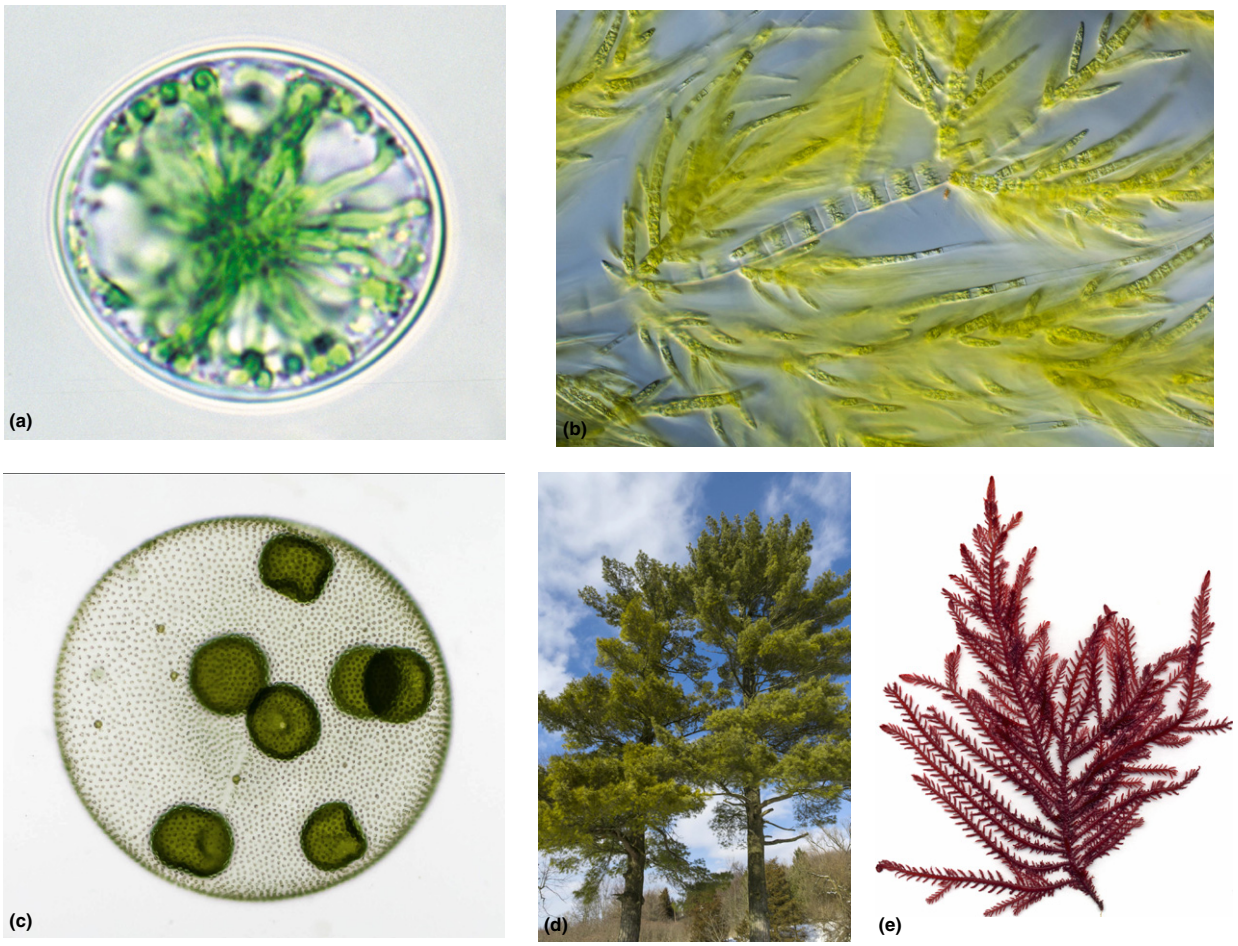

Figure 1 Unicellular glaucophyte, *Glaucocystis* (a); branching filamentous green alga, *Draparnaldia* (b); colonial green alga, *Volvox* (c); typical large land plant (pine tree) (d); red alga or seaweed, *Delisea* (e). All photographs copyright of L.W. Wilcox.

in mitochondria and plastids is a result of a large transfer of the genes they had when free-living into the nuclear genome, a process that occurred over the long period of time since their divergence from a common ancestor. This downsizing of these organellar genomes means the three DNA compartments in a cell form an integrated and interdependent evolutionary unit. Analysis of DNA sequences of many genes reveals a deep, underlying kinship among the many species of red and green algae and green plants and strongly supports the endosymbiosis hypothesis, which was proposed long before DNA was even discovered (Delwiche, 1999).

Morphology

The morphology of Archaeplastida exhibits a remarkable range of thallus or plant body types. The simplest are single cells for their entire existence (**Figure 1(a)**), while others such as many green algae form long chains of cells in linear strands or with sparse to profuse numbers of branches (**Figure 1(b)**). Such thalli are very common among the green algae and glaucophytes. More complex thalli are found in red and green algae, which form large colonies such as *Volvox* (**Figure 1(c)**), or three-dimensional tissues, internal architecture for transporting water and organic materials, and multicellular reproductive structures. The most complex plant bodies are found in the land plants, including trees, flowers, and shrubs, and among them are the largest living organisms on the planet.

The Glaucophyta (**Figure 1(a)**), the smallest group (about a dozen species in total) in the Archaeplastida, are unicellular, some swimming by means of flagella and others nonmotile. The plastids contain chlorophyll a only and have a peptidoglycan layer that is considered an evolutionary remnant of the walls of cyanobacteria made of this substance. Glaucophyte plastids have photosynthetic membranes studded with microscopic round structures called phycobilisomes, also found in cyanobacteria and red algae, that capture light outside the wavelengths absorbed by chlorophyll and channel the energy captured to the chlorophyll molecule. A close look at the plastids reveals a metallic blue-green hue. Once considered a variety of green algae, glaucophytes are now thought to be a distinct, albeit small, lineage in the Archaeplastida (Rodríguez-Ezpeleta *et al.*, 2007).

The red algae, or Rhodophyta, are also a distinct lineage of Archaeplastida, with about 6000 to 10 000 species, and probably many more to be discovered. They range in size from tiny unicells and microscopic filaments to larger leafy or bushy

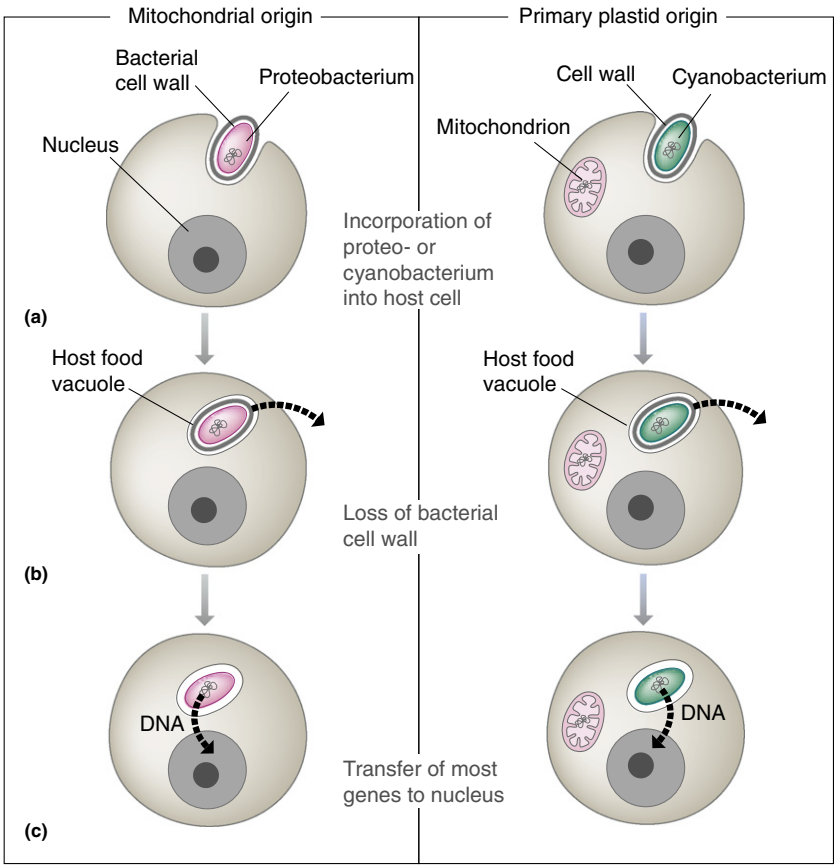

Figure 2 Endosymbiotic hypothesis for origins of mitochondria and plastids. A nucleated cell is hypothesized to have engulfed a proteobacterium (left) to produce a nonphotosynthetic protist; this then engulfed a cyanobacterium to form a photosynthetic eukaryotic cell. Reproduced from Figure 20.17 in Graham, L.E., Graham, J.M., Wilcox, L.W., 2015. Plant Biology, third ed. Madison, WI: LJLM Press.

seaweeds about the size of a small fern (**Figure 1(e)**). Most species of red algae are multicellular leafy seaweeds, although the unicellular forms and filamentous varieties are intensely studied because of their occurrence in extremely hot, acid habitats, such as the unicelluar *Cyanidium* (Yoon *et al.*, 2006), and their ancient fossil records (fossils of *Bangia* 1.3 billion years old, Butterfield *et al.*, 1990). The construction of the red algal thallus is basically filamentous, that is, even the large frondlike or gelatinous straplike species have thalli composed of microscopic filaments compacted and arranged into these larger structures. Red algae have a series of stages in their life cycle with varying numbers of chromosomes and complexity apparently correlated with more sets of chromosomes. The life cycle is complex for the most diverse group of red algae known as Florideophytes, but interestingly no red algae are known to produce cells with flagella, so the reproductive spores and gametes do not swim. Thus, though red algae are relatively large and complex, not to mention beautiful, they are not as diverse in species or habitats as green algae and plants.

Green algae (**Figures 1(b)** and **1(c)**) and land plants (**Figure 1(d)**) make up the third lineage, which is the largest in terms of number of species and organism size, as well as the most familiar to us, since the most diverse lineage of green plants surrounds us on land. There are two large groups of green algae. One is the Chlorophyta, or green algae sensu stricto, which includes most freshwater green algae, plus all of the green seaweeds. It is likely that there are many undiscovered species in this group, especially in the form of tiny unicellular species. The other main group is called the Streptophyta (Bremer, 1985), or Charophyta (Karol *et al.*, 2001), and it comprises several smaller groups of freshwater algae plus the huge evolutionary branch that we call land plants. Another term for land plants is embryophytes, because multicelluar embryos develop from the diploid zygote. This is in contrast to Chlorophyta and Charophyta, in which the single-celled zygote undergoes meiosis and produces a haploid life stage.

Scientists were surprised to find that not all green algae are each other's closest relatives. In fact, the Charophyta are actually more closely related to land plants than they are to the Chlorophyta and share a number of unique features with land plants. Technically speaking, this means the green algae do not form a monophyletic group. This surprising finding was initially discovered through studies of the internal structure of cells and how they divide (Pickett-Heaps, 1975), and it was later corroborated by analysis of DNA sequences (Karol *et al.*, 2001; McCourt *et al.*, 2004; Leliaert *et al.*, 2012). Therefore we now hypothesize that land plants descended from a small group of green algae about 450 million years ago (Lewis and

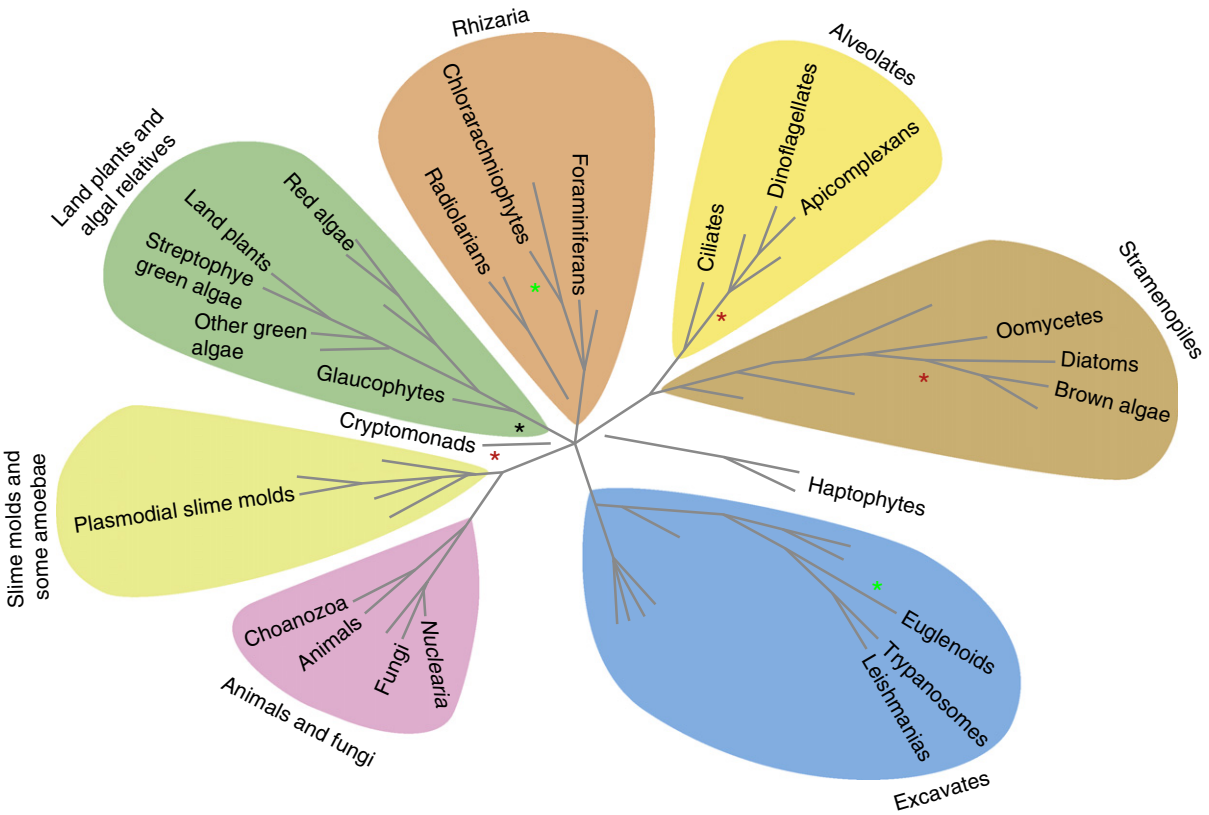

Figure 3 Evolutionary tree of eukaryotes showing the relationship of Archaeplastida to other photosynthetic organisms. The Archaeplastida are shown outlined in green. Shown on the tree with asterisks are branches on which occurred the endosymbiotic events described in the text. The black asterisk shows the acquisition of cyanobacteria in primary endosymbiosis. The red asterisks indicate three independent acquisitions of a red alga in secondary endosymbiosis (Chlorarachniophytes and Euglenoids) (there may have been more than three, Delwiche, 1999). The light green asterisks show independent acquisitions of green algae in secondary endosymbiosis. Modified from Figure 20.8 in Graham, L.E., Graham, J.M., Wilcox, L.W., 2015. Plant Biology, third ed. Madison, WI: LJLM Press.

McCourt, 2004; Leliaert *et al.*, 2012) (**Figure 3**), and this single lineage adapted to a dry existence and diversified into over 400 000 species of plants.

The Archaeplastida are indirectly responsible for even more of the photosynthetic life on Earth than just the three lineages mentioned above. This is because the Archaeplastida lineage is the source of plastids in several other important groups of algae, including diatoms, dinoflagellates, and brown algae (Delwiche, 1999). The plastids in these groups were acquired through endosymbiosis, but instead of taking in prokaryotic cyanobacteria, called primary endosymbiosis, host cells engulfed eukaryotic red or green algae in several independent lines of evolution, in a process called secondary endosymbiosis (**Figure 3**). Among these, the diatoms and dinoflagellates are very important primary producers in the sea, and are responsible for much of the oxygen we breathe.

Fossil Record

The fossil record of Archaeplastida is quite ancient. Fossil filaments very similar to living marine red alga *Bangia* (Butterfield *et al.*, 1990) have been found in rocks possibly older than 1.2 billion years. The finding of recognizable red algal filaments in rocks this much old means that the three main groups of Archaeplastida diverged much earlier. However, the delicate and fragile nature of most unicells and filaments means that they are less commonly fossilized than land plants (Stewart, 1993), and the age of the oldest Archaeplastida remains yet to be determined.

Habitats and Diversity

The three major lineages differ in their species diversity and modern day habitats. Very few glaucophytes are known, and they are all unicellular freshwater organisms. Red algae generally are larger, complex multicellular organisms, and more than 10 000 species are known to science. Most are found in salt water, and they make up the predominant plant life on some shorelines. A few species of red algae have successfully adapted to freshwater streams, and one group of red algae incorporate calcium carbonate into their thalli and are a major component of coral reefs in the tropics. The green algae and plants share a more recent common ancestor with each other than with the other Archaeplastida groups, and they are by far the most diverse of the three main lineages. More than 12 000 species of green algae are known (Lewis and McCourt, 2004),

found in both freshwater and marine ecosystems, with a significant but smaller number living in aerial habitats on land.

From molecular and fossil evidence, it is clear that multicellular algae evolved several times from unicellular ancestors. These independent origins of larger, complex algae led to a diversity in thallus types, from fleshy tubular marine algae, to branching freshwater species with the form of tiny trees, to colonial microscopic forms with thousands of component unicells (**Figure 1(c)**).

The Archaeplastida exhibit both asexual (vegetative) and sexual reproduction, although sex is apparently absent for many species. Asexual propagation of fragments of thalli or special asexual spores that may swim or lie dormant is common. Sexual reproduction involves gametes of two sexes or mating types. In many green algae the gametes are morphologically identical cells that swim by means of flagella. In some cases, one swimming gamete is larger, and in others one gamete is much larger and nonswimming (i.e., an egg) and the other much smaller and motile (i.e., the sperm). The latter process is called oogamy and is the universal pattern of sexual reproduction in the land plants that evolved from green algal-like ancestors. In the case of the most common land plants we encounter, conifers and flowering plants, the gametes are contained throughout the sexual process entirely within or closely associated with the tissue of the parent plant, i.e., within the flower or pollen grain or reproductive structures produced in the cone. The sperm lacks flagella (with a few exceptions, such as ginkgos and cycads), although the sperm cells do move through a pollen tube to the larger egg inside the female plant body. The trend towards dimorphic gametes that never leave the parental flower, pollen tube, or reproductive structures produced in the cone of the parent plant probably was important for the success and diversification of land plants through the provision of nutrients and materials from the parent plant to the large egg, the zygote, and the growing embryo.

Green Plant Origins and Diversification

Why only one green algal line of evolution produced the highly successful and diverse land plants is a particularly intriguing question. One hypothesis is that a shift in the reproductive life cycle allowed for greater complexity of form (Graham, 1993). The closest green algal relatives of land plants live in freshwater and for most of their life cycle, these algae are haploid. After sexual reproduction, a single-celled diploid zygote is produced, but a series of reduction divisions apportions the chromosomes into daughter cells such that they are all haploid. In the evolution of land plants, this single-celled zygote appears to have delayed these reduction divisions while a series of mitotic divisions occurred. The result was an intercalated multicellular diploid life cycle stage, a key part of the land plant life cycle. In the most complex land plants, this diploid stage, called a sporophyte, is the conspicuous plant that we are familiar with – woody trees, colorful and elaborate flowering plants, large fern fronds. The haploid phase, called the gametophyte, is often small and inconspicuous in land plants. In the most extreme cases, the haploid form is reduced to a growth stage of just a few cells that remain inside the parent sporophyte (e.g., inside flowers or pollen). One hypothesis is that the evolution of a plant stage with two sets of chromosomes released a flurry of genetic and morphological complexity in evolution and was a major factor in producing the diversity we see in land plants.

Another hypothesis on why the Charophyta were the green algae that gave rise to land plants is that during mitosis they produce a structure known as phragmoplast. This structure, which provides a scaffold for the new cell wall, leaves small channels that cross the wall between the new daughter cells. The phragmoplast is thought to have played a role in the evolution of size and complexity in land plants by allowing the movement of cellular material (proteins, hormones) from cell to cell, which produced a greater integration of plant form.

The evolution of desiccation resistant spores, first found in the Archaeplastid fossil record in the Charophyta green algal relatives of land plants, probably played an important role in enabling algae and their land plant descendants to survive periodic dry conditions at the margins of ponds. Such spores were likely present in the most ancient photosynthetic organisms that we would have recognized as land plants, which were probably similar to today's bryophytes, also known as mosses and liverworts (Graham, 1993). Bryophytes are often found in moist terrestrial habitats, and they lack some of the elaborate structures that are thought to have played a role in land plant diversification. For example, mosses have a small diploid sporophyte stage that grows upon and depends upon the large green mossy carpets made up of the haploid stage in the moss life cycle. Mosses also lack true vascular tissues that move water and sugars between roots and leaves, and only simpler tiny leaf-like and root-like rhizoids are found in bryophytes. Nevertheless, mosses are very successful, even in the driest habitats, although their diversity pales in comparison to that of true vascular plants.

Summary

The Archaeplastida are one of the major evolutionary lineages of photosynthetic organisms, and arguably the most important for animals, including humans, because the ancestors of one group (green algae and plants) was able to invade land and set the stage for the evolutionary movement onto land of many animal groups. Their great importance in the world's ecosystems and to human health and welfare are difficult to exaggerate. The three lineages of Archaeplastida contain most of the large algae, seaweeds, and plants that support life on Earth, and life as we know it would not be possible without Archaeplastida.

See also: Protist Diversification. Seedless Land Plants, Evolution and Diversification of

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Artificial Selection

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Glossary

Additive genetic variance The component of the phenotypic variance that causes offspring to resemble their parents.
Covariance A measure of the lack of independence between the values of two traits.
Effective population size (N_e) The size of an idealized population that would experience the same magnitude of genetic drift as the population of interest.
G-matrix The set of additive genetic variances (on the diagonal) and covariances for a group of traits.
Genetic correlation The standardized genetic covariance between a pair of traits.
Genetic covariance The component of covariation between two traits caused by variation at shared gene loci (pleiotropy) or pairs of genes (linkage disequilibrium).
Linkage disequilibrium A nonrandom relationship between the alleles present at two or more loci, which can cause a genetic correlation.
Narrow sense heritability A standardized measure of the degree of resemblance between offspring and their parents, defined as the additive genetic variance divided by the total phenotypic variance.
Pleiotropy It occurs when one locus affects more than one phenotypic trait, contributing to a genetic correlation.
Random genetic drift Fluctuations in allele frequency that occur by chance, particularly in small subpopulations, as a result of random sampling error in the gametes that form the next generation.
Response to selection The change in trait mean across one generation.
Selection differential A measure of the strength of directional selection equal to the mean of the selected group minus the mean of the entire population, or the covariance between relative fitness and trait values.

Introduction

Artificial selection is the process by which humans choose individual organisms with certain phenotypic trait values for breeding. If there is additive genetic variance for the selected trait, it will respond to the selection, that is, the trait will evolve. All of our domesticated species, including crop plants, livestock, and pets, are the products of artificial selection for desirable traits, such as seeds and fruits that do not disperse readily, increased meat and milk production, and docile behavior. The earliest artificial selection may have been unconscious, but it developed into a sophisticated science of plant and animal breeding; indeed, much of the field of quantitative genetics was developed to improve breeding programs.

The importance of artificial selection to the field of evolutionary biology dates back to Darwin, who was likely the first to use the term artificial selection in the '*Origin of Species*' (Darwin, 1859). Darwin used the obvious evolutionary results of domesticated species to show the power of selective breeding as an analogy to natural selection. One of the earliest uses of experimental artificial selection to address evolutionary questions was by Holtorp (1944). He selectively bred *Brassica* plants that produced an extra cotyledon and reported an increase in frequency of plants with three and even four cotyledons in subsequent generations. Similarly, Huether (1968) was able to increase and decrease the number of corolla lobes in *Linanthus* through five generations of artificial selection. These early studies established that even traits that are conserved at higher taxonomic levels could evolve.

Artificial selection differs from what has been called laboratory natural selection (Rose *et al.*, 1990) or controlled natural selection (Conner, 2003). In artificial selection the experimenter chooses specific phenotypic traits to select upon, while in controlled natural selection an environmental factor is manipulated and evolution of the populations in response to this selective agent is monitored. While artificial selection is certainly a form of experimental evolution, often the meaning of the term 'experimental evolution' is confined to controlled natural selection, excluding artificial selection (e.g., Kawecki *et al.*, 2012). Because artificial selection applies a known strength and direction of selection to specific phenotypic traits, it is one of the most powerful methods available for understanding the underlying genetic variation and thus evolvability of those traits; in controlled natural selection the strength and direction of selection cannot be determined by the investigator.

Fundamental Concepts

The rate of adaptive phenotypic evolution depends on the strength of natural selection and the amount of additive genetic variation for the trait in the population. This principle is encapsulated in the breeder's equation:

$$R = h^2 S \qquad [1]$$

in which R is the response to selection, defined as the evolutionary change in the trait mean across one generation, h^2 is the heritability, defined as the proportion of the total phenotypic variance due to additive genetic variance, and S is the selection differential, which measures the strength of selection. This form of the equation is useful for evolutionary biologists,

because it allows the prediction of short-term phenotypic evolution (R) from knowledge of the heritability and strength of selection. Additive variance is crucial, particularly in sexual species, because without additive variance a trait will not evolve; if the heritability is zero, so is R in the breeder's equation. The primary utility of artificial selection to evolutionary studies is that it provides the most powerful means of testing for presence of additive genetic variance for a trait. In artificial selection the selection differential is imposed experimentally and the response is measured, yielding an estimate of heritability for the selected trait, as shown by a rearrangement of the breeder's equation:

$$h^2 = R/S \qquad [2]$$

This is called the realized heritability (**Figure 1**).

A serious shortcoming of the breeder's equation for evolutionary studies is that it is univariate, that is, it relates to only a single trait. However, traits commonly covary genetically, which means they share gene loci in common (called pleiotropy) or that allele frequencies at loci that independently affect the two traits are correlated (called linkage disequilibrium; see Conner and Hartl, 2004 Chapter 5 for a fuller explanation). Genetic covariance is commonly standardized as the genetic correlation, just as additive variance is commonly standardized as the heritability. Genetic variances and covariances for a set of traits are expressed in matrix form, called the G-matrix; analogous to the univariate breeder's equation, the rate of evolution of a set of traits is the product of the G-matrix and the strength of direct selection on each of the traits (Conner and Hartl, 2004). If there are traits that covary genetically with a trait under selection, then those traits will also evolve, a phenomenon termed correlated responses to selection. Thus, artificial selection is an excellent way to test for genetic covariance between the selected trait(s) and other traits, in an analogous manner to how additive variance is tested, that is, by measuring phenotypic changes in other traits that were not subjected to artificial selection.

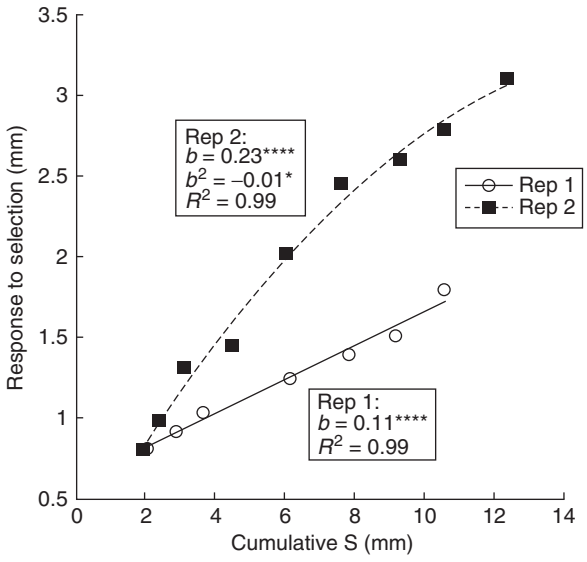

Figure 1 Realized heritability of anther exertion in wild radish, estimated as the slope (b) of the regression of response to selection (R) on the cumulative selection differential (S). Here the two replicates show quite different realized heritabilities, with Replicate 1 less than half that of Replicate 2. Reprinted from Conner, J.K., Karoly, K., Stewart, C., *et al.*, 2011. Rapid independent trait evolution despite a strong pleiotropic genetic correlation. American Naturalist 178, 429–441. © 2011 the University of Chicago.

Basic Design Considerations

Careful attention to design is important for producing a convincing outcome of any experiment, and artificial selection is no exception. Only some highlights of experimental design will be addressed here, as excellent detailed discussions are available (e.g., Bohren, 1975; Hill, 1980; Hill and Caballero, 1992). The main issue is to be able to definitively attribute the observed phenotypic response to the selection treatment, as opposed to random genetic drift or differences in the environment across generations. The latter can never be eliminated, even in a growth chamber. First, selection lines need to be replicated; because artificial selection can be time-consuming, often there are only two replicates of each selection treatment, but replication is necessary to separate the effects of selection from random genetic drift.

It is also ideal to include the same number of randomly mated (that is, unselected) control lines, which directly estimate phenotypic change due to drift and environmental differences combined; with replicated controls these two nonselective factors can be distinguished. However, selection can be separated from drift and the environment without control lines in the usual case where a trait is selected in opposite directions in separate replicated selection lines. This is because the response to selection can be tested between the high and low lines; if pairs of high and low lines are grown interspersed randomly in the same environment, the high–low phenotypic difference is due to selection, not the environment, and parallel high–low differences across replicate pairs are most likely due to selection rather than drift. In general, all the lines within one replicate (e.g., high, low, control) should be grown and measured together, so that average phenotypic differences among them are not due to environmental differences.

Besides replication to separate drift from selection, it is important to minimize drift by maximizing the effective population size (N_e) within each selection line, both to increase the precision of estimates of additive variance and also to minimize loss of fitness in multigeneration experiments. Thus, the design of an artificial selection experiment is a trade-off between the number of replicates, control lines, and the effective population size within each line. If the number of organisms that can be maintained is limited, then each set of lines within one replicate (e.g., high, low, and control) can be raised together, but separated in time from other replicates. The trade-off with this procedure is that each generation will take longer.

Another way to increase N_e without increasing the number of organisms is to perform within-family selection rather than mass selection. In mass selection, the most extreme individuals in each line are selected regardless of whether they are related to each other; if the trait is heritable, then families with alleles

for high or low values of the trait will be overrepresented. In within-family selection, a number of offspring of each family are raised and measured, and the same number (often one) of most extreme individuals within each family is selected for the next generation. This maximizes N_e; in fact, if one individual is chosen per family, and each selected individual has equal numbers of offspring in the next generation, then N_e is double the actual number of individuals selected. The main drawback of within-family selection is that the strength of selection will be weaker than with mass selection.

Weaknesses of Artificial Selection

The more common methods of testing for additive genetic variance and covariance use a breeding design to create known genetic relationships among organisms (parents and offspring, siblings, etc.), or estimating these relationships (i.e., the pedigree) in a natural population using genetic markers, in both cases without selection. Here the disadvantages of artificial selection compared to the more common methods are briefly discussed; the advantages will be made clear under 'Applications in Evolutionary Biology with Examples' section.

Perhaps the biggest disadvantage of artificial selection is that it requires fairly large numbers of individuals to be measured and mated, so this approach is not feasible for many, if not most, species. The traditional quantitative genetic breeding designs also require this, but molecular genetic markers make pedigree analysis possible for some wild populations, and this approach has been used in a few wild mammal and bird populations (e.g., Reale *et al.*, 2003; Kruuk *et al.*, 2000; Charmantier *et al.*, 2008). An advantage of any quantitative genetic study done in the field, pedigree-based or not, is that the G-matrix can be very dependent on the environment due to changes in the expression of additive genetic variances and covariances (e.g., Conner *et al.*, 2003). Artificial selection can in principle be conducted in nature especially in plants or birds in nest-box populations (Postma *et al.*, 2007), but this has rarely been done and for most organisms it will not be feasible.

Another disadvantage of artificial selection is that the number of traits that can be selected is very limited, usually to one or two at a time (but see Hine *et al.*, 2014). In a breeding or pedigree study the full G-matrix for as many traits as can be measured can be estimated. In artificial selection the additive variance for the trait selected upon can be estimated, as well as the genetic covariances between the selected trait and any others that are measured after selection, but the variances of, and covariances between, any traits that were not directly selected upon cannot be estimated. Thus, artificial selection is best reserved for cases where a particular trait is of interest, and to questions other than the full G-matrix (see Applications in Evolutionary Biology with Examples).

Applications in Evolutionary Biology with Examples

Evolutionary Constraints

As noted above, artificial selection is the most powerful test for additive genetic variation and covariation. This is because it is based on comparisons of means across generations rather than estimating variances and covariances from a breeding design or pedigree; means can be estimated with more precision than variances and covariances. In addition, artificial selection can be conducted over a number of generations, so that very small amounts of genetic variance and covariance can be detected as the response to selection accumulates across generations. This is very important in evaluating whether a lack of evolution, that is, an absolute evolutionary constraint, is caused by a lack of additive variance, because a lack of response to several generations of strong selection is more convincing evidence of constraint than a variance estimate that is not statistically different from zero. The pioneering artificial selection experiments of Holtorp (1944) and Huether (1968) were able to demonstrate additive variance for traits that were highly conserved, showing that this evolutionary conservation is not due to a lack of variance for those traits.

Most artificial selection experiments produce a significant response, suggesting that a lack of additive genetic variance rarely constrains evolutionary change. This is even true in most of a handful of studies that have selected perpendicular to the major axis of a genetic correlation, that is, in the direction of minimum variance in bivariate space (**Figure 2**; reviewed in Conner *et al.*, 2011). However, some have argued that a fully multivariate approach to constraints is needed, and that for groups of traits there will be directions in multivariate space (typically estimated as eigenvectors) that will lack variance (Blows and Hoffmann, 2005; Walsh and Blows, 2009). To test this hypothesis, Hine *et al.* (2014) applied artificial selection along all eight eigenvectors of eight cuticular hydrocarbons in *Drosophila serrata*; these hydrocarbons have been shown to be involved in mate choice. Significant responses to selection were found for all eight eigenvectors, and for the five eigenvectors that explained the most variance this response was significant for all three replicates. However, for the three eigenvectors that exhibited the least variance, the response was significant for only one or two of the three replicates.

Despite the fact that most artificial selection studies report a statistically significant response to selection, a few studies have not (e.g., Baer and Travis, 2000; Allen *et al.*, 2008; Dorn and Mitchell-Olds, 1991; Hoffmann *et al.*, 2003; Hall *et al.*, 2004). In, perhaps, the most convincing demonstration of a lack of additive variance in a wild species, Hoffmann *et al.* (2003) found no statistically significant response to 28 generations of selection for increased dessication resistance in a rainforest fly (*Drosophila burchii*; **Figure 3**), suggesting that adaptation to drier conditions predicted with global warming would be severely constrained. This study is convincing because the large number of generations of selection increases the ability to detect small responses in each generation, and because there was also no response in crosses among the different selected lines, making inbreeding depression caused by drift an unlikely explanation for the lack of response. However, there was an 11% increase in dessication resistance in two of the three selection lines; while this difference is not statistically significant, the presence of additive genetic variance in the population cannot be ruled out, illustrating the general difficulty in proving that something is absent.

Another important and less common role for artificial selection is investigating the short-term evolutionary effects of

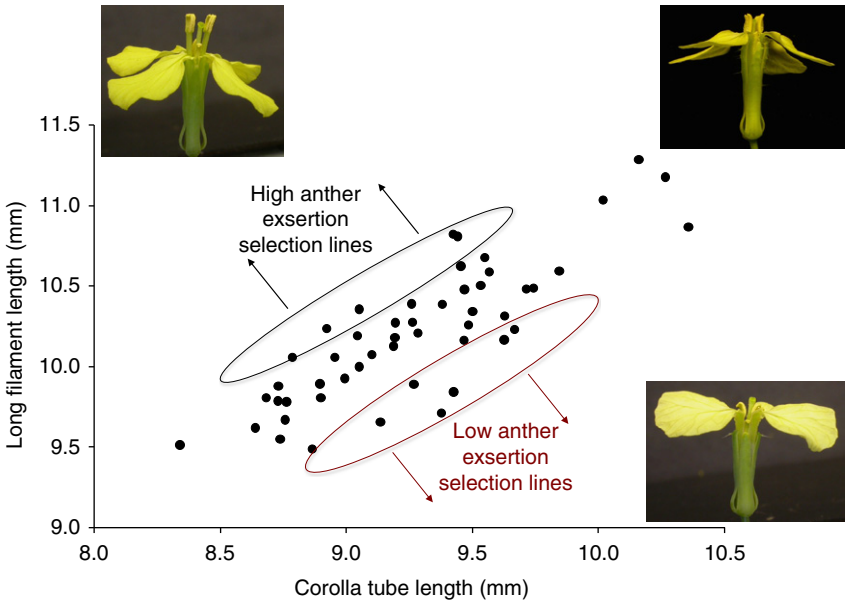

Figure 2 Graphical depiction of selection perpendicular to the major axis of variation for two traits. The genetic correlation of 0.85 between filament and corolla tube in wild radish flowers is depicted by the points in the graph. Anther exsertion is a composite trait defined as filament minus corolla tube, which describes how far the anthers protrude from the corolla tube. The picture at upper right shows a plant from the natural population with zero exsertion, with the bottom of the anthers at the opening of the corolla tube. Each set of arrows shows the direction of selection for one set of lines; in the high exsertion lines the individuals selected have long filaments relative to their corolla tubes (points enclosed by the black ellipse) while in the low lines individuals selected have long tubes relative to their filaments (red ellipse). The two sets of lines are separate, that is, high line individuals are mated to each other, not with low line individuals. Thus, this is directional selection in two directions on the composite trait of anther exsertion. Examples of flowers after each direction of artificial selection are shown in the upper left and lower right corners. Redrawn from Conner, J.K., Karoly, K., Stewart, C., *et al.*, 2011. Rapid independent trait evolution despite a strong pleiotropic genetic correlation. American Naturalist 178, 429–441.

selection on the genome. A major unresolved issue in evolutionary biology is the stability of the G-matrix across generations (reviewed in Arnold *et al.*, 2008); if the G-matrix is not stable, it is less likely to cause evolutionary constraint. Theory predicts that directional selection on a trait or combination of traits, which is what is almost always conducted in artificial selection studies, will eventually deplete additive genetic variance for that trait, which then constrains further response to that selection. This is one cause of what is called an evolutionary limit or plateau, that is, when a response to artificial selection ceases; however, a plateau can also be caused by natural selection in the opposite direction to the artificial selection, without a depletion of variance (**Figure 4**). Conner *et al.* (2011) showed that the selection depicted in **Figure 2** produced no consistent change in the G-matrix for these two floral traits in wild radish. Delph *et al.* (2011) conducted a different form of selection specifically designed to change the G-matrix. The negative correlational selection imposed significantly reduced the genetic correlation in flower size between male and female plants in two of three replicates. This selection was similar to that depicted in **Figure 2**, except that the high and low selected plants were mated to each other rather than mated within each group.

Understanding the Genetic Basis of Phenotypic Evolution

The examples discussed above are all of the most common use for artificial selection in evolutionary biology, that is, as a powerful method for detecting the presence of additive genetic variation for a trait or group of traits. Artificial selection can also be combined with quantitative trait locus (QTL) mapping to help identify gene loci responsible for genetic variation within a natural population, where adaptive evolution occurs. QTL designs generally use crosses between genetically and phenotypically divergent populations, which can be created from a single natural population by artificial selection. Chromosomal regions identified by a QTL analysis of a cross between these lines are those responsible for the response to artificial selection, and must have had allelic variation for loci affecting the trait in the natural base population. This approach was used to discover 26 QTLs for bristle number in *Drosophila melanogaster*, and 20 of these included likely candidate genes for this trait (Long *et al.*, 1995; Gurganus *et al.*, 1999; Nuzhdin *et al.*, 1999).

If there is already a strong candidate gene for a given trait, then allele frequency changes can be tracked across generations of artificial selection on that trait. For example, *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)* are loci affecting flowering time in the model plant *Arabidopsis thaliana*. Scarcelli and Kover (2009) selected for early flowering under growth chamber conditions simulating fall or spring germination, both of which occur naturally in this species. A large decrease in flowering time occurred in both growth conditions, and a concomitant change in *FRI* allele frequency also occurred, but only under spring germination conditions. *FLC* allele frequencies did not change significantly under either condition.

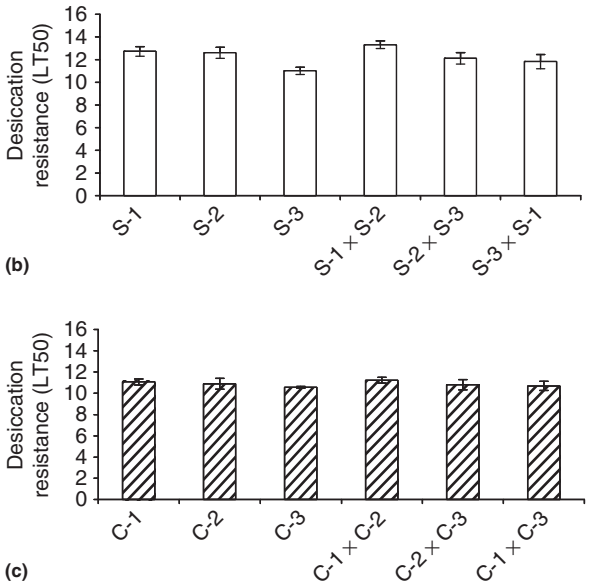

Figure 3 Lack of significant response to 28 generations of artificial selection for increased dessication tolerance. LT50 is the number of hours it took for 50% of the flies to die in the absence of water. Panel (b) shows the means for the three selection lines and all possible crosses between them, and (c) shows the same results for control lines. S-1 and S-2 show a higher dessication resistance than controls, but the overall difference between selected and control lines was not statistically significant. Reprinted with permission from Hoffmann, A. A., Hallas, R.J., Dean, J.A., Schiffer, M., 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science 301, 100–102.

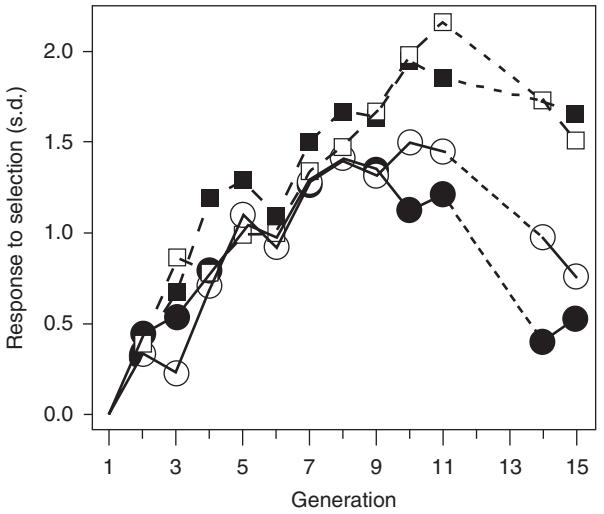

Figure 4 Reversal of response to artificial selection due to natural selection. Cuticular hydrocarbons in *Drosophila serrata* were artificially selected using two different treatments (circles and squares) with two replicates of each. The response to selection reached a plateau at generation 7 in the treatment denoted by circles, and all lines evolved back toward the means in the base population after the artificial selection was stopped ('relaxed'; dashed lines). This indicates that the artificial selection moved the populations away from their laboratory optima, and that the plateau was not caused by a depletion of additive genetic variance for the trait. Reprinted with permission from Hine, E., Mcguigan, K., Blows, M.W., 2011. Natural selection stops the evolution of male attractiveness. Proceedings of the National Academy of Sciences of the United States of America 108, 3659–3664.

Thus, variation in loci other than *FRI* and *FLC* were responsible for the phenotypic change under fall germination conditions, demonstrating the important point that expression of additive genetic variation for any trait (flowering time in this example) can depend on the environment (e.g., Conner *et al.*, 2003); this is one type of genotype–environment interaction. Thus, in combination with molecular genetic techniques, artificial selection can be used not only to demonstrate the presence of additive genetic variance, but also to understand what gene loci are responsible for this variance, and how this depends on the environment.

Understanding Natural Selection

The discussion and examples above make clear that artificial selection has most often been used to understand additive genetic variation, which is appropriate based on eqn [2]. By itself, artificial selection does not provide information about natural selection, but it can be used to create experimental populations with altered phenotypic means and variance for additional studies of adaptation and natural selection. If a trait is an adaptation, then we expect that the mean value for that trait will be near the optimum in natural populations due to past selection, assuming that the population is near equilibrium. Thus, artificial selection on an adaptive trait should move it away from this optimum, increasing the strength of natural selection to return the trait to the optimum. Laboratory artificial selection studies have produced many examples of selected traits that evolve back toward their original mean values when artificial selection is stopped (or 'relaxed'; Figure 4); this is good evidence that the trait means were moved away from their optimum in the lab environment (Falconer and Mackay, 1996). This same approach could be applied to study natural selection in the wild, by placing organisms that have been artificially selected for a putatively adaptive trait in the field and following their phenotypic evolution across multiple generations. This approach is most practical in sessile organisms like plants.

The approach above requires observing a response to natural selection over several generations. Another way to use the new phenotypic distributions created by artificial selection to understand natural selection is to combine divergent selection lines into a single experimental population that as a result has increased variance in the selected trait. This population can then be placed in the field and natural selection acting on the trait can be estimated as the slope of the regression of fitness on the trait (Lande and Arnold, 1983). It has long been recognized that studies of hypothesized adaptive traits are hampered by a lack of variance, because if a trait is adaptive, past directional or stabilizing selection will have removed the unfit variants from the population. Thus, the increased variance in the experimental population can increase the power of measurements of natural selection (Schluter, 1988; Haller and Hendry, 2014). This approach was used to show that different pollinators selected on different floral traits in wild radish (Sahli and Conner, 2011).

Summary and Future Directions

As this article has stressed, artificial selection is the most powerful way to test for additive genetic variance for a phenotypic trait, which in turn means that it is an ideal way to determine if a trait can evolve in response to selection. Molecular genetic techniques by themselves will not be able to accomplish this goal until the loci responsible for most of the additive variance in the trait are known, and it is known how each locus contributes to additive variance. However, as discussed above, the combination of artificial selection with molecular genetic techniques can greatly enhance our understanding of the mechanisms underlying genetic variance thus trait evolution, so more studies of this type are clearly needed.

A major challenge for future work is the environmental dependence of additive genetic variance for traits, evidenced by direct measurements of additive variance (e.g., Conner *et al.*, 2003 and references therein) as well as the environmental dependence of QTL (see the discussion of Scarcelli and Kover, 2009 above; Ungerer *et al.*, 2003). Ideally, artificial selection could be conducted in the field (Postma *et al.*, 2007), but this will prove very difficult for most organisms. A more feasible approach is to test the fitness and trait values of populations created by artificial selection in the field (e.g., Sahli and Conner, 2011; Galloway and Burgess, 2012; Kristensen *et al.*, 2007), but this does not test for the presence of additive genetic variance in the field environment.

One exciting future direction for the use of artificial selection in evolutionary biology would be to follow both phenotypic and genetic changes during artificial selection, with the latter done through whole-genome sequencing. This is an extension and combination of some of the approaches described above, enabling an unprecedented understanding of the molecular genetic underpinnings of a phenotypic response to selection. The resulting lines could then be placed in the natural population that the selection lines were derived from and tracked phenotypically and genetically for several generations. The prediction is that both phenotypic means and allele frequencies should evolve back toward those found in the natural population before artificial selection began, but interesting surprises seem likely.

Acknowledgments

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See also: Evolution and Agriculture II. Evolutionary Applications to Breeding. Evolvability, Quantitative Genetics of. Genotype-by-Environment Interaction. Multivariate Quantitative Genetics. Natural Selection, Measuring. Quantitative Genetics in Natural Populations. Quantitative Genetic Variation. Quantitative Genetic Variation, Comparing Patterns of

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B

Bacterial Diversity, Introduction to

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> Life on earth is such a good story you cannot afford to miss the beginning... Beneath our superficial differences we are all of us walking communities of bacteria. The world shimmers, a pointillist landscape made of tiny living beings.
>
> Margulis and Sagan (1986)

> What you see is that the most outstanding feature of life's history is a constant domination by bacteria. This is the age of bacteria. The bacterial mode, the mode being the most common form of life, is never altered in 3 1/2 billion years. We don't see it that way because bacteria are tiny and they live beneath us, but bacteria live in a wider range of environments.
>
> S.J. Gould (2006)

Introduction

Humans have probably always been aware that invisible, life-like agents influence living and nonliving phenomena, but the attribution of such influences to discrete life forms that we now call bacteria occurred recently – less than five centuries ago. The Italian scholar, Girolamo Fracastoro (1476–1553) is credited with the first to propose, in 1546, an atomistic theory of disease, linking epidemics to particulate contagion. The invention and use of microscopes by Antoni van Leeuwenhoek (1632–1723) initiated an unprecedented era of scientific discoveries in the field of bacterial diversity. However, the appreciation of the consequences of bacterial ecological diversity had to await theoretical and empirical advances, for example, through the works of John Snow (1813–1858) and Louis Pasteur (1822–1895) that finally overturned the prevalent 'miasma theory' in favor of the 'germ theory' of disease causation (Ogunseitan, 2005). Despite the remarkable progress in visualizing, dissecting, and analyzing bacteria, we continue to refine our understanding of the causes and consequences of bacterial diversity, including the current focus on the human microbiome and the role of complex communities of diverse bacteria, as opposed to individual specific pathogens (NIH, 2015).

Until relatively recently, there was a paucity of sensitive tools for investigating bacterial diversity in nature. Most of the accumulated evidence and literature on the generation and coexistence of various species dating from Darwin's (1859) original observations, focused on eukaryotes with phenotypes that are relatively easy to identify and count (Dykhuizen, 1990; Hubbell, 2001). Yet, studies of diversity in prokaryotes have yielded insights into the process and its outcomes that would otherwise be difficult, if not impossible to acquire (Dykhuizen *et al.*, 2009; Feldgarden *et al.*, 2003; O'Malley, 2014). With cultivation on artificial solid nutrient media, many bacteria form visible colonies that take on distinctive phenotypic features such as size, shape, color, texture, and odor. Historically, bacteriologists relied on colony morphology and its heuristic associations with desired functions such as fermentation or undesirable effects such as disease causation as the fundamental basis for bacterial systematics.

The advent of high-resolution microscopy added information on the biochemical reactions of cell wall (i.e., Gram stain) and the shape of individual cells such as coccoid, bacillary, or spiral; and the structure of micro-colonies such as clusters (i.e., *Staphylococcus*) or chains (*Streptococcus*). But scientific consensus on the definition of bacterial species remained elusive because of the somewhat fluid nature of morphological expression under various environmental conditions. Moreover, relatively recent applications of molecular genetic analyses, particularly the sequencing of 16S rRNA that has contributed to a rapid increase in the number of taxonomic groups described for prokaryotes over the past two decades (Cole *et al.*, 2003; Scholss and Handelsman, 2004). Advances in ecological sampling and meta-molecular studies (Renella *et al.*, 2014) have also challenged traditional species concepts for prokaryotes with major consequences for contemporary understanding of bacterial diversity. This article covers the main developments in the theoretical and empirical investigations of bacterial diversity with emphasis on the challenges and opportunities presented by studying isolated bacteria in the laboratory and bacterial communities in nature.

Theoretical Approaches to Describe Bacteria Diversity

Extinction, strictly defined in a planetary sense, has never been demonstrated for bacteria (Rankin *et al.*, 2010). Moreover, there is considerable evidence in the natural history of

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prokaryotes that creative mutualism, endosymbiosis, and nonsexual exchange of genetic materials have led to new taxonomic lineages, possibly in response to environmental stressors that might have resulted in natural selection toward extinction (Hillesland and Stahl, 2010; Margulis and Sagan, 2003; Ogunseitan, 2008). Therefore, for this group of organisms, it has been difficult to reach consensus on a universal measure of the unit of natural selection and its outcome in diversification of species, especially without common understandings of geographic scope, niche specificity, mechanisms through which variable environmental parameters influence population size and community diversity, and ultimately, of the parameters of speciation in prokaryotes (Fraser *et al.*, 2009; Gevers *et al.*, 2005; Retchless and Lawrence, 2007; Venter *et al.*, 2004; Woese and Fox, 1977). There have been numerous theoretical excursions to illuminate these issues, although they remain primarily unresolved topics of active research.

For example, after nearly a quarter of century, Baas-Becking (1934) often quoted adage "*everything is everywhere……*" continues to generate considerable debate, hypotheses, and excursions in bacterial geography. Most bacteriologists will agree that it is impossible to know by direct count the number of individual bacterial cells or the number of bacterial species existing on Earth at any given point in time. Nevertheless, we have credible theoretical estimates (Curtis *et al.*, 2002; Hughes *et al.*, 2001; Whitman *et al.*, 1998), which place the total count in the vicinity of 10^{30} and the number of species at 10^{9}. Theoreticians estimate that more than half a million bacterial species are present in thirty grams 30 g of forest soil because speciation is easy and extinction is difficult for this group of organisms. This implies an implausibly infinite increase in the number of species over time, with no real impact of natural selection, except perhaps under narrowly defined local situations (Dykhuizen, 1998). Achieving precision in these estimates is neither possible nor useful for practical purposes, except for the fact that our knowledge of Earth's biosphere and its potential for sustainability is remarkably inadequate, and will not improve without understanding prokaryotic diversity and its myriad linkages to fundamental ecosystem functions (Ogunseitan, 2005).

Attempts to measure the rate of production of new bacterial species have focused on assessments of quantitative change in species richness over time, or the change in the magnitude of a phenotype, for example, enzyme activity, speed of motility, and output of bioluminescence, in response to selective pressure. The theoretical underpinnings of such measurements derive from attempts to quantitatively reconstruct the historical influence of natural selection or to determine the most vulnerable stages in the life cycle of organisms (Haldane, 1930). For example, natural selection intensity for a trait that follows statistically normal distribution in a population is computed as:

$$I = \log_e[s_o \div S]$$

where I is Haldane's intensity of natural selection, s_o is the chance (percentage) of survival of an individual (species) with the optimum trait (phenotype), and S is the mean chance of survival in the population (community).

There have been numerous renditions of this equation, depending on a variety of assumptions regarding mortality rates and the statistical profile of the distribution of the trait under selection within the population (Demetrius *et al.*, 2007; Lipstich and Sousa, 2002; Marcus, 1964; O'Donald, 1968).

Measuring the level to which natural selection increases the mean generational fitness has also been a popular topic for theorists. That quantity, often described as the additive genetic variance of fitness (V_w^2), resulted from the so-called 'fundamental theorem of natural selection' (FTNS) (Fisher, 1930) redefined for clarity as 'the rate of increase in the mean fitness of any organism at any time ascribable to natural selection acting through changes in gene frequencies is exactly equal to its genetic variance in fitness at that time' (Edwards, 1994). V_w^2 and its attributes have been popular for theoretical analyses regarding change in trait distribution following natural selection, but it has been notoriously difficult to measure, in part due to the long generation periods of model eukaryotic organisms with easily tracked phenotypes (Burt, 2000; Crow, 2002). Hence, there is a distinct advantage for using bacteria in studies of fitness and genetic variance. In this regard, the interaction of bacteria and bacteriophage, including genetic transduction, is one of the most thoroughly investigated and measured natural selection processes.

For example, mathematical models have been developed to describe the role of transduction (bacteriophage-mediated gene transfer from donor to recipient bacteria cells) in the selection and establishment of new phenotypes in bacterial communities. The model describes the selective advantage of beneficial genes under the selective pressure of, for example, antibiotics. The ratio of transductants to total cell numbers (T/N) at time zero and at a later time t, modified by a selection factor f is given by:

$$(T/N)_t = (T/N)_0 f$$

when new transductants are added to the population, and provided transductants have a selective advantage ($f > 1$), the ratio of transductants to total cell numbers after g generations is given by:

$$(T/N)_g = f_g\{(T/N)_0 + \Delta(T/N)_{trans} \div f - 1\}$$

$$-\Delta(T/N)_{trans} \div f - 1,$$

where $(T/N)_{trans}$ represents new transductants. Therefore, it is possible to predict the trend of accumulation of transductants, given assumptions about the selective pressure acting on specific genotypes. This mathematical model can predict stable transduction of specific antibiotic resistance genes in environments contaminated with antibiotics (Ogunseitan, 2005).

The small physical size of bacteria, their relatively brief generation periods and susceptibility to selective pressures that can be calibrated renders them attractive for use as model organisms for conducting experiments that test the predictions of evolutionary theories including natural selection. But as noted, there are several difficulties against unsophisticated interpretation of the results and conclusions based on of the outcomes of artificial selection experiments conducted with isolated bacterial cultures under laboratory conditions.

Experimental Approaches to Describe Bacteria Diversity

Despite relative anatomical and metabolic simplicity, bacteria exhibit complex characteristics that have been investigated for the purpose of advancing empirical knowledge of natural selection. Such features include susceptibility to bacteriophage infection, plasmid content, transposable elements, toxin production, motility, and pigmentation. The sensitivity of these features to natural selection has been exploited to engender major industrial processes. However, until recently, experimentation with bacteria on solid, batch and continuous cultures have not been very prominent in informing the theoretical advances about natural selection (Dykhuizen, 1990).

Sergie Winogradsky did not explicitly set out to investigate the theoretical underpinnings of bacterial diversity, but his invention – the 'Winogradsky Column' – has become a classic model for experiments on selection in bacterial ecology (Zavarin, 2006). By creating a nutrient gradient, the column facilitates the enrichment of certain species of bacteria at the expense of other species across the gradient. The inventor's statement on the purpose of the selective culture approach epitomizes the experimental manifestation of Darwin's (1859) principle of 'survival of the fittest.'

> The culture will be elective if it is favorable for the detection of the sole function … By supporting the microbe in question, engaged in the life contest with other microbes, we will have a significant prevalence of this microbe in our cultures …The application of pure cultures eliminates the most essential ecological factor, I mean competition, since it is precisely this factor that determines the distribution of the processes implemented by microbes in nature, and automatically directs the succession of these processes (Winogradsky, 1952).

Adoption of the selective enrichment culture has contributed greatly to the expansion of our knowledge of bacterial diversity and the discovery of new species that are crucially important for ecological processes. However, the approach is not easy to query at the fine level of quantitative detail needed to pursue specific questions about bacterial diversity. Instead, the pure culture approach, so detested by Winogradsky, has come to be regarded as indispensable for investigating the diversity of bacteria at the species and molecular levels.

For example, the bacterium *Escherichia coli* has long been the quintessential model for various investigations of bacterial diversity, physiology, molecular genetics, and evolution. A remarkable example of niche colonization and bacterial geography is the ubiquitous presence of *E. coli* in the lower intestines of warm-blooded organism regardless of the geographical distribution of the host. There is also a remarkable diversity of organisms collectively grouped as *E. coli* – whereas most members of the specie are harmless commensals, many can cause life-threatening diseases among humans. Although several mechanisms have been proposed on one hand as the causes of molecular diversity in *E. coli*, other mechanisms are also known to reinforce the common features that select for a certain level of homogeneity easily recognizable among strains within the species (Blattner *et al.*, 1997; Hayashi *et al.*, 2001; Jackson *et al.*, 2011; Noble *et al.*, 1998).

Experiments with long-term laboratory cultivation of *E. coli* have been among the most well described that address questions related to the emergence of polymorphism and variability in the context of natural selection and ecological opportunities for expansion or restriction (Barrick and Lenski, 2013; Plucain *et al.*, 2014; Kawecki *et al.*, 2012; Wiser *et al.*, 2013). Experiments with bacteria are particularly suited for querying causal issues in natural selection, including gene-environment interaction, how specialization leads to ecological diversification, and negative frequency-dependent selection (Feldgarden *et al.*, 2003). Despite the remarkable success of this approach, questions frequently arise about whether the small size of bacteria limit the application or generalizability of results from experiments conducted with them to test evolutionary principles. For example, it has been pointed out that there is considerable disparity between the genomic mutation rates observed in laboratory culture experiments compared with the rates estimated by sequence comparisons (Ochman *et al.*, 1999). In part to resolve these discrepancies, the use of artificial life or digital organisms to investigate natural selection has gained increasing attention (Clune *et al.*, 2012; Lenski *et al.*, 2003; Wilke *et al.*, 2001). In this regard, the *Avida* and *Tierra* software artificial life platforms are among the most developed for studying 'evolution' of self-replicating programs (Adami, 1998). Among the surprising finds from the series of studies of digital organisms is the potentially constructive role of deleterious mutations in adaptive evolution (Covert *et al.*, 2013).

Implications and Applications of Bacteria Diversity

There is possibly no greater threat to the global healthcare system than the one posed by the emergence of resistance to multiple antibiotics among pathogenic bacteria that cause diseases in humans. This threatening phenomenon results from the natural processes that generate and maintain bacterial diversity. In its first-ever comprehensive report on surveillance and information on antibacterial resistance at country-level worldwide, the World Health Organization declared that 'very high rates of resistance have been observed in bacteria that cause common healthcare associated and community-acquired infections (e.g., urinary tract infection, pneumonia) in all WHO regions' (World Health Organization, 2014). The United States Centers for Disease Control and Prevention, in its first-ever snapshot of the burden of antibiotic resistant bacteria noted that each year in the United States at least 2 million people are infected with bacteria that are resistant to antibiotics, resulting in at least 23 000 deaths as a direct result of the infections (CDC, 2013). This 'reversal of fortune' is the outcome of a global experiment in natural selection following large-scale manufacture and use of antibiotics hailed less than a century ago as magical interventions in public health and agriculture. We know now that many genes encoding for antibiotic resistance are located on mobile genetic elements that can rapidly disseminate in bacterial populations through well-studied genetic exchange processes of conjugation, transduction, and transformation (Ogunseitan, 1995). There is an extensive literature on the quantification of genetic exchange processes in natural bacterial communities, particularly on the rates of transmission of antibiotic resistant genes. However, there is an urgent need to further explore theoretical

frameworks, including group theory, for the purposes of generating predictive models of bacterial diversity, the limits of genetic dispersal, and phenotype stability (Ogunseitan, 2008).

The understanding of qualitative and quantitative boundaries of bacterial diversity is also applicable to the investigation of coevolutionary processes governing the interactions between pathogenic bacteria and human, animal, and plant hosts. Several theoretical models have been developed to explain the outcome of bidirectional selective pressures on bacterial diversity, emergence of genomic pathogenicity islands, and host immune response. Exploratory research in this direction includes testing among the predominant models, including balanced polymorphism ('trench warfare model'), arms race (red queen model), and positive selection on favorable alleles (Toft and Anderssen, 2010). The extent to which infection by pathogenic bacteria exerts selective pressure and influences natural selection in humans is also a rich area of research, especially with respect to the geography of human diseases. There are very few quantitative measures of this hypothesis, with the exception of attempts to explain the decline in mortality from pulmonary tuberculosis in Europe and North America just before the emergence of antibiotic therapy (Lipstich and Sousa, 2002).

In agriculture, there is increasing interest in understanding the vulnerability of critical crop–bacterial interaction and the possibility of local extinction of, for example, nitrogen-fixing *Rhizobium* species due to natural or anthropogenic pressures (Marco *et al.*, 2009). Remarkably, the experimental evolution approach has been used to investigate and confirm the transformation of a plant pathogen to mutualistic relationship between leguminous plants and Rhizobia (Marchetti *et al.*, 2010).

Finally, measuring natural selection in bacteria is increasingly important for understanding global environmental change phenomena, including attempts to mitigate climate change through manipulating the role of bacteria in the global biogeochemical cycles. Work on this topic includes experimental evolution to investigate the emergence of mutualism between methanogenic bacteria and sulphate-reducing bacteria (Hillesland and Stahl, 2010). Adaptation is life's most persistent question, and natural selection is the executioner's forum whereby death and extinction result for individuals and species not prepared to respond correctly. Through their remarkable ability to generate new forms and functions and to adapt constantly, bacteria are the only group of organisms that have probably inhabited the planet continuously since the emergence of life on Earth. This group of organisms have survived numerous episodes of climate change and various other kinds of selective pressures, and they have successfully navigated the natural selection forum. We have much to learn about and from bacterial diversity.

See also: Bacterial Species Concepts. Biogeography, Microbial

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Bacterial Species Concepts

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Species – A Special Level of Taxonomy?

Life's diversity is organized into clusters of related organisms that are similar in structure, function, and genomic properties. These phenotypic and genetic clusters are found from the most complex organisms to the prokaryotes and at all levels of life's diversity, from the domains and phyla to species (Caro-Quintero and Konstantinidis, 2012; Mallet, 1995). Between these clusters are gaps that represent intermediate phenotypes that we can imagine but do not actually exist in the natural world (Cohan, 2013). This pattern of clusters and gaps reflects the genealogical continuity of all organisms, taking into account that some lineages have been extremely successful while nearly every lineage that has ever existed has gone extinct (**Figure 1**).

Systematists and evolutionary biologists have taken a special interest in diversity at the species level. Above the species level, systematists have widely agreed that the taxa they demarcate are merely categories of convenience (McDonald *et al.*, 2012). Many believe the same about species (Hey, 2001; Doolittle and Zhaxybayeva, 2009), but others hold that there is something special about species – that they hold certain dynamic properties that transcend human attempts at classification (Cohan and Perry, 2007; Mayr, 1942; de Queiroz, 2005). Among these proposed properties are that each species is ecologically distinct and irreversibly separate from other species, that each species is cohesive in that some force constrains diversification within a species, and that each species is recognizable as a DNA sequence cluster for genes that they share (de Queiroz, 2005; Kopac *et al.*, 2014). Whether all these species properties apply to newly divergent lineages in the prokaryotic world is not yet resolved.

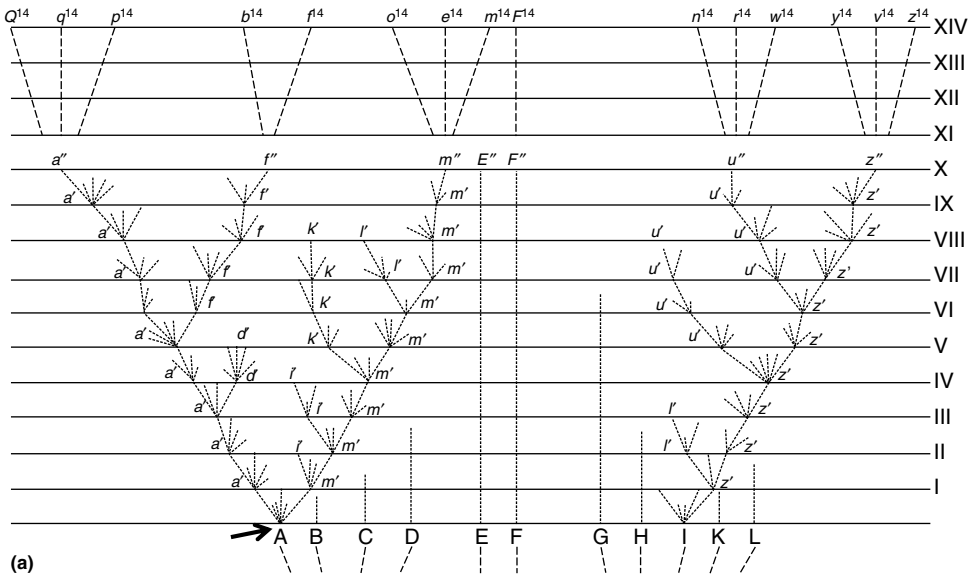
Prokaryotic Systematics: Species as Clusters of Similar Organisms

Prokaryotic systematists have aimed to identify species as closely related groups of organisms that differ in their ecological roles in biological communities, their ecological relationships with other organisms, and their physiological capacities (Rosselló-Mora and Amann, 2001). To this end, systematists have defined species as clusters of close relatives separated by large gaps in phenotypic and molecular characters (Rosselló-Mora and Amann, 2001; Vandamme *et al.*, 1996). This cluster-based approach has proved remarkably robust, even as the criteria for defining prokaryotic species have changed over the decades from exclusively phenotypic characteristics (usually metabolism) to include various molecular properties, such as multilocus sequences, whole-genome content, and whole-genome average nucleotide identity (Rosselló-Mora and Amann, 2001; Kopac and Cohan, 2011; Konstantinidis and Tiedje, 2005; Goris *et al.*, 2007) (**Figure 2**).

At present, systematics demands that members of a species share at least 70% of their genes, as determined by DNA–DNA hybridization, have at least 98.7% sequence identity at the 16 S rRNA gene, and differ from other species in at least one diagnostic (non-overlapping) phenotypic character (Stackebrandt and Ebers, 2006; Stackebrandt *et al.*, 2002). With the knowledge that genome content more reliably yields the number of shared genes than DNA–DNA hybridization, and that whole-genome average nucleotide identity more accurately predicts relatedness than a single gene, many taxonomists are demanding change in the criteria for demarcating species in systematics (Goris *et al.*, 2007; Vandamme and Peeters, 2014). Systematists have traditionally attempted to describe the phenotypes of new species as comprehensively as possible (Tindall *et al.*, 2010), and it appears that comparisons of whole-genome content may be the most efficient and reliable route to this goal. For example, in silico mapping of genomes to phenotypes has resulted in much more comprehensive species descriptions than was possible before genomics (Vandamme and Peeters, 2014; Amaral *et al.*, 2014; Thompson *et al.*, 2014). Beyond describing the phenotypes, systematics might also prioritize as fundamental a description of the habitats of isolation of any new taxon, since habitat descriptions have proved essential for characterizing the ecological and physiological differences among close relatives (Yilmaz *et al.*, 2011; Hunt *et al.*, 2008; Becraft *et al.*, 2011; Martiny *et al.*, 2009).

The species clusters recognized by systematics typically hold an enormous amount of diversity in genome content, physiology, biochemistry, and ecology (Manning *et al.*, 2008; Smith *et al.*, 2006; Dykhuizen *et al.*, 2008; Walk *et al.*, 2009; Hunt *et al.*, 2008; Koeppel *et al.*, 2008; Connor *et al.*, 2010; Sikorski, 2008; Ward, 1998). This presents a practical problem for microbiologists outside of systematics. The animal ecologist G. Evelyn Hutchinson suggested that a species' membership should be homogeneous in its physiological, biochemical, morphological, and ecological characteristics because this would yield the useful property that the characteristics of any individual classified to a species could be easily predicted (Hutchinson, 1968). If species could be defined as homogeneous units, the total membership of a pathogenic species, for example, would have the same disease-causing properties, the same tissue tropisms, the same transmission properties, and the same host range, while organisms with significantly different properties would be recognized as different species. The reality of prokaryotic species taxonomy is that much ecological diversity is subsumed within a recognized species (Ellegaard *et al.*, 2013).

The broad species demarcations of prokaryotic systematics can lead to errors in population genetic estimates of population sizes and the rates of processes such as migration (Kopac and Cohan, 2011). The problem is that many of these analyses require focus on a single, ecologically homogeneous population (Volz, 2012; Ho and Shapiro, 2011), but microbial population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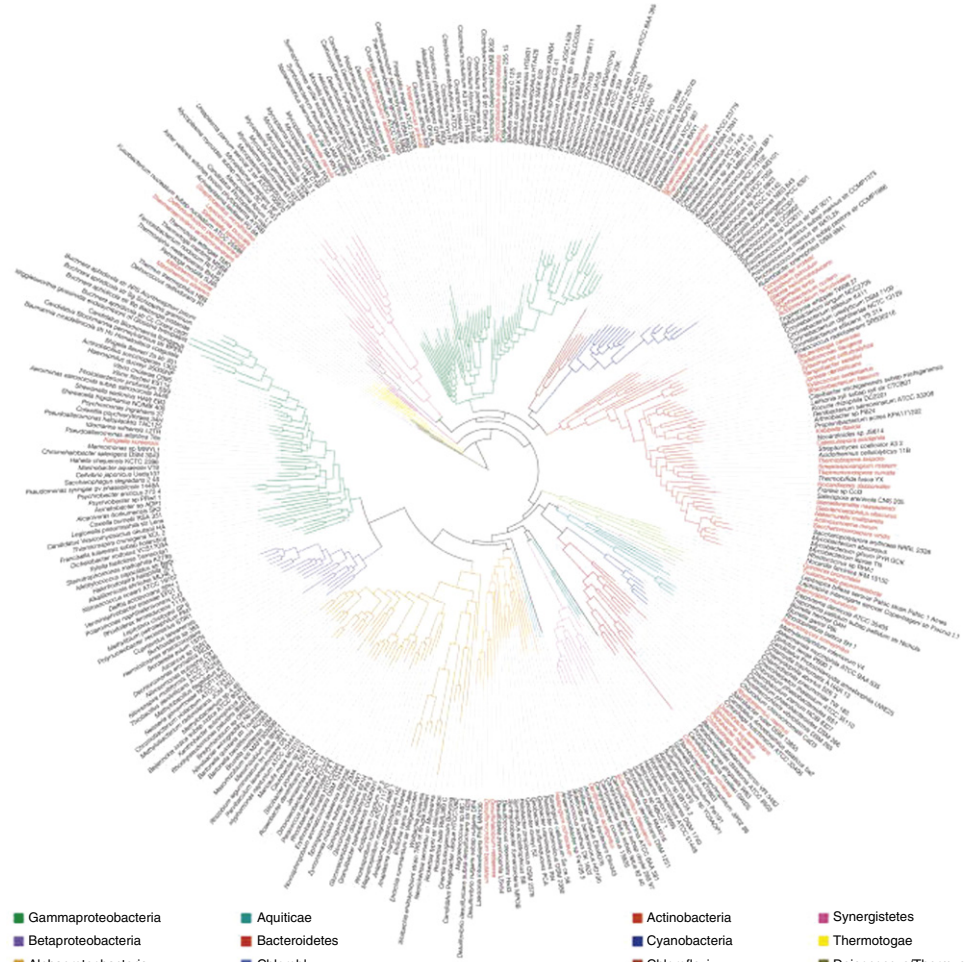
(a)


Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Deltaprotecbacteria
Epsilonproteobacteria
Acidobacteria
Aquiticae
Bacteroidetes
Chlorobl
Chlamydiae/vemucomicrobia
Planctomycetes
Spirochaetes
Actinobacteria
Cyanobacteria
Chloroflexi
Firmicutes
Tenericutes
Fusobacteria
Synergistetes
Thermotogae
Deinococcus/Thermus

(b)

biologists may implicitly and wrongly assume that the diverse members of a prokaryotic species taxon in a particular community are equivalent and interchangeable. Taxonomy can be a false friend to microbial population biologists.

By defining species so broadly, prokaryotic systematics does not encourage full exploration of all the ecological, physiological, and genomic diversity among close relatives that is subsumed within a species taxon. How might we arrive at a systematics of species that recognizes more finely demarcated groups that may differ in important properties? One possibility would be simply to reduce the fixed level of molecular divergence allowed within a species. Alternatively, systematics could aim to identify more finely demarcated populations that hold the dynamic, evolutionary properties ascribed to species outside of microbiology (Cohan and Perry, 2007).

Bacterial Species as Dynamically Defined Groups

Most systematists outside of microbiology have understood species to be more than closely related groups separated by gaps, that species have a reality beyond human attempts at classification (de Queiroz, 2005; Mayr, 1982). The property of cohesion has come to be understood as a quintessential aspect of species (de Queiroz, 2005; Mayr, 1982; Templeton, 1989). In this view, species are real because they are the largest groups whose diversity is constrained by a force of cohesion. In the case of the highly sexual animals and plants, the force constraining diversity within species is understood to be genetic exchange (Mayr, 1963). In Mayr's Biological Species Concept, the origin of species by one species splitting into two (speciation) requires that newly divergent populations break free of cohesion caused by recurrent, high-frequency genetic exchange; speciation has therefore been understood to be rare.

Recently, however, zoologists and botanists have questioned whether animal and plant species are really cohesive across their geographic ranges, and whether cohesion by genetic exchange actually prevents speciation (Mallet, 2008; Schluter, 2009). Similarly, microbial ecologists have debated whether genetic exchange has a role in preventing speciation in prokaryotes (Wiedenbeck and Cohan, 2011; Cadillo-Quiroz *et al.*, 2012; Retchless and Lawrence, 2010), whether bacterial speciation is rare or difficult (Hunt *et al.*, 2008; Doolittle and Zhaxybayeva, 2009; Cohan and Perry, 2007), and even whether bacterial species are cohesive at all (Cohan, 2011).

Genetic Exchange and the Nature of Prokaryotic Species

Several aspects of genetic exchange may affect the character of species and speciation in prokaryotes. First, recombination is not tied to reproduction as it is in many eukaryotic groups. Instead, recombination occurs only when an opportunity arises through uptake of DNA in the environment or when a virus or plasmid (a vector of recombination) takes up DNA from one cell (the donor) and then infects another cell (the recipient), allowing for genetic transfer of DNA from donor into recipient; in addition, some archaea can exchange genes through cell fusion (Naor *et al.*, 2012; Cohan and Aracena, 2012). Consequently, homologous recombination in prokaryotes occurs at an extremely low rate, usually not much greater than the rate of mutation (Vos and Didelot, 2009; Wiedenbeck and Cohan, 2011).

One consequence of such low recombination rates is that recurrent recombination appears unlikely to hinder adaptive divergence. The principle is that when niche-specifying genes from another population are introduced only rarely, natural selection in the recipient population can successfully eliminate these genes (Wiedenbeck and Cohan, 2011; Vos, 2011; Haldane, 1932) (**Figure 3**). Given that recombination even among very close relatives is already extremely low, reduction of recombination from this already-low rate (i.e., evolution of sexual isolation) is not likely a necessary milestone for ecological diversification and speciation (Wiedenbeck and Cohan, 2011).

Recombination is not known to have reversed the adaptive divergence between any ecologically distinct bacteria, although recombination was originally understood to have reversed the adaptive divergence between two *Campylobacter* taxa (Sheppard *et al.*, 2008). However, recombination was later interpreted to have effected an adaptive change within the recipient population that did not undo the ecological distinctness of the populations (Sheppard *et al.*, 2011).

One caveat to the conclusion that recombination cannot constrain speciation is that perhaps recombination might be more frequent than has been estimated, especially among the closest of relatives. It is possible that studies on recombination rates have pooled multiple, ecologically distinct populations that recombine at a reduced rate because they live in different microhabitats (Shapiro *et al.*, 2012).

Another consequence of rare recombination is a phenomenon known as 'periodic selection,' in which the entire genome-wide diversity within an ecologically homogeneous population is purged by natural selection. Because of the rarity of prokaryotic recombination, nearly the entire genome of the original adaptive mutant will reach 100% frequency before the adaptive gene can be recombined into other genetic backgrounds (**Figure 4**). Dynamic models of selection and recombination have shown that genome-wide purging within an ecologically homogeneous, prokaryotic population is inevitable whenever an adaptive mutation appears (Cohan, 2005).

Nevertheless, various empirical studies of sequence diversity have concluded that genome-wide sweeps do not occur.

Figure 1 Clustering of organisms as a property of the genealogy of life, as well as extinctions of nearly every group that has ever lived. (a) The only figure in Darwin's *Origin of Species* (Darwin, 1859). There is continuity of all life as we look one generation at a time into each organism's past until the ancestry of any two organisms coalesces into a common ancestor (e.g., the continuity of the *a* and *m* lineages through their common ancestor, indicated by the arrow), while there is discontinuity among living organisms because many intermediates have gone extinct (e.g., lineages *d*, *l*, *k*, *l*, and *s*). (b) Clustering and lack of intermediates among the bacterial phyla. Here each phylum (in a unique color) is clearly indicated as a sequence cluster, based on 31 shared genes (Wu *et al.*, 2009). Beyond the clustering by sequence, each phylum also clusters as a group with unique architectural properties that are profoundly divergent (Cohan, 2010). While clustering is most evident here at the phylum level, all taxonomic levels from the divisions to species also show clustering. Reproduced with permission from Nature Publishing Group.

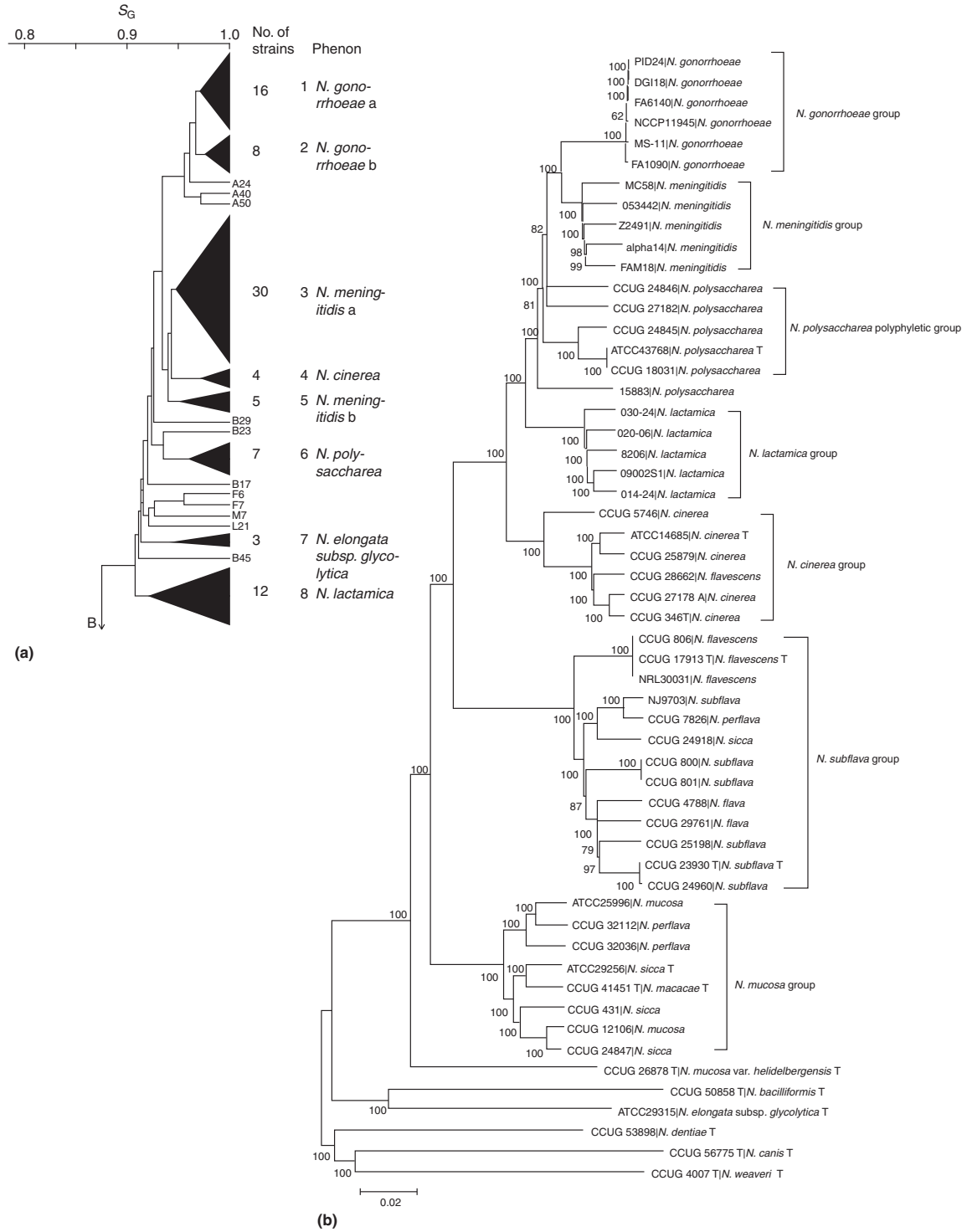


Figure 2 Species as morphological and molecular clusters, as shown in the genus *Neisseria*. (a) Clustering of close relatives of *N. meningitidis* by 155 phenotypic, largely metabolic, characteristics (Barrett and Sneath, 1994). (b) Clustering by a multilocus approach, here a concatenation of 246 shared genes from fully sequenced genomes (Bennett *et al.*, 2012). The groups recognized as species can be observed as clusters by either morphological or molecular criteria. Note that within many taxa the molecular criteria suggest multiple clusters, and that it is only by convention that we recognize a particular level of clustering to demarcate species. Reproduced with permission from the Society for General Microbiology.

They showed that a single chromosomal region increased in frequency over a set of ecologically distinct populations without purging genome-wide sequence diversity (Guttman and Dykhuizen, 1994; Papke *et al.*, 2007; Shapiro *et al.*, 2012). However, these studies did not take into consideration that genome-wide sweeps are predicted only within a single

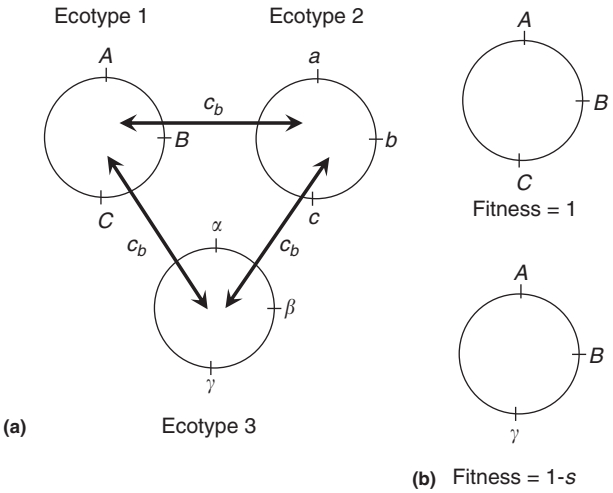


Figure 3 Why recurrent recombination is unlikely to prevent adaptive divergence in prokaryotes? (a) Model of recurrent recombination between closely related, adaptively divergent populations (ecotypes). Here the ecological niche differences are determined by homologous alleles at three loci, where capital alleles confer the adaptation for Ecotype 1, lower case alleles for Ecotype 2, and so on. Recombination occurs recurrently between populations at the rate c_b. (b) Fitness penalty for having a niche-determining allele from another population at one locus. (c) The equilibrium frequency of the maladaptive foreign allele is determined by c_b and s. The rate of recombination among closest relatives within the same population has been estimated at 10^{-6} per gene per organism per generation; so even if the rate of recombination between populations is as great as that within them, the equilibrium frequency of maladaptive foreign alleles is predicted to be negligible (Wiedenbeck and Cohan, 2011).

population of ecologically homogeneous organisms (**Figures 5–6**) (Kopac and Cohan, 2012). While some have argued that periodic selection does not occur in nature (Cordero and Polz, 2014), a metagenomic approach recently demonstrated genome-wide sweeps of diversity within various bacterial populations in nature (Bendall *et al.*, 2016).

The origin of bacterial species is also impacted by the ability of prokaryotes to acquire genes across extremely distantly related groups. A single genetic acquisition (by horizontal genetic transfer) can dramatically alter the ecological niche of a recipient organism, and some have argued that as a result prokaryotes speciate at a prodigious rate (Doolittle and Zhaxybayeva, 2009).

Recombination-Based Approaches to the Origin of Bacterial Species

In many studies of the origin of prokaryotic species, recombination has been viewed as a rampant process that can erode the distinctness of nascent species (Doolittle and Zhaxybayeva, 2009). Speciation theories based on Mayr's Biological Species Concept see the breaking of recombination between populations as a critical step in population divergence: populations can diverge ecologically and irreversibly only after their genetic exchange is blocked (Coyne and Orr, 2004).

Several studies have concluded that reduced recombination is necessary for the origin of ecological divergence in prokaryotes (Cadillo-Quiroz *et al.*, 2012; Ellegaard *et al.*, 2013). For example, in *Sulfolobus islandicus* isolated from the same hot spring, rates of recombination were higher within each of two ecologically distinct clades than between them (Cadillo-Quiroz *et al.*, 2012). In such studies, ecological divergence between sexually isolated clades was taken as evidence that recombination must be lowered to allow irreversible divergence among close relatives into ecologically distinct populations.

However, these studies did not address whether ecological divergence may have also occurred *within* each clade, where recombination rates are higher. One recent study, on *Synechococcus* in Yellowstone hot springs, revealed ecological diversification within clades that included the closest known relatives, which showed the highest rates of recombination (D. M. Ward, pers. comm.). While recombination is often reduced among ecologically distinct clades that are easily distinguished as sequence clusters, it appears that the reduction in recombination may follow, rather than precede, their ecological divergence (Mallet, 2008), especially if the clades prefer different microhabitats (Shapiro and Polz, 2014).

Consider next how ecological divergence among populations may reduce their recombination. First, random, non-adaptive sequence divergence that accumulates between populations directly reduces the recombination between them. This is because homologous recombination between a donor segment and the recipient cell's chromosome requires near-identity between donor and recipient at one or both ends of the donor segment, resulting in exponential decay of recombination rate with sequence divergence (Majewski and Cohan, 1999b; Vulić *et al.*, 1997).

Also, adaptive divergence between populations can reduce their recombination, especially if they have diverged by integrating unique sets of novel genes that improve adaptation to their particular niches. In the 'fragmented speciation' hypothesis (Retchless and Lawrence, 2010), homologous recombination between populations is reduced in each chromosomal region where one population has acquired an adaptive gene that the other population lacks.

Recombination between already-divergent populations will be further reduced if populations become adapted to different microhabitats (Shapiro and Polz, 2014). Another possibility is that ecological divergence will result in differences in the vectors of recombination, whereby the viruses and plasmids that infect one population cannot infect the other (Jensen *et al.*, 1998). Systems analyses of genetic transfers among hundreds of fully sequenced genomes have revealed two primary obstacles to recombination: divergence in habitat and phylogenetic distance (Popa and Dagan, 2011).

Ecological Speciation in Bacteria

Theories of ecological speciation hold that ecological divergence is the critical, initial step in the origin of diversity, and that patterns of recombination play only a minor role in

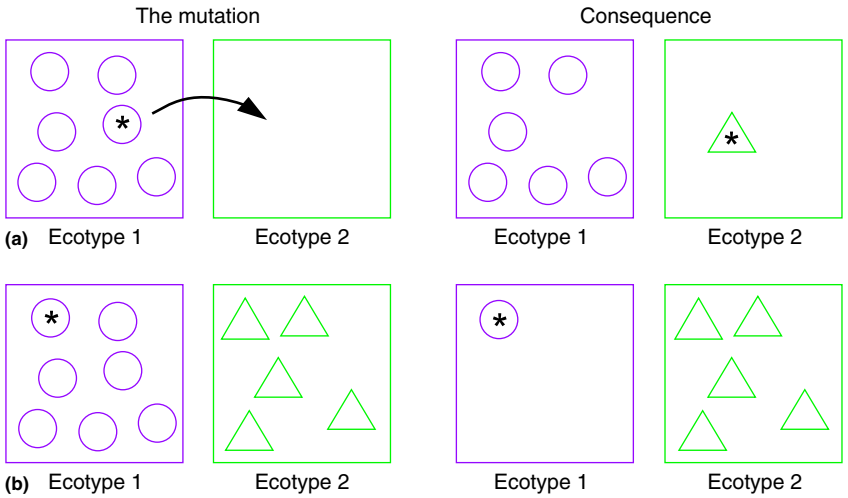


Figure 4 The dynamics of ecotype formation and periodic selection within an ecotype. Circles represent different genotypes, and asterisks represent adaptive mutations. (a) Ecotype formation event. A mutation or a recombination event allows the cell to occupy a new ecological niche, founding a new ecotype. A new ecotype can be formed only if the founding organism has undergone a fitness trade-off, whereby it cannot compete successfully with the parental ecotype in the old niche (Wiedenbeck and Cohan, 2011). (a) Periodic selection event. A periodic selection mutation improves the fitness of an individual such that the mutant and its descendants outcompete all other cells within the ecotype; these mutations do not affect the diversity within other ecotypes because ecological differences between ecotypes prevent direct competition. Periodic selection leads to the distinctness of ecotypes by purging the divergence within, but not between ecotypes (Cohan, 2005). Reproduced with permission from Landes Publishers.

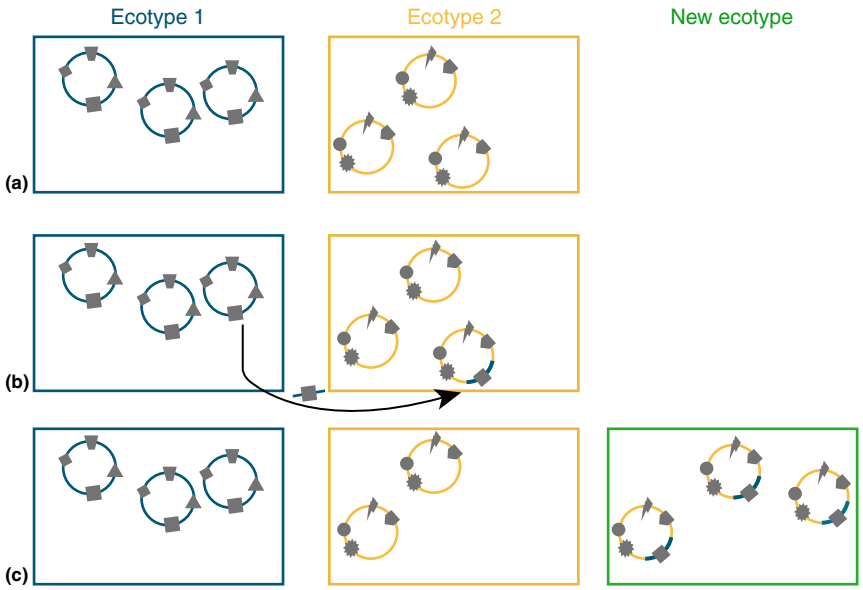


Figure 5 The consequences of an adaptive genetic transfer between ecologically distinct populations when an adaptive transfer creates a new ecotype. The niche-determining genes for each ecotype are represented as unique symbols on the circular chromosome (e.g., a trapezoid in Ecotype 1), which is a unique color for each ecotype. The addition of the 'square' gene to the genome of Ecotype 2 creates a new ecotype, which can coexist with both pre-existing ecotypes. Because the adaptive gene is transferred on a small segment, the part of the chromosome that is homogenized across ecotypes is short (Majewski and Cohan, 1999a; Kopac and Cohan, 2012).

the subsequent fate of newly split, ecologically distinct populations (Van Valen, 1976; Mallet, 2008; Schluter, 2009). Evidence for ecological speciation is accumulating even for animals and plants where recombination is obligately tied to reproduction every generation. Recombining populations of animals or plants in adjacent microhabitats are able to diverge, owing to the modest (but apparently sufficient) reduction in genetic exchange across habitats, allowing natural selection to eliminate niche-specifying genes entering from the adjacent population (Mallet, 2008). A fortiori, ecological speciation is

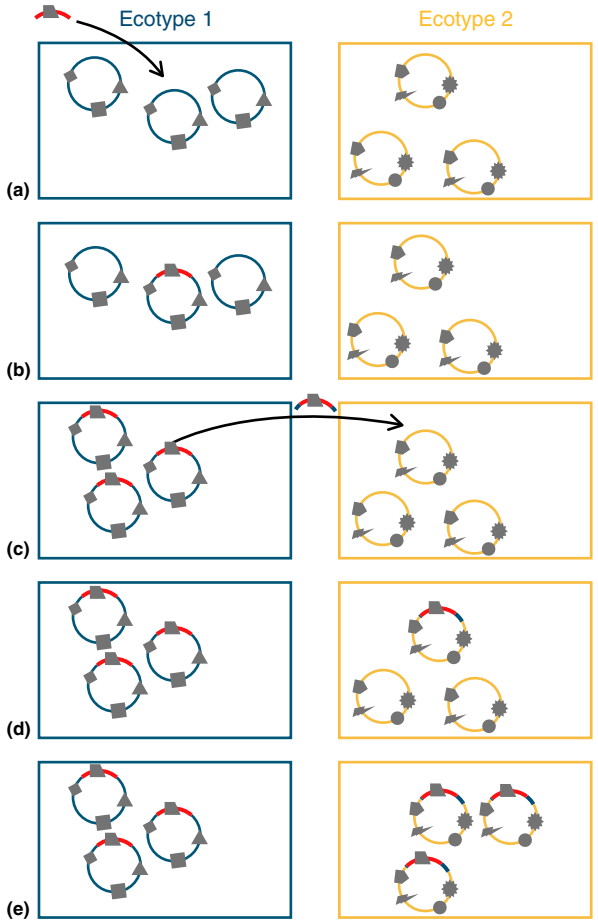


Figure 6 The consequences for adaptive genetic transfer between ecotypes when the adaptive transfer creates a periodic selection event in the recipient ecotype. (a) and (b) A 'trapezoid' gene, which is of adaptive value across different ecotypes, enters Ecotype 1 from another ecotype (whose chromosome is marked as red), and creates a recipient individual that can outcompete all the other organisms within Ecotype 1. (c) The recombinant creates a periodic selection event within Ecotype 1, yielding a genome-wide purging of diversity within the ecotype. (d) The generally adaptive 'trapezoid' gene then transfers into Ecotype 2, and creates a recipient individual that can outcompete all other members of Ecotype 2. (e) This causes a genome-wide purging of diversity within the ecotype. Note that even though there is genome-wide purging of diversity accompanying periodic selection events within each ecotype, the only chromosomal part that gets homogenized across ecotypes is the short segment that transfers across ecotypes (Kopac and Cohan, 2012).

all the more likely in prokaryotes, given the intrinsically very low, mutation-like frequencies of recombination in prokaryotes (Wiedenbeck and Cohan, 2011).

Geographic isolation (and the ensuing reduction in recombination between populations) is therefore not expected to be essential for ecological divergence between prokaryotic populations (Cohan, 1994). Indeed, recent studies have shown sympatric diversification of one population into multiple, ecologically distinct, indefinitely coexisting populations, in both laboratory studies (Treves *et al.*, 1998; Blount *et al.*, 2012; Rainey and Travisano, 1998; Koeppel *et al.*, 2013) and in nature (Connor *et al.*, 2010; Hunt *et al.*, 2008; Becraft *et al.*, 2011; Denef *et al.*, 2010).

It is not yet known which of the species-like properties attributed to species of animals and plants follow from ecological speciation in prokaryotes. To identify these properties for newly divergent prokaryotic lineages, it is useful to define these lineages in a way that does not presuppose the properties they will have beyond ecological divergence. To this end, 'ecotypes' may be defined as lineages that are ecologically distinct, such that the ecotype lineages can coexist indefinitely as a result of their ecological divergence; while there may also be ecologically distinct lineages *within* an ecotype, their differences are not sufficient to allow their indefinite coexistence (Kopac *et al.*, 2014). Microbial ecologists studying the origins of ecological diversification have focused on the origin of ecotypes (or similarly defined, newly divergent ecological populations) rather than the broadly defined species of taxonomy (Hunt *et al.*, 2008; Connor *et al.*, 2010; Sikorski, 2008; Shapiro *et al.*, 2012).

What species-like properties follow from ecological divergence? In the 'stable ecotype' model of speciation, the origin of new ecotypes is rare, and each ecotype is recurrently purged of diversity, genome-wide, through periodic selection events over its long lifetime (Cohan and Perry, 2007) (**Figure 7(a)**). Different ecotypes have the species property of being irreversibly separate because, owing to their ecological differences, periodic selection cannot prevent divergence among newly split ecotypes; moreover, ecological divergence between ecotypes is not prevented by recurrent genetic exchange (Wiedenbeck and Cohan, 2011; Vos, 2011). Stable ecotypes are discernible as sequence clusters because longstanding ecotypes have had opportunity to accumulate neutral sequence divergence at every locus, while diversity within ecotypes is recurrently purged (Cohan and Perry, 2007) (**Figure 7(a)**). In summary, slow speciation and indefinite coexistence of emergent species yields all the species-like properties attributed to animal species.

However, depending on the rates of ecotype formation and extinction, as well as the magnitude of ecological divergence, ecotypes may hold only a subset of species-like characteristics. In the 'speedy speciation' model, species are subject to recurrent cohesion by periodic selection, but speciation is so rapid that species are not discernible as sequence clusters (**Figure 7(b)**). In the 'nano-niche' model, the most newly divergent, ecologically distinct populations are only *quantitatively* different (**Figure 7(c)**). Here each ecologically distinct population has no unique resources but utilizes the same set of resources in different proportions (Cohan and Perry, 2007; Wiedenbeck and Cohan, 2011; Kopac *et al.*, 2014). This pattern of ecological divergence is common in animal and plant speciation (Schluter, 2000) and extends to some bacteria. A recent comparison of genomes within *Bacillus subtilis* has suggested nano-niche diversification (Kopac *et al.*, 2014). Nano-niche populations are predicted to be ephemeral because each is vulnerable to competition to extinction by another population using the same set of resources, and thus nano-niche populations are not irreversibly separate (Cohan, 2005). Hence, nano-niche populations do not meet the ecotype criterion that ecotypes must be able to coexist indefinitely as a result of their ecological divergence. Individual nano-niche populations likely have the species-like property of cohesion.

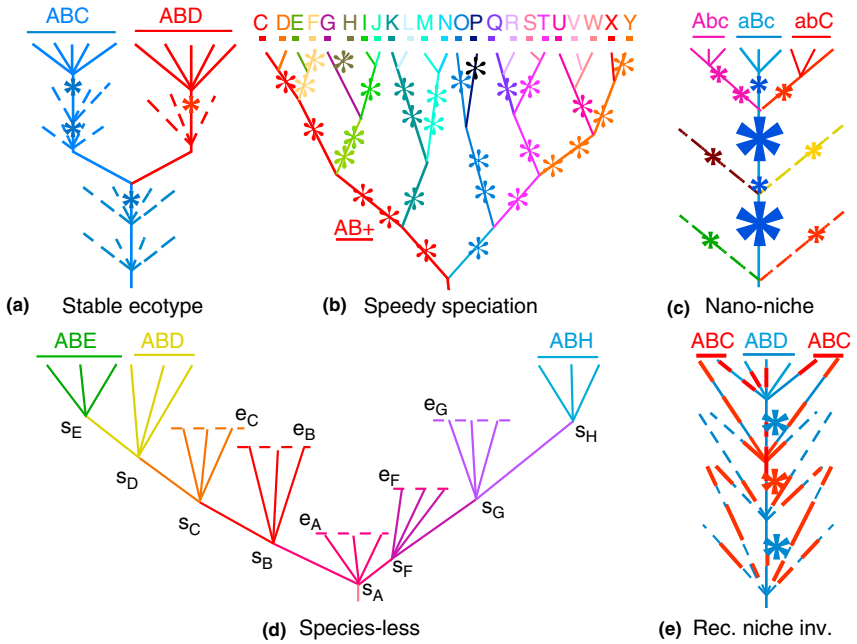


Figure 7 Models of ecological speciation in prokaryotes. Ecotypes are represented by different colors; periodic selection events are indicated by asterisks, and extinct lineages are represented by dashed lines. The letters at the top represent the resources that each group of organisms can utilize. In cases where ecotypes utilize the same set of resources but in different proportions, the predominant resource of each ecotype is noted by a capital letter. (a) In the Stable Ecotype model, each ecotype endures many periodic selection events during its long lifetime. The Stable Ecotype model generally yields a one-to-one correspondence between ecotypes and sequence clusters. The ecotypes are able to coexist indefinitely because each has a resource not shared with the others. (b) The Speedy Speciation model is much like the Stable Ecotype model, except that speciation occurs so rapidly that most newly divergent ecotypes cannot be detected as sequence clusters in multilocus analyses. (c) The Nano-Niche model. In the figure, there are three Nano-Niche populations that use the same set of resources but in different proportions (noted by Abc, aBc, and abC). Each Nano-Niche population can coexist with the other two because they have partitioned their resources, at least quantitatively. However, because the ecotypes share all their resources, each is vulnerable to a possible speciation-quashing mutation that may arise in the other populations. This could be a mutation that increases efficiency in utilization of all resources. These speciation-quashing mutations are indicated by a large asterisk; each of these extinguishes the other Nano-Niche populations. Thus, in the Nano-Niche model, cohesion can cut across ecologically distinct populations, provided that they are only quantitatively different in their resource utilization. (d) In the Species-Less model, the diversity within an ecotype is not limited by periodic selection but instead by the short time from the ecotype's invention as a single mutant until its extinction. The origination and extinction of each ecotype *i* is indicated by s_i and e_i, respectively. In the absence of periodic selection, each extant ecotype that has given rise to another ecotype is a paraphyletic group, and each recent ecotype that has not yet given rise to another ecotype is monophyletic. (e) In the Recurrent Niche Invasion model, a lineage may move, frequently and recurrently, from one ecotype to another, usually by acquisition and loss of niche-determining plasmids. In the figure, the red lines indicate the times in which a lineage is in the plasmid-containing ecotype; the blue lines indicate the times when the lineage is in the plasmid-absent ecotype. Periodic selection events within one ecotype extinguish only the lineages of the same ecotype. For example, in the most ancient periodic selection event shown, which is in the plasmid-absent (blue) ecotype, only the lineages missing the plasmid at the time of periodic selection are extinguished, while the plasmid-containing lineages (red) persist. Ecotypes determined by a plasmid are not likely to be discoverable as sequence clusters (Kopac *et al.*, 2014). Reproduced with permission from the American Society for Microbiology.

If both ecotype formation and extinction are frequent, quotidian events, the 'species-less' model can apply (Ward and Cohan, 2005; Cohan and Perry, 2007) (**Figure 7(d)**). Here, each ecotype lives only briefly before going extinct, so the diversity within an ecotype is constrained by the short time of its existence, from its founding by a single mutant or recombinant cell until the ecotype's early extinction. There is no time for cohesion by recurrent forces like periodic selection. Related to the 'species-less' model is the 'opportunitroph' model, where various generalist lineages adapt convergently to similar types of ephemeral particles (Polz *et al.*, 2006). The only species-like property that applies for the species-less model is ecological divergence (Kopac *et al.*, 2014).

Another kind of rapid diversification of bacterial lineages is fostered by plasmids (**Figure 7(e)**). These are infectious circles of DNA that can provide an ecological adaptation for the bacteria that host them. For example, a given 'symbiosis' plasmid confers to its *Rhizobium* host the abilities to infect a particular legume species and fix nitrogen. So, a particular *Rhizobium* lineage can change its ecological niche by acquiring a new symbiosis plasmid and losing its old one (Kumar *et al.*, 2015). In this 'recurrent niche invasion' model of bacterial speciation, lineages can change ecotypes very quickly (Kopac *et al.*, 2014). The lineages of different plasmid-based ecotypes are not irreversibly separate, as lineages are recurrently moving from one ecotype to another. However, the ecotypes themselves may persist indefinitely. Plasmid-determined ecotypes may be cohesive, since diversity within these ecotypes can be limited by periodic selection.

A longstanding question is whether the rate of ecological diversification is always so fast that even the closest relatives

are usually ecologically distinct (e.g., the species-less model), or whether diversification may be so slow that ecotypes hold huge populations of interchangeable organisms (stable ecotype model) (Doolittle and Zhaxybayeva, 2009). One possible determinant of the rate of diversification is metabolic plasticity (Cohan, 2016). Generalist heterotrophs such as *Bacillus* can easily change their ecological niche by acquiring a new gene or metabolic pathway and may thus speciate rapidly (Kopac *et al.*, 2014). On the other hand, photoautotrophs of the Cyanobacteria and Chlorobi utilize few organic resources and so have many fewer dimensions in which to diversify. Indeed, these two groups of photoautotrophs do appear to have diversified at a much slower rate than *Bacillus* (Becraft *et al.*, 2015; Bendall *et al.*, 2016).

It is not straightforward to identify the most newly divergent products of ecological speciation. Owing to the importance of horizontal genetic transfer in ecological speciation of prokaryotes (Gogarten and Townsend, 2005), one cannot anticipate the ecological dimensions by which ecological divergence occurs (Cohan and Perry, 2007). One promising approach to identifying ecotypes is based on finding closely related phylogenetic groups that are significantly different in the microhabitats from which they are isolated (Hunt *et al.*, 2008; Becraft *et al.*, 2011; Connor *et al.*, 2010). This approach is limited, however, by the investigator's knowledge about the ecological dimensions of speciation and whether speciation occurs principally through changes in microhabitat (Cohan and Koeppel, 2008).

Alternatively, systematists can attempt to identify the most newly divergent ecotypes even without knowing the ecological dimensions of divergence. Provided that there are periodic selection events purging the diversity within but not among ecotypes, ecotypes can be identified as sequence clusters for any gene(s) in the genome, using various algorithms (Francisco *et al.*, 2014; Koeppel *et al.*, 2008; Corander *et al.*, 2008; Barraclough *et al.*, 2009). Whether the most newly divergent ecotypes can be discovered as sequence clusters depends on the rate of speciation. In groups with extremely rapid speciation, such as *Bacillus* and perhaps other generalist heterotrophs, the resolution of the entire core genome may be required (Kopac *et al.*, 2014). However, in groups with slow speciation, such as *Synechococcus* and *Chlorobium*, even a single gene may provide the resolution to discover the newly divergent ecotypes (Becraft *et al.*, 2015).

Two genome-based approaches combine the advantage of providing molecular resolution to identify newly divergent ecotypes, while not requiring advance information about the ecology of speciation. First, if two lineages differ in their histories of positive selection on genes they share, one can reasonably conclude that the lineages are ecologically distinct (Vos, 2011; Kopac *et al.*, 2014). Alternatively, one can infer ecological distinctions among lineages that differ in the content of their genes, particularly when the dimensions of ecological divergence are implied from the genes unique to each lineage (Guttman *et al.*, 2014; Shapiro *et al.*, 2012).

These approaches do not require the cultivation of the species we seek to identify. Single-cell genome sequencing of uncultivated organisms has yielded the diversity of ecological species in *Prochlorococcus* (Kashtan *et al.*, 2014; Malmstrom *et al.*, 2013), and high-throughput sequencing of DNA from the environment (metagenomics) has yielded evidence of groups with species-like properties in *Chlorobium* (Bendall *et al.*, 2016).

Should all of the ecotypes in the prokaryotic world be named as species? This may be possible in groups where the origin of new ecotypes is slow, such as the photoautotrophs and perhaps the chemolithotrophs, with little metabolic plasticity. However, in rapidly speciating groups such as generalist heterotrophs and perhaps pathogens, the naming of diversity may have to be limited to ecological variants of particular interest to microbial ecology, public health, or biotechnology (Kopac and Cohan, 2011), and that are expected to have some permanence (Thompson *et al.*, 2014).

Conclusion

Species may be demarcated as clusters with a predetermined level of diversity, as in the practice of systematics. The high threshold of diversity stipulated by systematics has yielded unhelpfully diverse species, but the threshold could be changed to yield more homogeneous species taxa. Alternatively, demarcating species as units of recombination or as ecologically distinct populations yields homogeneous groups with at least some of the dynamic properties attributed to species. In either case, genomes provide an efficient way to discover diversity at the species level and to infer the ecological roles of each species.

See also: Bacterial Diversity, Introduction to. Recombination in Bacterial Populations. Species Concepts and Speciation

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Basic Science and Evolutionary Biology

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Glossary

Evolutionary trap When there is a phenotype–environment mismatch (see below), organisms can behave in maladaptive ways that can pose a serious threat to species survival.

GMO Genetically modified organism (GMO) is an organism whose genes have been altered using genetic engineering techniques.

Phenotype–environment mismatch This occurs when the environment in which an organism has evolved changes quickly, significantly, or both, so that the phenotype is no longer adapted to it.

Phylogenetic methods A broad category of statistical methods employed to determine the relatedness of species or types. These analyses can be done with morphological and/or genetic traits.

In 1993, an estimated 2.5 million people worldwide had AIDS (acquired immune deficiency syndrome), and many of the few lucky enough to get treatment were dying. Zidovudine, one of the first antiretroviral drugs developed to treat AIDS, would induce resistance in HIV, the virus that causes AIDS, and render the drug useless. However, by 1996, combination therapy of multiple antiviral medicines was dramatically increasing survival of HIV-infected patients. This standard combination therapy relied on knowledge that resistance mutations are rare and arise independently for each type of antiviral treatment. Using many treatments at once minimizes the chances that any one virus can evolve resistance in all fronts. This new standard of care, in use today in almost a third of the 35 million people infected with HIV, emerged as the direct consequence of our knowledge of evolution, in particular, how organisms adapt to their environment.

This is just one example among many of the relevance of evolutionary biology to society. The work of evolutionary biologists involves studying the mechanisms and consequences of evolutionary processes. However, as illustrated in the example above, their work is not entirely abstract. Beyond helping to redefine the boundaries of knowledge, understanding evolutionary principles is central to our health care, food safety, and biodiversity conservation.

Surprisingly, despite the direct connections between quality of life and application of evolutionary principles, these principles are not known or accepted as fact by some percentage of citizens in many countries around the world, but particularly in the USA, where only 30% of adults express a definite belief in evolution (Miller *et al.*, 2006). At the same time, government support for evolutionary biology research and other basic sciences is stagnant in the USA and Europe, barely keeping up with inflation (Brennan *et al.*, 2014a,b; Global R&D forecast by Batelle, 2014).

There is, therefore, a disconnect between the impact of basic evolutionary science, the government support that goes into it, and the public understanding of how knowledge of basic evolutionary principles affects their daily life. This article discusses the distinction between basic and applied science, the current state of funding for basic science, and gives some examples of the impact of evolutionary research, to make these connections explicit.

Impacts of Evolutionary Biology

In 2008, the National Research Council's Board on Life Sciences convened a committee to examine the current state of Biology, and make recommendations on how to use biological advances to further societal well-being. The report identified four areas where such advances are both necessary and achievable: increasing food security by developing new locally adapted plant crops, increasing the maintenance of ecosystems and biodiversity in a changing environment, expanding non-fossil fuel energy, and developing an individual approach to health care (Carroll *et al.*, 2014). A recent review on applied evolution identified two major causes of global challenges: rapid contemporary evolution and a phenotype–environment mismatch (Carroll *et al.*, 2014). Rapid contemporary evolution results in the emergence of pesticide and antibiotic resistance, while phenotype–environment mismatch contributes to low yield of food crops in a changing climate, and loss of biodiversity. Evolutionary biology principles that allow us to understand how organisms adapt to environmental change, and how they diversify, have played and will continue to play an important role in solving challenges in all these areas (Hendry *et al.*, 2011; Losos *et al.*, 2013; Carroll *et al.*, 2014).

Food Security

Feeding a growing population given current climatic unpredictability is a central challenge of the twenty-first century. The emergence of high yield crops in the 1950s was made possible by the application of evolutionary principles to plant breeding. However, the use of monocultures and genetically modified (GMO) crops, bred to produce higher yields, requires heavy pesticide use, and in recent years, yields have suffered from the emergence of pesticide resistance in species that attack such crops (Hendry *et al.*, 2011; Losos *et al.*, 2013; Carroll *et al.*, 2014). Heavy pesticide use has well-known detrimental effects to the environment and public health (Wilson and Tisdell, 2001). Further intensifying food production by continuing the current monoculture approach of both GMO and non-GMO crops is likely to cause more environmental damage. Therefore, an agricultural approach based on evolutionary theory and ecological principles that takes into account how the

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relationships between species affect their evolution offers a safer alternative for the environment. Such an approach can minimize the evolution of pesticide resistance and preserve biodiversity, while maintaining long-term productivity (Bommarco *et al.*, 2013).

At the same time, selective breeding of crops that can cope with unpredictable climatic conditions resulting from global climate change will be crucial to producing enough crops to feed the world (Hendry *et al.*, 2011; Losos *et al.*, 2013; Carroll *et al.*, 2014). This can involve the production of drought and flood tolerant genetically modified crops, as well as selective breeding and hybridization of crops that already have some resistance traits, such as flood-resistant rice currently cultivated in Bangladesh and India (Carroll *et al.*, 2014).

An interesting and relatively new avenue for evolution to affect food production is to apply selection at the level of the group rather than the individual. This approach decreases the individual fitness, but increases overall group productivity by decreasing selfish genes. This concept was applied to the breeding of poultry, where group selection to lower competitive interactions among laying hens resulted in higher egg-laying rates and lower mortality rates, and can also be applied to other domesticated animals (e.g., Wade *et al.*, 2010). In food crops, genes that produce individually larger roots or leaves can be selected against to favor smaller roots and leaves that can result in a higher yield for the group (Carroll *et al.*, 2014).

Preserving Biodiversity, and Predicting and Mitigating the Effects of Global Climate Change

The challenge of preserving biodiversity can be addressed by protecting habitats that are identified as being crucial to several species, or those that serve as nurseries or refuges for many species (e.g., mangroves and wetlands); by establishing captive breeding programs that specifically reduce inbreeding and maximize genetic diversity; and by recognizing which species are most likely to be threatened by changes in the environment given their low adaptability (Hendry *et al.*, 2011; Losos *et al.*, 2013; Carroll *et al.*, 2014). This latter approach can be particularly useful when considering the potentially negative effects of global climate change in wild populations. While some species may thrive, others will undoubtedly be at risk.

Non-Fossil Fuel Energy

One of the challenges of expanding alternative fuel sources such as wind and solar energy is anticipating and mitigating their potential impacts on wildlife. Collisions with wind turbines are a known cause of mortality for birds and bats, and avian mortality at solar panel farms has also been reported. Knowledge of how animals behave can help solve these potential problems. A recent report suggested that the impact to wildlife in terms of habitat changes and the turbines themselves is likely to be minimal (American Wind Wildlife Institute, 2014 report). However, knowledge that bats are more active at low wind speed has resulted in policy recommendations to switch off turbines when wind speed is low (Arnett *et al.*, 2010). A recent report on avian mortality at three solar powered facilities in California suggested that solar panels may be acting as 'evolutionary traps,' if they look like water that attracts insects and birds. A diverse array of evolutionary-based solutions can be implemented to reduce avian mortality at these sites, once the conditions that lead to mortality are known.

Health

In 2013, the CDC (Centers for Disease Control and Prevention) issued a report on antibiotic resistance threats in the United States. The list of potential threats is long, and divided into urgent, serious, and concerning microbial species. The much-publicized MRSA (methicillin-resistant *Staphylococcus aureus*) only reaches the serious level, while the relatively new CRE (carbapenem-resistant Enterobacteriaceae) makes the top of the list of urgent concerns (CDC, 2013). CRE comprise multiple bacteria species that have become resistant to carbapenems, the strongest antibiotics currently in use. Similar to the beginnings of the MRSA pandemic, CRE are currently limited to health care settings, hospitals, and elderly homes. However, in contrast to the 20% morbidity rate of MRSA, CRE causes deadly infection in 50% of those affected (CDC, 2013). These and many other antibiotic resistant superbugs constitute a serious health threat. Our best hope of managing not only their spread, but the emergence of further resistant strains is to apply evolutionary principles to our knowledge of their biology. For example, highly adaptable organisms when placed under strong selection can quickly evolve to mitigate the effects of selection on their survival. Rather than applying a continuous homogeneous source of strong selection, such as long-term single pesticide or antibiotic use, a better strategy to control the emergence of resistance often includes varying the selection regime in space or time (Hendry *et al.*, 2011; Losos *et al.*, 2013; Carroll *et al.*, 2014). Bacteria are highly adaptable because they can replicate quickly, and are adept at horizontal gene transfer, so individuals can pass on resistance genes to other unrelated individuals in the population, not only their descendants. For this reason, regulating and monitoring the use of the strongest antibiotics and using combination therapies as described for the treatment of AIDS can help to slow down the evolution of resistance (Carroll *et al.*, 2014).

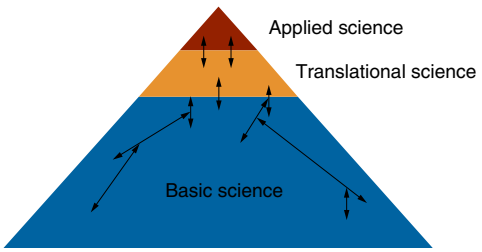


Figure 1 Applied science build upon the foundation of basic science. Though not all basic science projects contribute directly to an application, all applications require basic science. Translational science occurs at the interface between the two where targeted questions require specific basic scientific knowledge.

In addition, evolutionary medicine is helping us to understand and work with the body's naturally evolved defense mechanisms when faced with disease, and is even helping to understand aging and degenerative diseases (Wallace, 2005; Losos *et al.*, 2013). For example, morning sickness affects many pregnant women during the first trimester of their gestation, at the time when the fetus is most vulnerable to potential teratogenic compounds and infection in the mother could cause miscarriage (Profet, 1995). The types of food aversions that are typically shown by pregnant women ensure that foods that can potentially make them sick or affect the fetus are not consumed during this window, and women who suffer from morning sickness are known to have lower rates of miscarriage (Flaxman and Sherman, 2000; Flaxman and Sherman, 2008). This evolutionary understanding should prevent unnecessary treatment and help women understand the potential utility of morning sickness.

Justice System

Evolutionary methods relying in genetic analyses of samples have been used in trials in recent years. Phylogenetic analyses are now an accepted method to establish facts according to the United States criminal system (Losos *et al.*, 2013). This method has been used to determine whether intentional inoculation of HIV virus into victims occurred at the hand of doctors, by analyzing the relatedness of viral strains while accounting for their evolution in the body of infected patients (Metzker *et al.*, 2002; Scaduto *et al.*, 2010).

Basic and Applied Science

The benefits of science to the advance of society are undeniable, and yet sometimes it can be difficult to see the benefit of

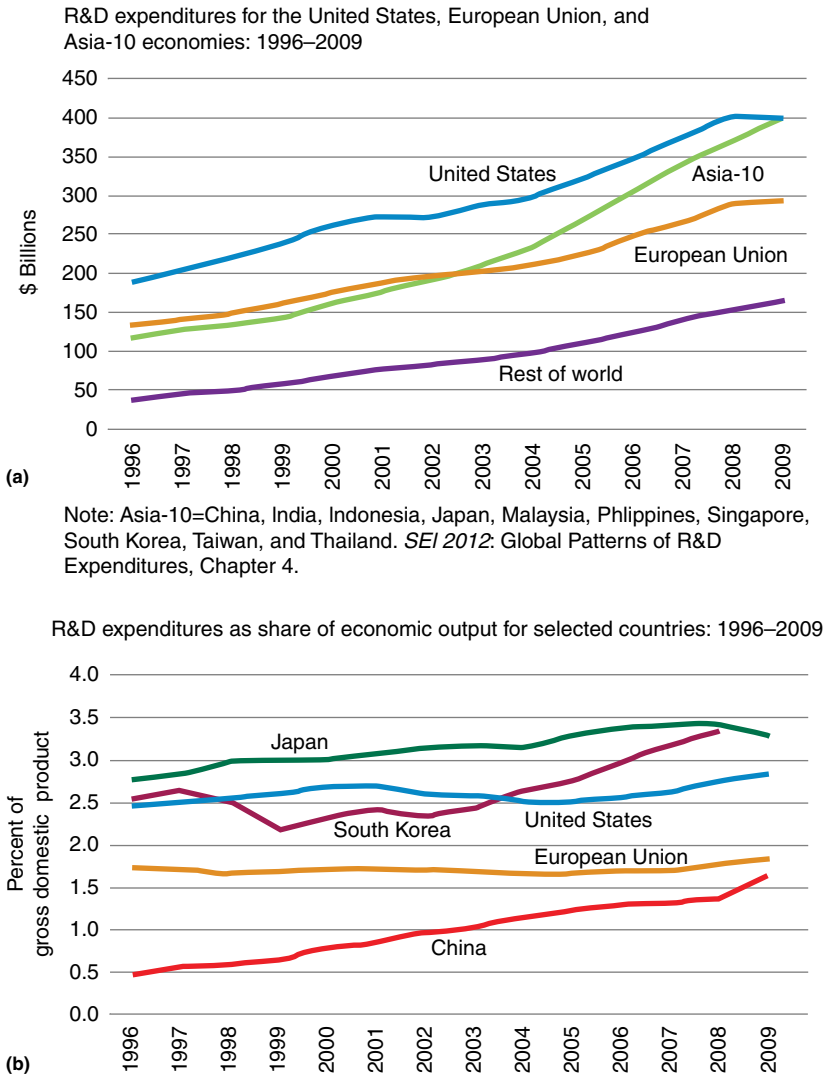


Figure 2 USA expenditure in research and development both in absolute number of dollars (a) and as a percentage of GDP (b). Reproduced from National Science Board's Science and Engineering Indicators Digest, 2012. Available at: http://www.nsf.gov/statistics/digest12/nsb1202.pdf (accessed 05.10.15).

individual studies. This is because many scientific projects are fundamental or basic science, that is, they are driven only by the desire to increase knowledge and redefine its boundaries. This fundamental knowledge is the basis upon which applied sciences are built. Applied science, on the other hand, is problem driven, seeking to find solutions to specific problems. At the interface between the two is translational (also known as developmental) science, that targets promising fundamental science for development into an application. Both translational and applied science result in incremental advances and progress because they seek to answer specific questions in a targeted manner. In contrast, basic science that does not have a specific problem to solve can have a transformative effect and result in groundbreaking innovation: completely novel ideas that fundamentally change or create new fields of inquiry, or result in a completely new technological approach.

The relationship between basic, translational, and applied science can be illustrated by a pyramid graphic (**Figure 1**). The base of the pyramid is basic science, comprising the majority of our scientific knowledge, and the apex illustrates the applications. The applications are built upon the basis of fundamental science. Though not all of basic science contributes to applied science, all of applied science 'requires' the existence of a broad base of basic knowledge, and critically, it is impossible to predict a priori which basic science will in fact turn out to be useful in solving a particular problem. An alternative scenario is to imagine a tree, where the fruits are the application of science, and the rest of the tree, the basic knowledge required to produce the fruit. Regardless of what the most fitting illustration of these connections may be, the important message is that without basic science, there would be no progress. Because we can't predict which projects will be used in solving a specific problem, the best strategy is to continue to support basic science that expands our knowledge of how nature works.

Scientific unpredictability derives in part from the interconnected nature of science. Many scientific breakthroughs occur because of unforeseen connections between unrelated projects within a discipline, or between unrelated fields. Recognition of the unpredictable relationship between basic and applied science led to the recent creation of the Golden Goose Award by the American Association for the Advancement of Science (AAAS), in partnership with other organizations. Dr. Paul Schanberg and members of his lab, as well as Dr. Tiffany Field, were awarded the Golden Goose Award in 2014. The work of Dr. Schanberg involved studying growth of rat pups. His team found that when they separated rat pups from their moms, they stopped growing. After a series of experiments, they identified that mechanical stimulation from the mom licking the babies was needed to elicit growth. Dr. Field was a psychologist studying growth in human infants, and after a chance meeting with Dr. Schanberg, she decided to explore the possibility that touch may also be important for human baby growth. Her research lead to the now well-established infant massage technique, that introduced regular massage and contact to babies born prematurely. This technique has saved the lives of countless babies, as well approximately $4.7 billions on healthcare costs. Dr. Schanberg would have never predicted that his work would result in such a remarkable application.

Another example is the work of Professor Craig Tovey, who inspired by a talk on the biology of honeybees went on to develop an algorithm to determine how close to optimality the bees were at gathering nectar. He found that through their 'waggle dance' behavior to recruit foragers to the nectar sources, honeybees were within 8% of their optimal nectar gathering ability. Almost 10 years after he concluded this research, Sunil Nakrani, a PhD student from Oxford came to seek Prof. Tovey's advice in solving the problem of developing a web-hosting algorithm to optimally allocate bandwidth to different client's needs. Prof. Tovey immediately saw the potential use of his honeybee research to solve this problem. The honeybee algorithm that Tovey and Nakrani developed, improved the efficiency of server allocation drastically, leading to an increase of 5–25% in revenue generation by the servers (Nakrani and Tovey, 2007). Due to the competitive advantage gained through the implementation of such algorithms in industry, it is hard to quantify the economic impact of Prof. Tovey's research on honeybee foraging. However, algorithms similar to the honeybees are continuously being developed for many different applications. What started as a curiosity has become fundamental to the efficient use of the internet.

The National Research Council's (US) (2009) report on Biology for the twenty-first century explicitly sought out to apply current knowledge of biology to some of the most pressing societal problems. However, it explicitly acknowledged that its success depends on fundamental research. Even as evolutionary biology has been widely applied for the health

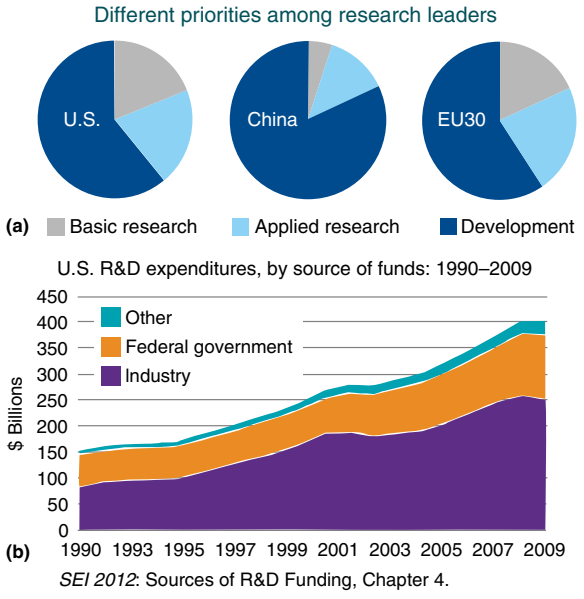


Figure 3 Investment in Basic Science is much smaller in China, the only nation with rapidly increasing investment in R&D. (a) Reproduced from Global Research and Development Forecast, 2014 (Funding sources for all R&D in the USA) Produced by Batelle. Available at: http://www.battelle.org/docs/tpp/2014_global_rd_funding_forecast.pdf (accessed 05.10.15). (b) Reproduced from National Science Board's Science and Engineering Indicators Digest (2012). Available at: http://www.nsf.gov/statistics/digest12/nsb1202.pdf (accessed 05.10.15).

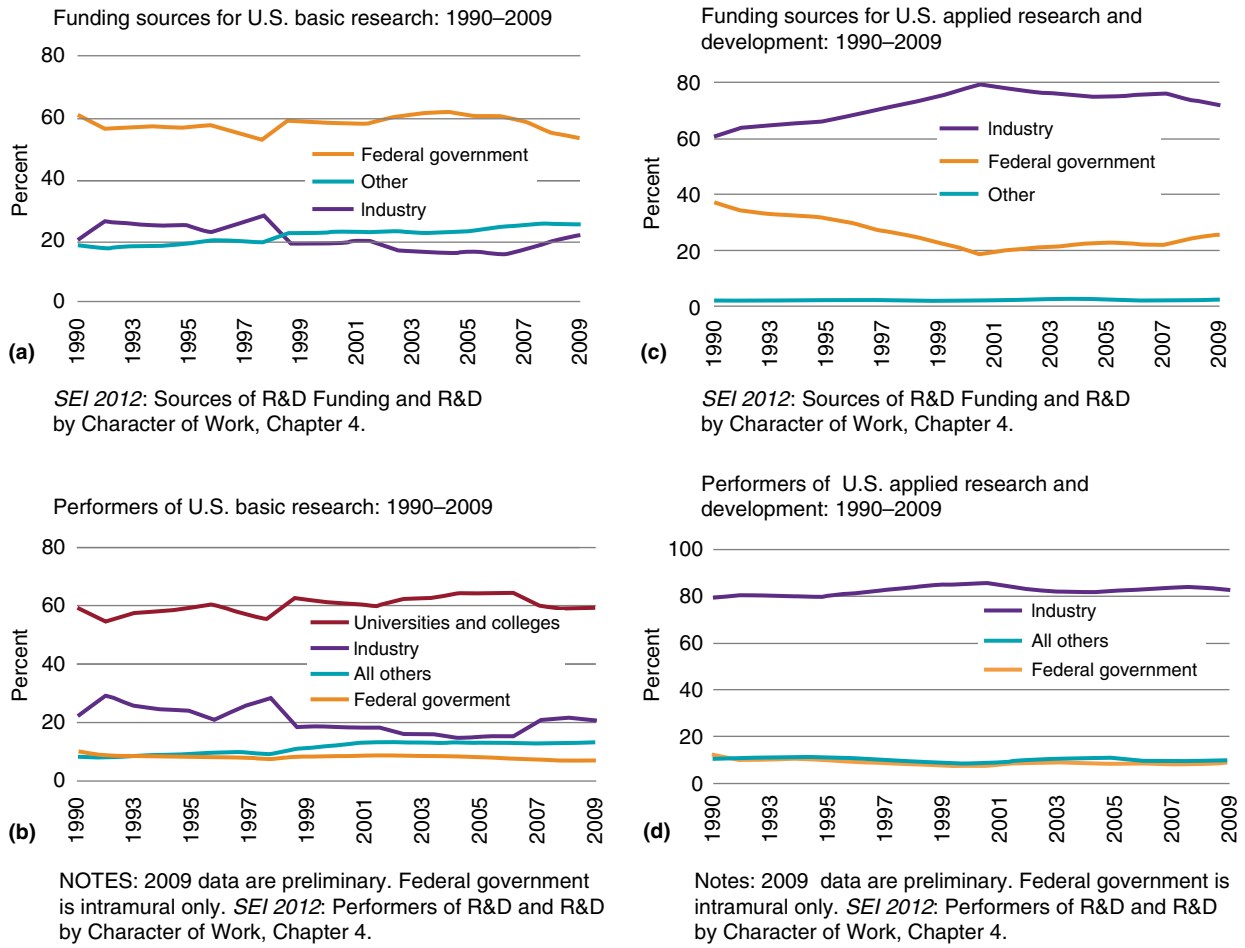
and economic benefit of society as discussed above, most research is conducted without such applications in mind.

Funding for Basic Science

The USA has traditionally invested more money than any other country in research and development (**Figure 2(a)**). However, the percent of gross domestic product (GDP) that the USA invests in research and development (R&D) has been steadily declining over the past few years (**Figure 2(b)**), and currently nine other nations invest a higher percentage of their GDP in R&D, including Israel, Finland, Sweeden, South Korea, and Japan. China, India, and Brazil, the world's fastest growing economies, have been steadily increasing their investment in R&D. With the current trends, they will surpass USA investment within a decade (National Science Board Digest, 2012; Global R&D forecast by Batelle, 2014). Europe has also been steadily declining its investment in R&D, and the current weak economic trends in both the USA and Europe suggest that increased investment is unlikely in the near future (Global R&D forecast by Batelle, 2014). Both the USA and Europe have typically invested close to 20% in basic science, while Chinese investment in basic science is barely 5% (Global R&D forecast by Batelle, 2014, Batelle Institute; **Figure 3(a)**). Therefore, lack of growth of R&D funding in the USA and Europe will disproportionately impact basic research around the world.

The money the USA invests in R&D includes investment by industry, the federal government, as well as a small (but growing) proportion being spent by private individuals (**Figure 3(b)**). In 1990, the federal government in the USA contributed nearly 50% of the R&D expenditure of $150 billion. In 2009, the figure was closer to 30% of the nearly $400 billion spent ($135 billion) (National Science Board Digest, 2012), and even this reduced investment has elicited political battles in Washington D.C.

In the USA, the federal government is the source of funding for the majority of the basic science, particularly through funding the National Science Foundation (2012). However, NSF is not charged only with funding basic science, but many scientific projects at large, many of them translational. Most basic research is performed at universities and academic institutions. Therefore, cuts and reduced growth of the basic science budget primarily affect academia (**Figures 4(a)** and **4(b)**). In contrast, applied science is funded and performed largely by industry (**Figures 4(c)** and **4(d)**). Out of the $144



SEI 2012: Sources of R&D Funding and R&D by Character of Work, Chapter 4.

SEI 2012: Sources of R&D Funding and R&D by Character of Work, Chapter 4.

NOTES: 2009 data are preliminary. Federal government is intramural only. *SEI 2012*: Performers of R&D and R&D by Character of Work, Chapter 4.

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Figure 4 Funders and performers of basic and applied science. Most basic science is funded by the government (a), and performed at academic institutions (b), while most applied science is funded by industry (c), and performed by industry (d). Reproduced from National Science Board's Science and Engineering Indicators Digest, 2012. Available at: http://www.nsf.gov/statistics/digest12/nsb1202.pdf (accessed 05.10.15).

billion allocated to the R&D budget in 2014, only $6.3 billion were allocated to NSF. The Biological directorate budget went from $511.14 million in 2001 to $678.93 million in 2013, which is equivalent to $514.83 million in 2001 (Brennan *et al.*, 2014a,b).

Despite the low priority that basic science funding is getting from the government, a recent Pew Research Center survey found that 71% of the public believes that government investment in basic science pays off in the long run. However, even as 60% of adults surveyed stated that government investment in science is essential for progress, a full 34% believe that private investment will be enough. This section of the public is likely unaware of the distinction between basic and applied science, and that private funds already contribute the majority of the money spend in science in the USA as shown in Figure 4. Government investment in science is needed not only to fund basic science performed in universities and academic institutions, but this science is also generally free of special interests, and available for the benefit of the general public.

Even as funding for basic science and the Biological science directorate at the NSF has stagnated, government-led programs to increase the appeal of STEM fields have been very successful in the USA. In 2011, roughly 36 000 STEM PhDs were awarded in the USA, almost double the number in 1982 (19 000 PhDs) (Schillebeeckx *et al.*, 2013). As a result of this increase of available scientists in STEM fields, competition for the shrinking pool of money at NSF (and the National Institutes of Health, NIH) has become fierce, and funding rates in some programs are as low as 3.5% when the number of submitted pre-proposals is taken into account. According to the NSF's Fiscal Year 2012 report: "The number of investigators submitting proposals has grown steadily over the past decade at a rate that exceeds the rate of growth of NSF's normal appropriation in inflation adjusted dollars. Consequently, the success rate of PIs has declined … The number of PIs who submitted proposals in 2010–2012 was 42% higher than the number in 2001–2003." As a result of increased competition for funding in the USA, many excellent scientists have found it difficult to continue their research programs, leading to both laboratory closures (Wadman, 2009; Startford, 2013), and the emigration of young scientists to pursue better research opportunities abroad (Rae-Dupree, 2013; Non-defense discretionary science survey, 2013). Many scientists around the world (36% in the USA and 29% elsewhere) cite lack of external funding as the main challenge to conducting research (Global R&D forecast by Batelle, 2014).

Public Perception of Evolutionary Science

A Pew research center poll in 2009 found that 61% of the general public in the USA agrees that humans and other creatures have evolved over time, but only 32% agree that this change is the result of natural processes, while 22% believe the change to be directed by a supreme being, and 31% believe that everything has existed in its present form since the beginning of time. A more recent Pew Research Center survey (2015) on public and scientists' views of science and society revealed large gaps between the percentage of scientists who believe science-related statements, and the percentage of the public who do. When asked whether humans have evolved over time, 98% of scientists responded yes, whereas only 65% of adults from the general public did, not much of a change since 2009. The United States has the lowest agreement with evolution than any other developed country in the world, but most countries have some percentage of evolution deniers (Figure 5).

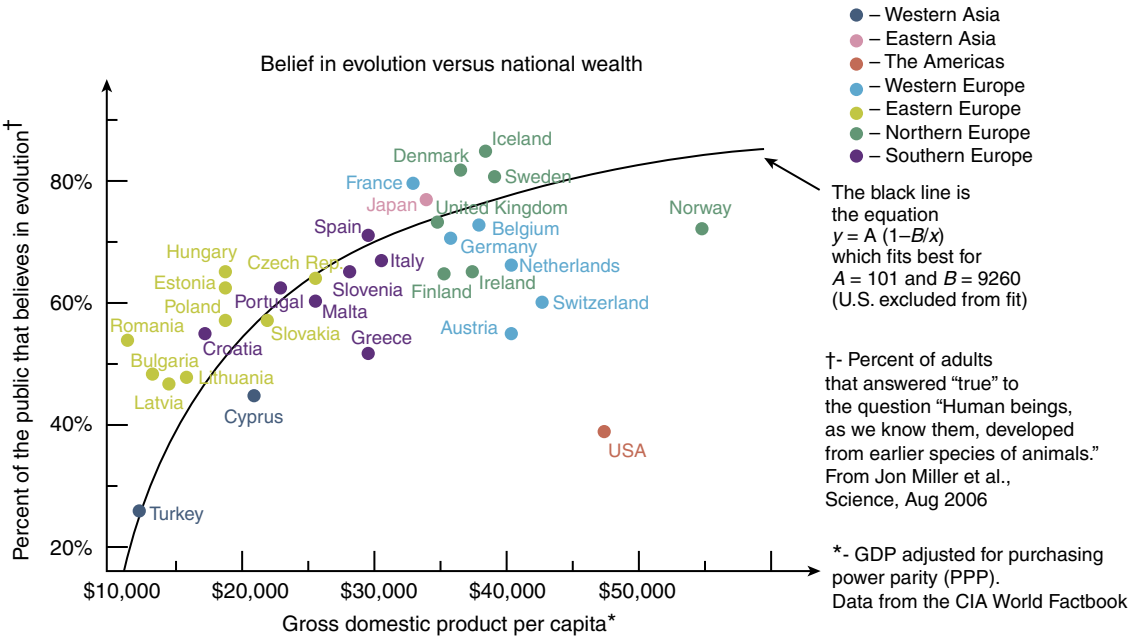

Figure 5 The USA has the lowest belief in evolution of any developed nation in the world. Reproduced from http://www.data360.org/image_view.aspx?Uploaded_Image_Id=1246 (accessed 08.10.15).

Perhaps the perception that there is not complete agreement among scientists over all the details of how evolution proceeds, contributes to public skepticism of science as shown in debates over climate change (Aklin and Urpelainen, 2014). In response, perhaps it is best to simply quote Adam Gopnik: "Darwinism is true in the complex sense that scientific theories always are – not fixed in its particulars, immutable and imposing, but rich, changing, and evermore explanatory" (Gopnik, 2015).

Evolutionary biology is a fundamental scientific field of study that has impacted and will continue to impact every major area of human well-being. Basic evolutionary research is crucial to our continued ability to improve human health, agriculture, and conservation.

See also: Evolutionary Computation. Evolutionary Medicine I. An Overview and Applications to Cancer. Evolutionary Medicine II. Use of the Comparative Method and The Animal Model. Evolutionary Medicine III. Mismatch. Pest Management, Evolution and. Philosophy, Evolutionary Biology and. Responses to Climate Change, Evolution and. Security, Evolution and

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Bayesian Phylogenetic Methods

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Introduction

Bayesian statistics has the unique feature that uncertainties in all unknowns (such as the unknown parameters in a model or the competing hypotheses for explaining the same data) are described using statistical distributions. In classical statistics (such as the maximum likelihood method), parameters and hypotheses cannot be assigned distributions. Suppose one wants to analyze the data (x) to estimate the unknown parameter θ under a model. In a Bayesian analysis, one assigns a distribution on θ before the analysis of the data. This is called the 'prior distribution' and reflects one's knowledge or belief about the possible values of θ. The Bayesian analysis of the data then produces the distribution of θ given the data, $f(\theta|x)$, called the 'posterior distribution.' The two are related through the Bayes theorem

$$f(\theta|x) = \frac{f(\theta)f(x|\theta)}{f(x)} \propto f(\theta)f(x|\theta) \quad [1]$$

Here the probability of the data given the parameter θ, $f(x|\theta)$, is the likelihood, and represents the information about the parameter θ in the data x. The marginal probability of the data, $f(x) = \int f(\theta)f(x|\theta)\mathrm{d}\theta$, is a normalizing constant, and its role is to ensure that $f(\theta|x)$ is a proper statistical distribution and integrates to 1. Equation [1] thus says that the posterior is proportional to the prior times the likelihood, or equivalently, the posterior combines information in the prior and in the data sample.

Note that the likelihood function is the basis for classical statistical methods, especially the maximum likelihood method. Thus all models developed for the maximum likelihood method can be implemented in the Bayesian framework. In analysis of large datasets, the two methods often produce numerically very similar results even though the interpretations differ. However, different results may be obtained by the two methodologies if the data are not informative, and in particular, if the focus of the analysis is on model selection.

In molecular phylogenetics, the data x is an alignment (or alignments) of sequences of nucleotides, codons, or amino acids from several species. Here, we assume that the sequences are already aligned and we ignore alignment errors. Our focus is the phylogenetic tree, which consists of the tree topology (τ) and the lengths of branches (denoted collectively as $\boldsymbol{b}$). The branch length is measured by the expected number of substitutions per site, and quantifies the amount of evolution along the branch. Given the tree, the sequence data at the tips of the tree (for extant species) are the product of the process of sequence evolution along the branches. This process is typically described by a continuous time Markov chain (Felsenstein, 1981). The model of substitution may include additional parameters, denoted ϕ, such as the relative substitution rates between nucleotides and the equilibrium frequencies of the nucleotides. More complex models may include parameters to describe the rate variation across sites in the sequence or the nonsynonymous/synonymous substitution rate ratio in comparisons of protein-coding gene sequences (Yang, 1993; Goldman and Yang, 1994). For more details on various models used in molecular phylogenetics.

The posterior distribution of the tree topology, branch lengths, and substitution parameters is then given by eqn [1] with parameter θ replaced by τ, $\boldsymbol{b}$, and ϕ:

$$f(\tau,\boldsymbol{b},\phi|x) \propto f(\phi)f(\tau,\boldsymbol{b})f(x|\tau,\boldsymbol{b},\phi) \quad [2]$$

Here $f(\phi)$ is the prior distribution on substitution parameters, $f(\tau, \boldsymbol{b})$ is the prior on tree topology and branch lengths, while $f(x|\tau, \boldsymbol{b}, \phi)$ is the likelihood or probability of the sequence data given the tree topology and branch lengths, given by the model of sequence evolution (Felsenstein, 1981).

The Bayesian approach to molecular phylogenetics was introduced by Rannala and Yang (1996), Yang and Rannala (1997), Mau and Newton (1997), and Li *et al.* (2000). The early studies used simple models of sequence evolution and assumed a constant rate of evolution (the molecular clock). Nowadays, we have several Bayesian phylogenetic programs that implement a wide range of complex models that account for various aspects of the sequence data. General Bayesian programs for phylogeny reconstruction include MrBayes (Ronquist *et al.*, 2012), BEAST (Drummond and Rambaut, 2007), and PhyloBayes (Lartillot *et al.*, 2009). A number of Bayesian programs are also available for estimating species divergence times incorporating information in both fossils and molecules, such as MCMCTREE (Yang, 2007) and DPPDIV (Heath *et al.*, 2012).

For an extensive discussion of Markov chain Monte Carlo (MCMC) algorithms used in Bayesian phylogenetics, see Chapters 7 and 8 of Yang (2014). The edited book by Chen *et al.* (2014) summarizes recent developments, especially concerning model selection in Bayesian phylogenetics.

Priors

The prior distribution is supposed to summarize one's objective information (according to 'Objective Bayesian') or personal beliefs (according to 'Subjective Bayesian') about the likely values of the model parameters. In Bayesian phylogenetics, the tree topologies (τ) represent discrete statistical models, the branch lengths ($\boldsymbol{b}$) are continuous parameters that are defined only on specific trees, while the substitution parameters (ϕ) are often defined for all possible trees. The parameter space of the inference problem is high-dimensional and also complex. Specification of the prior is thus a nontrivial task. Indeed, a few cases have been identified in which innocent-looking priors adopted in common Bayesian programs lead to unreasonable extreme results.

Here we describe a few commonly used prior distributions in Bayesian phylogenetics. First we consider the prior on the tree topology. Most phylogenetic analyses are conducted

without assuming the molecular clock and use unrooted trees. The total number of unrooted trees T_n for n species is

$$T_n = (2n-5)(2n-7)\cdots 1 \quad [3]$$

It is common to assign a uniform prior on all possible trees, with each assigned the probability $1/T_n$.

If the species are closely related, the evolutionary rate may be roughly constant among species. One can then use the molecular clock to infer rooted trees. Rooted trees are also used to infer species divergence times in the so-called molecular clock or relaxed-clock dating analysis. A prior distribution over the rooted trees and node ages (branching times) can be generated using a model of cladogenesis. For example, a birth–death process conditioned on the number of observed or sampled species can be used to describe the biological process of speciation and extinction, and to generate a prior on the rooted tree topologies and node ages. The birth–death process includes the Yule pure-birth process as a special case. Parameters for the birth–death model include the birth rate, the death rate, and the sampling fraction (the proportion of extant species that are actually included in the data). Those parameters in the prior can be changed to assess the impact of the prior on the posterior inference, or they may be estimated from the data by assigned prior distributions on them (called 'hyper-priors').

For DNA sequences sampled from the same species, Kingman's (1982) coalescent process provides a prior distribution for the gene genealogies. However, this is not a suitable prior model for inferring species phylogenies.

Next, we consider the prior for branch lengths. A binary unrooted tree for n species has $2n-3$ branches. Given each unrooted tree topology, the $2n-3$ branch lengths can be assigned independent and identical distributions (i.i.d.) such as the uniform or exponential. In the case of the uniform, an upper bound (such as 100) is specified by the user. However, those i.i.d. priors on branch lengths have been found to be problematic, as they may be very informative and unreasonable about the tree length (sum of branch lengths) (Rannala *et al.*, 2012). For example, a tree of 100 species has 197 branch lengths. If each is assigned the uniform prior U(0, 100), the tree length will have the prior mean 9850 and the 99% prior interval (8806, 10 894), with ~10 000 substitutions at an average site. When the data are not very informative (as is the case when the sequences are highly similar), this unreasonable prior can overwhelm the Bayesian analysis and leads to unreasonably long trees with large tree lengths (Brown *et al.*, 2010). An alternative has been suggested to fix this problem (Rannala *et al.*, 2012; Zhang *et al.*, 2012), in which a gamma prior is assigned to the tree length and then the sum is partitioned into branch lengths according to a uniform Dirichlet distribution (a multivariate extension of the uniform distribution).

Markov Chain Monte Carlo

Note that the normalizing constant $f(x)$ in eqn [1] involves an integral. When there are many parameters in the model, this integral will be multidimensional and may be very hard to compute. Modern Bayesian inference is often achieved through a computational algorithm called MCMC. This is an iterative simulation algorithm that generates a sample from the posterior distribution $f(\theta|x)$.

Here we illustrate the main features of the MCMC algorithm by applying it to the simple phylogenetic problem of estimating the distance θ between two sequences under the JC69 model (Jukes and Cantor, 1969). The data consist of the human and orangutan mitochondrial 12S rRNA genes, with $x=90$ differences at $n=948$ sites. The parameter θ is the expected number of nucleotide substitutions per site between the two sequences. Given θ, the likelihood or the probability of observing the data is given by the binomial probability

$$f(x|\theta) = p^x(1-p)^{n-x} = \left(\tfrac{3}{4} - \tfrac{3}{4}e^{-4\theta/3}\right)^x \left(\tfrac{1}{4} + \tfrac{3}{4}e^{-4\theta/3}\right)^{n-x}, \quad [4]$$

where $p = \frac{3}{4} - \frac{3}{4}e^{-4\theta/3}$ is the probability that a site is occupied by two different nucleotides in the two sequences separated by a distance θ. We assign a uniform prior on θ in the range (0, 1) so that $f(\theta)=1$ for $0<\theta<1$. The posterior is then given by eqn [1] as

$$f(\theta|x) = \frac{1}{f(x)} f(\theta) f(x|\theta) = \frac{1}{f(x)} \left(\frac{3}{4} - \frac{3}{4}e^{-4\theta/3}\right)^x \left(\frac{1}{4} + \frac{3}{4}e^{-4\theta/3}\right)^{n-x} \quad [5]$$

The following algorithm generates a sample from this posterior distribution.

1. Initialize: $n=948$, $x=90$, $w=0.25$. Set initial state: $\theta=0.1$, say.
2. Loop
   a. (Propose a new value θ^*.) Generate $u \sim U(0, 1)$ and set $\theta^* = \theta + w(\frac{1}{2} - u)$. Note that θ^* is a uniform random variable over the interval $U(\theta - \frac{w}{2}, \theta + \frac{w}{2})$. If $\theta^* < 0$, set $\theta^* = -\theta^*$.
   b. (Accept or reject the proposed value.) Compute the posterior density ratio $\alpha = \frac{f(\theta^*|x)}{f(\theta|x)} = \frac{f(\theta^*)f(x|\theta^*)}{f(\theta)f(x|\theta)}$. If $\alpha > 1$, accept θ^*. Otherwise accept θ^* with probability α. This can be achieved by drawing another random number $v \sim U(0, 1)$, and accepting θ^* if and only if $v < \alpha$. If θ^* is accepted set $\theta = \theta^*$. Otherwise set $\theta = \theta$.
   c. Print out θ.

It is easy to see that the algorithm simulates a Markov chain; the next θ value the algorithm will visit depends on the current θ only, but not the θ values visited in the past. Second, the algorithm tends to visit θ values with high posterior more often than θ values with low posterior. Indeed, the probability that the visited θ value is in the interval $(\theta, \theta + \Delta\theta)$ is $f(x|\theta)\Delta\theta$. In other words, the θ values generated by the algorithm constitute a sample from the posterior distribution $f(x|\theta)$. Lastly, there is no need to compute the normalizing constant $f(x)$ of eqn [5] since it cancels in the calculation of the posterior ratio α in Step 2b. This is the feature that allows us to avoid the calculation of the high-dimensional integrals, making it possible to implement sophisticated parameter-rich models that may not be feasible for maximum likelihood implementation.

Figure 1(a) shows the paths of two Markov chains from two runs of the algorithm, using different starting positions. Figure 1(b) shows the histogram and smoothed density estimate of posterior using a large sample from a long chain.

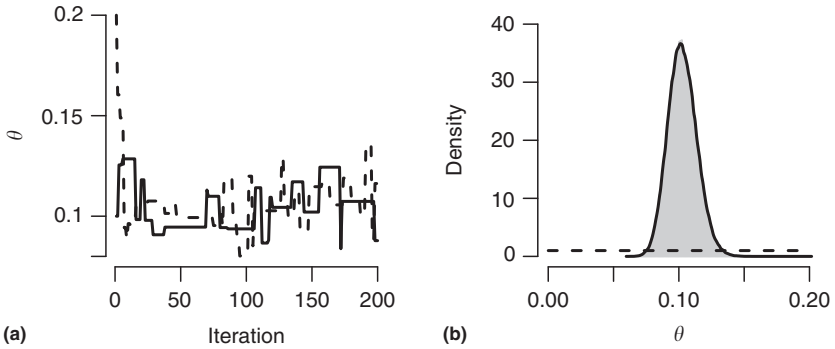

Figure 1 Markov chain Monte Carlo algorithm to sample from the posterior for the JC69 distance θ between two sequences. (a) Trace plot of two chains which started from different positions (0.1 and 0.2), each run over 200 iterations. (b) Histogram (shaded area) and smoothed density (solid curve) of the posterior sample obtained by running the algorithm over 10^6 iterations. The prior (dashed line) is shown as well for comparison.

The posterior mean is 0.1027, standard deviation is 0.0110, and the central 95% posterior credibility interval is (0.0824, 0.1253). In comparison, the famous JC69 distance formula gives the maximum likelihood estimate to be $\hat{\theta} = 0.1015$.

In phylogenetic reconstruction, the parameter space consists of several components: the tree topology τ, the branch lengths $\boldsymbol{b}$, and the substitution parameters ϕ. In each iteration, the different components may be updated in turn. For example, variants of tree search algorithms such as nearest neighbor interchange (NNI) and subtree pruning and regrafting (SPR) can be used to update the tree topology. The branch lengths and substitution parameters can be updated using sliding windows, as in the simple MCMC algorithm above. See Chapter 8 of Yang (2014) for a detailed discussion of MCMC proposal algorithms in phylogenetics. The phylogenetic MCMC algorithm generates a sample from the joint posterior distribution of the tree topologies (τ), the branch lengths ($\boldsymbol{b}$), and the substitution parameters (ϕ).

Output Analysis from Simulation

The MCMC sample from the posterior distribution can be summarized in different ways.

For scalar parameters such as branch lengths ($\boldsymbol{b}$) and substitution parameters (ϕ), the posterior means or medians are often used, together with the 95% posterior credibility intervals (CIs). Two types of intervals are commonly used. The 95% central (equal-tail) CI lies between the 2.5% and 97.5% quantiles of the posterior sample. The highest posterior density (HPD) CI includes values that make up 95% of the posterior probability and that have the highest posterior density. When the data are informative so that the posterior of the parameter is nearly symmetrical, the two intervals will be nearly identical. Otherwise they can be very different. The HPD interval is generally preferred over the equal-tail interval since it has the shortest length and includes only the most likely parameter values.

For the tree topology, a simple summary is the 'maximum a posteriori' (MAP) tree, which is the tree topology with the highest posterior probability (that is, the tree topology that is most visited during the MCMC algorithm). This gives a point estimate of the true tree. However, when the data are not very informative, the MAP tree may have a very low posterior probability, and is a poor summary. We also have an analogue of interval estimates for trees. The 95% credible set of trees contains those trees that have the highest posterior probabilities such that the total probability of the entire set is at least 95%. However, if this set contains a large number of trees, it will not be very useful.

The most commonly used summary is the so-called majority-rule consensus tree. Note that each internal branch defines a split (a bipartition) of the species. The majority-rule consensus tree includes splits that appear in at least half of the trees sampled, with the posterior probability of each split indicated on the internal branch of the tree. For more details on constructing consensus trees.

See also: Consensus Methods, Phylogenetic. Directed Evolution, History of. Maximum Likelihood Phylogenetic Inference. Molecular Evolution, Models of. Phylogenetic Invariants. Searching Tree Space, Methods for

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Biogeography, Conservation

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Conservation Biogeography – Origins and Aims

The field of Biogeography is devoted to understanding the spatial distribution of species and their shifts over time. As such, Biogeography is central to conserving biodiversity on Earth (Heaney and Lomolino, 2009). Conservation Biogeography emerged from the fusion of Biogeography and Conservation Biology, as an applied science seeking to preserve biodiversity through informed management decisions at regional, continental, and global scales (Whittaker *et al.*, 2005). To this end, Conservation Biogeography applies biogeographical methods, principles, and theories to case studies related to the conservation of biodiversity (Richardson and Whittaker, 2010). From its onset, Conservation Biogeography has focused on the development of context-explicit plans, especially maps, to identify priority areas for protection based on patterns of species distribution and biodiversity metrics (Dinerstein *et al.*, 1995; Long *et al.*, 1996). Data and methods that measure and map species diversity, endemism, and human-imposed threats have been central to the establishment to the discipline (e.g., Olson and Dinerstein, 1998; Myers *et al.*, 2000).

Insights from biogeographical studies have long been applied to conservation. The 1970s, for instance, witnessed a lively debate about the equilibrium theory of island biogeography and its application to Conservation Biology (MacArthur and Wilson, 1967). At that time, particular focus was given to whether the theory supported the establishment of large biological reserves worldwide, as opposed to several small protection areas of the same total size (the SLOSS debate; Simberloff and Abele, 1982). On one hand, biogeographic principles were used to justify an expectation of reduced levels of extinction in large reserves (Wilson and Willis, 1975; IUCN, 1980). On the other side, multiple small protected areas, when dispersed across geography, were known to complement each other in species composition and collectively hold more species. It was only in the mid-1980s, when species-specific ranges and population sizes became broadly acknowledged as important determinants of extinction (and hence central for reserve planning; Soulé and Simberloff, 1986), that the emphasis on a 'one-size-fits-all' approach to reserve design was abandoned.

Large-scale efforts to map species distributions and guide management and policy started in the mid-1990s, driven by nongovernmental organizations actively engaged in conservation (see Dinerstein *et al.*, 1995; Long *et al.*, 1996; Myers *et al.*, 2000, for studies by The World Wildlife Fund, BirdLife and Conservation International, respectively). At that time, much attention was given to how and where to design biological reserves while ensuring the maximum amount of biodiversity representation and complementarity under small social and economic costs (Csuti *et al.*, 1997; Pressey *et al.*, 1997; Edwards *et al.*, 1998). At the core of those analyses laid a vision to compile and combine information about the diversity and the geographical distribution of many large groups of species – providing general snapshots of the state of biodiversity. Mapping efforts such as those have been flagged as one of the most influential products of Conservation Biology (Whittaker *et al.*, 2005). By guiding management through targeted fund raising, landscape restoration, or reserve implementation, maps of diversity patterns and diversity metrics have effectively bridged scientific knowledge and action on the ground, liaising scientists, policy-makers, and stakeholders (Dalton, 2000; Myers and Mittermeier, 2003).

Yet it did not take long for Conservation Biogeography to expand within the academic world. The early 2000s witnessed an explosion of studies of the distribution of biodiversity at local and regional scales, often targeting comparatively small taxonomic groups in well-defined yet understudied ecosystems. The diversity of datasets compiled and analyzed in the beginning of the new century was noteworthy: from analyses of amphibians of the Brazilian Cerrado (Diniz-Filho *et al.*, 2006) to vertebrates of Northeast India (Pawar *et al.*, 2007), snakes of China (Chen, 2009), oak trees in Middle America (Torres-Miranda *et al.*, 2011), Mediterranean Islands butterflies (Dapporto and Dennis, 2009), and terrestrial and marine species in Antarctica (Terauds *et al.*, 2012), to mention a few. As a result of these efforts and of the pioneer NGO initiatives, government agencies worldwide began to employ maps of biodiversity metrics, human impacts, and biogeographical realms into conservation and prioritization plans (see MMA, 2002 for protocols applied in Brazil, for instance). Collectively, these academic and case studies built from and contributed to an unprecedented increase in the amount and quality of georeferenced locality data from species across the globe (several now publically available through online repositories such as The Global Biodiversity Information Facility, Flemons *et al.*, 2007; Vertnet, Guralnick and Constable, 2010; among others), worldwide environmental descriptors (e.g., Bioclim, Hijmans *et al.*, 2005; CliMond, Kriticos *et al.*, 2012), and novel correlative methods to model species distributions based on environmental features (e.g., Phillips *et al.*, 2006). Importantly, however, the growth of Conservation Biogeography over the past 10–15 years allowed the field to expand its methodological approaches, aims, and ties with other scientific disciplines.

Evolving Maps

The biodiversity metrics and variables mapped by Conservation Biogeography studies have unquestionably evolved during the last decade. Going beyond analyses of species diversity, recent research demonstrated the relevance of mapping the distribution of phylogenetic diversity in geographic space, assessing, for instance, the proportion of the Tree of Life represented in a given area or reserve system (see Carvalho *et al.*, 2010 for a study of Cerrado mammals, McGoogan *et al.*, 2007 for African primates). Estimates of phylogenetic diversity and

phylogenetic (even phylogeographic) endemism have also expanded the classic use of species diversity and endemism measures (Chen, 2009; Carnaval *et al.*, 2014).

By incorporating historical thinking into Conservation Biogeography, a vast number of studies also began to look beyond present-day mechanisms to explain and predict spatial patterns of biodiversity. Past (Late Quaternary) habitat stability, as inferred through correlative distribution models projected into paleoclimatic scenarios, have been reported to predict species richness more effectively than contemporary conditions in the Australian Wet Tropics (Graham *et al.*, 2006) and the South American Savannahs (Werneck *et al.*, 2012). Former climates have likewise been shown to explain spatial patterns of intraspecific lineage diversity (Carnaval *et al.*, 2009) and lineage endemism (Carnaval *et al.*, 2014) in Atlantic Rainforest vertebrates (**Figure 1**). Moreover, the use of species distribution data and community composition analyses to delimit biogeographic regions for conservation (e.g., Chen, 2009; Terauds *et al.*, 2012) has been expanded to incorporate information about the evolutionary history of regional biotas. As a result, maps of spatial turnover in the phylogenetic composition of communities are becoming increasingly available (see Holt *et al.*, 2013 for a global study of the vertebrate biota).

Biodiversity metrics in Conservation Biogeography have also moved beyond species and genes. Novel studies map the distribution of functional diversity in geographic space, and integrate new and exciting data about the ecological roles of communities into conservation guidelines and reserve planning (Carvalho *et al.*, 2010; McGoogan *et al.*, 2007). The mapping of non-biodiversity elements relevant for reserve design has also advanced: new studies incorporate refined landscape measurements of anthropogenic impacts on natural habitats such as estimates of human population size, crop production, and cattle ranching (Diniz-Filho *et al.*, 2006).

Evolving Approaches

Small-scale investigations – both spatially and taxonomically – have also been recently attributed to the realm of Conservation Biogeography. Within those, important insight emerged from studies of the biogeography of invasive species – a timely topic in the face of human-facilitated movement of species (see Tolley *et al.*, 2008 for an example of how molecular data can pinpoint potential sources of invasions in South African frogs). Other examples include the development of models of environmental favorability for endangered top predators and their prey (Real *et al.*, 2009), and species-specific models of distribution under future climatic scenarios (Hu and Liu, 2014).

Going beyond the compilation and analysis of empirical information from ecosystems and biological groups, studies in Conservation Biogeography have also incorporated new types of data. Simulations, for instance, were recently used to evaluate temporal changes in the composition of species and to examine what biodiversity metrics best describe community response to habitat changes (Devictor and Robert, 2009). The results argue for a need to expand the dimensions of biodiversity targeted by Conservation Biogeography, showing the

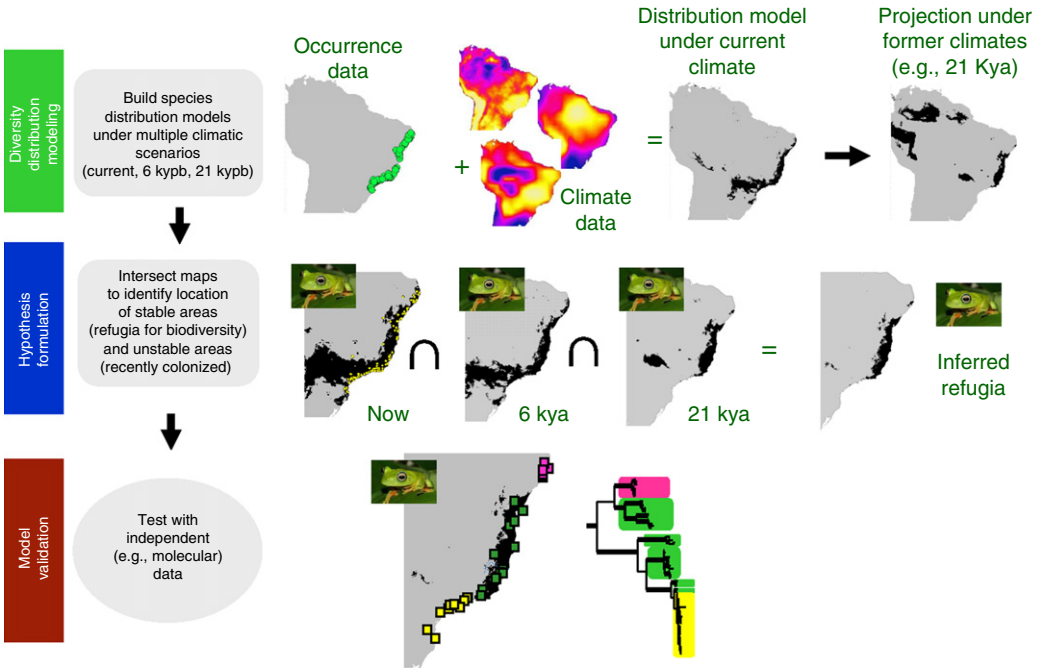

Figure 1 Integrative framework to map species-specific areas of climatic stability (refugia), where genetic diversity may be expected to accumulate over time (see Carnaval *et al.*, 2009). In step 1, information on species occurrence and present-day climate are combined into a correlative model of the distribution of the species, which is projected into the past given available reconstructions of former environmental conditions. In step 2, models of species distribution under multiple time periods are intersected to identify areas that have been suitable for the species over time (stable areas, or refugia). Extinction is expected to be low (hence genetic diversity is expected to be high) in these areas. In step 3, independent data (for instance, sequence data from molecular markers) are used to validate those hypotheses.

power of metrics that reflect the ecological traits and ecological role of species within trophic cascades (e.g., proportions of generalists vs. habit specialists). Empirical data from distinct ecosystems also support this view: species of distinct ecological and life history traits show different spatial distribution patterns; the inclusion of ecological data improves resolution of community analyses (Pawar *et al.*, 2007).

Evolving Aims

Conservation Biogeography has moved beyond the mere mapping of present-day patterns of diversity to become increasingly model-based and predictive. Expanding on the concept of distribution congruence across large numbers of species (see Diniz-Filho *et al.*, 2006; Pawar *et al.*, 2007), recent studies have explored correlations between biodiversity measurements, environmental descriptors, and geography to model spatial turnover in the taxonomic (Ferrier *et al.*, 2007), phylogenetic (Rosauer *et al.*, 2014), and functional structure of communities (Swenson *et al.*, 2011). Moreover, novel approaches are validating the use of genetic data from wild populations, when analyzed in a spatially-explicit context, to estimate unknown demographic parameters (e.g., migration rates, carrying capacity) for use in simulations of the geographic distribution of lineage diversity under future environmental conditions (Brown *et al.*, 2016).

Evolving Cross-Disciplinary Links

Conservation Biogeography currently feeds off high quality data on species identification (taxonomy), species occurrences (distribution), species history (phylogenetics and phylogeography), and their roles in the environment (ecology), as well as fine-scale environmental information. As a result of its interdisciplinary nature, the lines separating the realm of Conservation Biogeography from those of Ecology, Macroecology, Evolution, Macroevolution, and Environmental Sciences have somewhat blurred over time. 10 years ago, the spatial scale of focus was said to render Conservation Biogeography studies distinct from those at the intersection of Biogeography and Ecology (which traditionally focused on metapopulation dynamics, short-term effects of habitat fragmentation, and impact of matrix composition on species assemblages), as well as those at the intersection of Biogeography and Macroecology (Whittaker *et al.*, 2005). Yet, as biogeographical research became more integrative over time (Riddle *et al.*, 2008; Morrone, 2009), so did Conservation Biogeography.

This integrative aspect poses challenges. The limited quality and availability of data on species attributes (Diniz-Filho *et al.*, 2006; Loiselle *et al.*, 2008), our incomplete understanding of the processes governing biodiversity structure (Whittaker *et al.*, 2005; Olden, 2006; Diniz-Filho *et al.*, 2010), the inherent difficulties of integrating information from multiple data sources (Richardson and Whittaker, 2010), and the uncertainties associated with future climate predictions (Ackerly *et al.*, 2010) all need to be addressed as the discipline continues to evolve. Yet, it is precisely the complex and integrative aspect of Conservation Biogeography that makes this such a fascinating field of study – and one with the potential to help guide us through the drastic environmental changes that are fast approaching.

See also: Biogeography, Evolutionary Theories in. Conservation Biology, Evolution and. Phylogeography

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Biogeography, Ecological Theories in

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Glossary

Geographic range The total geographic area covered by the populations of a species.

Species abundance distribution (SAD) The function describing probability of observing a species with a given abundance. In theory this function can be represented by a mathematical equation. Empirically the SAD refers to the collection of abundances for all species in a sample and thus the probability of a species with an abundance n is simply the frequency of species in the sample with abundance n.

Species area relationship (SAR) The relationship between the number of species found in a sample and the geographic area over which that sample is taken. This relationship inevitably increases (more area intuitively harbors more species) but the exact rate at which species number increases with area varies across ecosystems. Often this relationship is approximated as $S = cA^z$ where S is species number, A is area and c and z are constants that depend on the system under study.

Imagine you travel to a tropical archipelago and on each island you painstakingly search out and record the identity of every unique ant species. After months of fieldwork your data begin to reveal patterns. Islands that are more isolated have fewer ant species and islands that are larger have more ant species. Perhaps from your training as an ecologist you are not entirely surprised by this finding, but you are intrigued to know how general it is: do plants, birds, mammals, and other arthropods all follow similar patterns across similar island archipelagos? Indeed the fact that species richness increases with area is a well-known phenomenon in ecology. Arrhenius in 1921 documented that for a diverse array of plants and animals a tenfold increase in area yields approximately a tenfold increase in the number of species found in that area. If this pattern is so pervasive what processes might causes it? We can imagine these were the questions running through the head of biogeographer and ant systematist Edward O. Wilson and ecological theoretician Robert H. MacArthur as they pondered the data that Wilson had collected that would inspire the groundbreaking theory of island biogeography (MacArthur and Wilson, 1967).

The goal of theory in any science is to identify the core set of processes responsible for producing persistent patterns. Simple assumptions are a core feature of scientific theories, which seek to be as general as possible and thus cannot consider every minor detail of possible mechanisms in a diverse world. In the case of biogeography, the patterns of interest concern repeatedly documented patterns in the distribution of species and biodiversity across geographic space. These patterns include the increase of species richness with area, decrease with isolation, increase toward the tropics, and the prevalence of rare species across space.

Theories of Species Richness and Composition

The following set of theories seek to explain the species richness of a given area based on its geophysical characteristics and/or the commonness or rarity of the species comprising that area's ecological community.

The Equilibrium Theory of Island Biogeography

The young systematist Edward O. Wilson brought his observations of ant species composition to a young ecologist Robert H. MacArthur and together they developed a theory that would revolutionize the fields of ecology and biogeography and help found the field of conservation biology. MacArthur and Wilson (1967) proposed that the number of species present on an island results from a balance between the number of new immigrant species that arrive to the island from the mainland and the number of species lost due to extinction on the island. If immigration depends on isolation (more isolation, less immigration) and extinction depends on area (more area, less extinction; **Figure 1**) then the theory of MacArthur and Wilson (1967) predicts the pervasive pattern that species richness increases with area and decreases with isolation. The increase of species richness with area (the species area relationship, SAR) had captured the interest of biologists for decades and so predicting it from simple first principles constituted a huge advance in theoretical ecology. This simple theory transformed ecology and biogeography from sciences of description to sciences of prediction and paved the way for future conservation practitioners to begin planning for land conservation based on the expected number of species they could preserve (Diamond, 1975; Diamond *et al.*, 1976; Gilpin and Diamond, 1980).

MacArthur and Wilson's (1967) theory is said to predict a dynamic equilibrium because it predicts a constant number of species through time that arises from a balance between immigration and extinction. MacArthur and Wilson posited that, as the number of species on an island increases, immigration rate will drop due to the fact that the number of unique (i.e., not already on the island) colonists from the source pool will become limited. Similarly they proposed that extinction would increase with the number of species on the island both due to each species' per capita extinction rate contributing to the next extinction rate, and decreased population sizes per species if, as they assumed, the total number of individuals on the island was fixed by resource constraints. As species richness increases on an island eventually immigration rate will equal extinction rate (**Figure 1**) and at this point the island is in

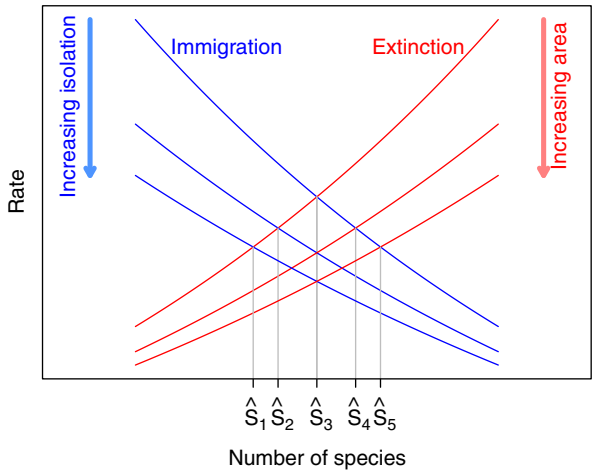

Figure 1 Graphical representation of MacArthur and Wilson's (1967) theory of island biogeography. Across the *x*-axis is the species richness of a hypothetical island. Several potential immigration rates are shown in blue while potential extinction rates are shown in red. Because immigration rate decreases and extinction increases as the number of species on an island increases a stable equilibrium in species number can occur where these two rates intersect. Several such equilibria are denoted by $\hat{S}_i$. The different rates correspond to different island characteristics, with more isolated islands experiencing less immigration and larger islands experiencing less extinction. Thus MacArthur and Wilson's (1967) theory predicts a positive relationship between island area and equilibrium species richness (e.g., $\hat{S}_3$ is the species richness of a small island and is less than, $\hat{S}_4$ of a bigger island and less still than $\hat{S}_5$ of an even bigger island). Their theory also predicts a negative relationship between island isolation and equilibrium species richness (e.g., increasing isolation leads to less species richness going from $\hat{S}_3$ to $\hat{S}_2$ to $\hat{S}_1$.

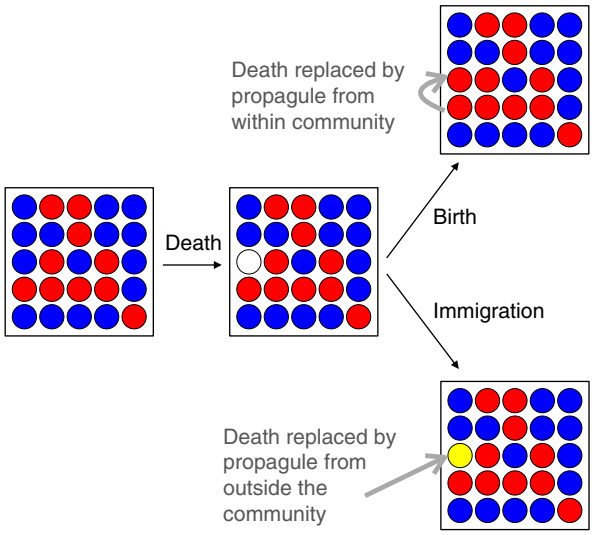

Figure 2 Graphical representation of Hubbell's (2001) Unified Neutral Theory of Biodiversity and Biogeography. In the model community (depicted as two species: red and blue, with dots corresponding to individual organisms) death occurs at random and is replaced either by a birth event (the parent chosen at random from within the community) or an immigration event (a propagule arrives from outside the community) which could introduce a new species to the community as indicated by the yellow individual. The dynamics of the metacommunity are similar except the process of immigration is replaced by the process of speciation.

equilibrium. This equilibrium is 'dynamic' because while the number of species may remain constant, the actual identities of species on an island is predicted to change as new species arrive and replace others going extinct.

This theory was most famously tested by Simberloff and Wilson (1970) in an experiment in which Simberloff defaunated mangrove islands of all their arthropod occupants and observed the colonization process. In accordance with MacArthur and Wilson's equilibrium theory, the number of species increased over time until species number reached an equilibrium level.

While the theory of island biogeography (MacArthur and Wilson, 1967) was originally developed explicitly for islands, it has seen broad applications in other systems. Researchers have applied the idea of balancing immigration and extinction to continuous landscapes by supposing that local communities can be approximated as island-like systems and receive immigrants from the surrounding landscape, now treated as a separate 'mainland,' much the same way as islands receive immigrants from actually separate mainlands (Hubbell, 2001; Leibold *et al.*, 2004).

Researchers have also identified exceptions to and shortcomings of MacArthur and Wilson's theory. One particularly interesting exception occurs when equilibrium cannot be achieved due to shifting landscapes. Brown (1971) identified such 'non-equilibrial' communities in the mountaintop refugia of the great basin (mountaintops here are equivalent to islands of montane habitat) where changing climate since the last glacial maximum cutoff dispersal routes for montane-adapted mammals, thus the current dispersal potential for these mammals does not match the current conditions and so their observed diversity is out of equilibrium compared to theoretical predictions.

The equilibrium theory of MacArthur and Wilson (1967) also ignored the speciation process, which can be critical in the assembly of island biotas (Gillespie, 2004; Losos, 2009; Losos and Schluter, 2000). Speciation adds to the species richness of an island in a similar way as immigration does, but speciation can be independent from the source pool of potential species colonists (Losos, 2009; Losos and Schluter, 2000). Losos and Schluter (2000) showed that speciation drastically changes the shape of the SAR, increasing the rate of species accumulation with area.

The Unified Neutral Theory of Biodiversity and Biogeography

Hubbell (2001) was inspired by the challenge of incorporating speciation into the theory of MacArthur and Wilson (1967) and in turn spurred a new renaissance in theory development in ecology and biogeography. Hubbell's (2001) Unified Neutral Theory of Biodiversity and Biogeography (**Figure 2**) assumes a local community functions like an island but in addition to immigration from the surrounding metacommunity, speciation can add species richness. Hubbell's additional inclusion of an explicit demographic process or birth and death enabled him to also predict the actual abundances of

species in a community leading to new predictions to be tested with data. Hubbell (2001) made the controversial assumption that all individuals across all species have equal probabilities of birth and death or giving rise to a new species. This assumption of 'neutrality' received substantial criticism because it refuted the historically central role of species differences (i.e., niches) in determining species composition and abundance (Hutchinson, 1959; Leibold, 1995). However, the Unified Neutral Theory of Biodiversity and Biogeography predicted the species abundance distribution (SAD) of many communities surprisingly well, leading to ongoing research as to what mechanisms, niche or neutral, underlie the patterns of diversity, rarity, and commonness we observe in nature (Rosindell *et al.*, 2011).

Brown (1995) and Maurer (1999) each proposed kindred niche-based theories of species richness and abundance through geographic space. Both built from the *n*-dimensional niche concept of Hutchinson (1959) which assumes that each species' fitness is limited by a unique set of environmental constraints. Brown (1995) and Maurer (1999) observed that such constraints should vary in space and thus a species cannot be equally abundant everywhere, but instead will experience areas of high and low population density.

The Maximum Entropy Theory of Ecology

One alternative to the mechanistic views put forward by neutral theory (Hubbell, 2001) and its niche-based counter arguments (e.g., Brown, 1995; Maurer, 1999) is the view that patterns in biodiversity may arise not because of uniquely biological mechanisms but simply the statistical properties of large aggregations of things (Preston, 1950; Nekola and Brown, 2007; Harte, 2011). Nekola and Brown (2007) showed that the diversity patterns of many systems completely removed from biology (e.g., cars in parking lots) follow patterns that can be well described by ecological theories, implying that the mechanisms put forward by these theories may not be uniquely biological. This line of thinking builds off the idea that if any generalities are to be found in biodiversity, the cause of these generalities must not be one prevailing biological mechanism, as a plethora of documented mechanisms have been recorded across the vast array of earth's ecosystems, but instead emerge in spite of that diversity of locally relevant mechanisms (Preston, 1950; Rosenzweig, 1998). This thinking is best formalized in the application of principles from statistical physics in ecology and biogeography. Harte (2011) put forward the maximum entropy theory of ecology (METE) which is able to accurately predict the SAD and the SAR, all while making no assumptions about the processes by which species enter a community, reproduce, and die. Harte achieves this by using the principle of maximum information entropy (Jaynes, 1982) which seeks to predict metrics (such as the SAD) given state variables (such as the total number of species and the total number of individuals) in a system. This is the same principle that can be applied to ideal gasses to derive predictions of their behavior (Jaynes, 1957).

Among other predictions, METE predicts the shape of the SAR with great accuracy compared to data (Harte *et al.*, 2009). In fact, METE predicts that all SARs should follow the same form across systems, which is simply a function of the ratio of the number of species to number of individuals in the systems. This reduction of a seemingly intractable diversity of patterns (i.e., all the variety of shapes and rates of SARs) to one simple rule is known as a scale collapse in that multiple observations from across scales collapse into one pattern when the salient variables are considered.

The theories we have thus far considered concern themselves with the geography of species richness and abundance. They seek to explain observed patterns of diversity, commonness, and rarity using simple mechanistic assumptions (such as balanced immigration and extinction) or in fact reject the mechanism all together and seek to explain observed patterns as statistical inevitabilities of large aggregations of things.

Theories of Geographic Range Size and Geographic Gradients

So far, the theories discussed have focused on the geographic distribution of species richness and abundance. Another central focus of theoretical biogeography is the distribution of species themselves – that is where in space do species live and how can we predict the properties of that spatial distribution (referred to as the geographic range)? A central focus through the development of biogeographic theory has been the study of the size of geographic ranges and how this predictably changes according to the geographic position of the range. The size and geography of ranges has implications for both the accumulation of species in space (the SAR) and the geographic pattern of species richness.

The average size of a species' geographic range is not constant across geographic space. Species ranges tend to be smaller in the tropics and at lower elevation (Stevens, 1989, 1992; Brown, 1995). This observation was formalized by Stevens (1989) who named the patterns 'Rapoport's rule' after earlier work by Rapoport (1982). However, why this pattern is so consistently observed still eludes explanation. One leading hypothesis was put forward by Janzen (1967) who argued that because the tropics are more climatically stable, species should evolve narrower climatic tolerances and thus their smaller range size naturally follows. A complementary hypothesis posits that species interactions are more intense in the tropics (Pianka, 1966) which could constrain species to the geographic region in which they have coevolved strategies for dealing with competitors, predators, and mutualists (Thompson, 2005).

The architecture of geographic ranges also bears on the shape of the SAR. Intuitively, more small ranges should yield a steeper SAR. Storch *et al.* (2012) developed this intuition into another form of SAR scale collapse, showing that after accounting for the mean geographic range size of a clade, its constituent SARs collapse onto a single curve.

The fact that geographic ranges are smaller in the tropics is also related to the latitudinal diversity gradient; there are more species in the tropics than in temperate regions (Pianka, 1966). Smaller geographic ranges in the tropics should intuitively lead to higher diversity, especially higher β-diversity (i.e., greater turnover in species composition across space). Whether β-diversity is indeed higher in the tropics is still under active

debate (Kraft *et al.*, 2011; Qian *et al.*, 2013) but the higher overall diversity is undeniable (Pianka, 1966). Thus, whether the higher diversity is the cause or consequence of smaller geographic ranges in the tropics also warrants further study.

Theories of biogeography address the spatial distribution of richness and abundance, both through considering the dynamics of assemblages of species as in the equilibrium theory of island biogeography (MacArthur and Wilson, 1967), and by trying to understand patterns in the geographic ranges of species and their consequences. Much research remains until we can fully understand how general biogeographic patterns (such as the SAR) emerge and whether these are the result of simple but pervasive biological mechanisms (e.g., those posited by Hubbell's (2001) neutral theory) or are in fact near statistical inevitabilities (Harte, 2011). Understanding whether geographic range patterns are the cause or consequence of species diversity patterns also remains a fruitful area for future theoretical development.

See also: Biogeography, Conservation. Biogeography, Evolutionary Theories in. Biogeography of Interactions. Biogeography of Islands, Lakes, and Mountaintops; Evolutionary. Biogeography, Patterns in

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The Brown Laboratory.

Biogeography, Evolutionary Theories in

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Introduction

One of the most commonly invoked yet persistently controversial causal mechanisms underlying the formation of new species is allopatric divergence. This is thought to occur with landscape reconfiguration and/or climate change that fracture species ranges into multiple isolated refugia divided by newly formed zones of unsuitable habitat. Over time, the lack of dispersal between formerly continuous populations results in the emergence of new sister populations or species (Ford *et al.*, 1949; Mayr, 1954; Cracraft, 1974; Lande, 1980; Losos and Glor, 2003). When this process of allopatric speciation is driven by reconfigurations of the landscape such as the separation of continents by plate tectonics, the uplift of mountains, the emergence of an Isthmus, desertification, or the formation of a large river, it is referred to as vicariance in the parlance of biogeography. Moreover, it has been seen as a mechanism of central importance because it can potentially subdivide whole communities of co-distributed species into co-distributed species-pairs, with these large-scale events potentially multiplying substantial portions of preexisting levels of biodiversity (Cunningham and Collins, 1994; Lessios, 1998; Bacon *et al.*, 2015).

This idea was perhaps first articulated by Alexander von Humbolt where he argued that if the separation of continents occurred after the development of living organisms, we should find similarities between animals on the opposing coastlines of such continents (von Humboldt and Bonpland, 1807). This idea was later more fully developed by Joseph D. Hooker who argued that the present-day distribution of plants in the southern hemisphere is the remains of a historically more widespread flora that was broken up by geological and climatic processes. Although he proposed that mechanisms causing community-wide allopatric isolation and diversification involved land-bridges between continents that have subsequently subsided (Hooker, 1853), an accumulation of observational evidence led to more general formalization of a theory of vicariance. For example, in the early twentieth century David Starr Jordan brought together such evidence from an array of geographic contexts to argue that the emergence of geographical barriers were the dominant drivers of speciation (Jordan, 1905, 1908), and this general idea has been long since supported by modern geological and climatic evidence (Moss and Wilson, 1998). Not only has vicariance been long considered to be important drivers of biological diversification across much of the tree of life (Rosen, 1978; Mayden, 1988; Cracraft, 1982; Joseph *et al.*, 1995; Riddle *et al.*, 2000; Gamble *et al.*, 2008), there has even been strong arguments in favor of advocating this mode of speciation to be an a priori assumption or null model (Wiley, 1988) leading to biased inference (Briggs, 2009).

As simple and indisputable as this may seem, an alternative hypothesis of allopatric speciation involving dispersal over preexisting areas followed by isolation and speciation also has old roots (Ortmann, 1902; Darlington, 1938; Adamson, 1932). This idea has seen a resurgence and many of the more recent emerging examples come from oceanic islands lacking in histories with connections to a mainland continent (de Queiroz, 2005; McCartney *et al.*, 2000; Winkworth *et al.*, 1999), as well as larger islands with ancient mainland connections such as Madagascar and New Zealand (McGlone, 2005; Yoder and Nowak, 2006).

As the debate about which of the two processes underlie the Earth's biodiversity continues (Wiens and Donoghue, 2004; Zink *et al.*, 2000; Bowen *et al.*, 2013), in parallel a number of different methodological advances have emerged that have the potential to more rigorously evaluate competing biogeographic scenarios given genetic data that is becoming increasingly sampled at the scale of partial genomes and multiple individuals across each extant species.

Ancestral Area Reconstructions

Traditionally, research and methodology to infer and reconstruct the ranges of species within a phylogenetic context have relied on pattern-based nonstatistical approaches such as parsimony and cladistic biogeography (Morrone and Carpenter, 1994; Donoghue and Moore, 2003). Only recently have inferences based on statistical frameworks such as maximum likelihood and Bayesian approaches been used to test alternative geographical hypotheses of diversification (Ronquist and Sanmartín, 2011). Such model-based parametric methods make inference using time-calibrated phylogenies under various models such as Dispersal–Vicariance analysis (Ronquist, 1997), Dispersal–Extinction–Cladogenesis (Ree and Smith, 2008), and ones that model the ancestor–descendant range evolution based on stochastic dispersal, vicariance and local extinction along all nodes and tips of a phylogeny (Goldberg *et al.*, 2011; Webb and Ree, 2012; Landis *et al.*, 2013; Matzke, 2014). These flexible methods allow the incorporation of spatial and temporal information as constraints, such as from fossils, sea levels changes, climate shifts, and continental drift (Clark *et al.*, 2008; Mao *et al.*, 2012; Meseguer *et al.*, 2015). Other advances include the incorporation of changes in speciation rates, sympatric speciation, instantaneous long distance founder speciation from ghost taxa, as well as being able to accommodate classical vicariance.

While these methods have provided opportunities to test biogeographical hypotheses with greater complexity, this comes with a cost of requiring more data. Although fossils can provide information for dating biogeographic events, they are often insufficient for resolving the geographical range evolution at ancient nodes in the phylogeny. Irregardless, integrating multiple sources of information such as ecological species distribution models (SDMs) can yield well resolved biogeographic histories given well-resolved phylogenies across

a diverse range of taxa and timescales (Meseguer *et al.*, 2015; Matzke, 2014).

Despite these advances, such model-based inferences all rely on the correct inference of a phylogenetic tree. However, two notable advances for phylogenetics have made this aspect of the inference much improved. First, there is the recent ability to generate data from hundreds or even thousands of places in the genome with the advent of new DNA sequencing technologies (Brito and Edwards, 2009). Secondly, phylogenetic models have recently incorporated theory from population genetics such as the coalescent, which accommodates the stochasticity across unlinked gene trees and ancestral polymorphism (Edwards, 2009). Indeed, with this new perspective, there becomes a clear link between historical geographic demography, the speciation process, and the history of phylogenetic relations, such that one could combine these types of inference within a global framework to reconstruct biogeographic history.

Inference of Biogeographic Histories with Nonspatial Population Genetic Models

Ancestral area reconstruction allows inference of the geographic mode of diversification by estimating the ancestor–descendant range evolution along all nodes and tips of a phylogeny, yet this type of inference is most powerful and reliable across the most recent nodes of divergence. Moreover, using coalescent-based models and population genetic data, one can obtain highly resolved inference of complex geographic histories of isolation, colonization, and/or admixture (Ortego *et al.*, 2015; Veeramah and Hammer, 2014). Leveraging the greater inferential power of these complex historical demographic models with substantially greater amounts of genomic data will increasingly play a role in understanding the geographic component of diversification (Edwards *et al.*, 2015).

Not only can coalescent models be used to test various hypotheses within lineages or species with greater amounts of inferential complexity being achieved with greater amounts of data (Excoffier *et al.*, 2013) or by integrating across various approaches (Metcalf *et al.*, 2014), these approaches also allow testing traditional biogeographic hypotheses such as vicariance and colonization. For example, if one adds effective size change under the often used *Isolation and Migration* model (Hey and Nielsen, 2004), this additional parameter directly relates to the demography of colonization by small founder populations (Hey, 2005) or conversely a vicariant history where an ancestral species is more evenly split into daughter species (Locke *et al.*, 2011). As is the case for methods for ancestral area reconstruction, these coalescent model-based strategies for testing classical biogeographic hypotheses can be aided by integrating with other approaches such as ecological species distribution modeling (Lacey Knowles and Alvarado-Serrano, 2010; Carnaval *et al.*, 2009; **Figure 1**).

Another strategy for improved statistical strength and achieving a better understanding of how regional climate and landscape change can shape the geographic history of speciation across a whole assemblage within the context of these classical hypotheses of vicariance and colonization is to deploy population genetic splitting models within a coalescent-based

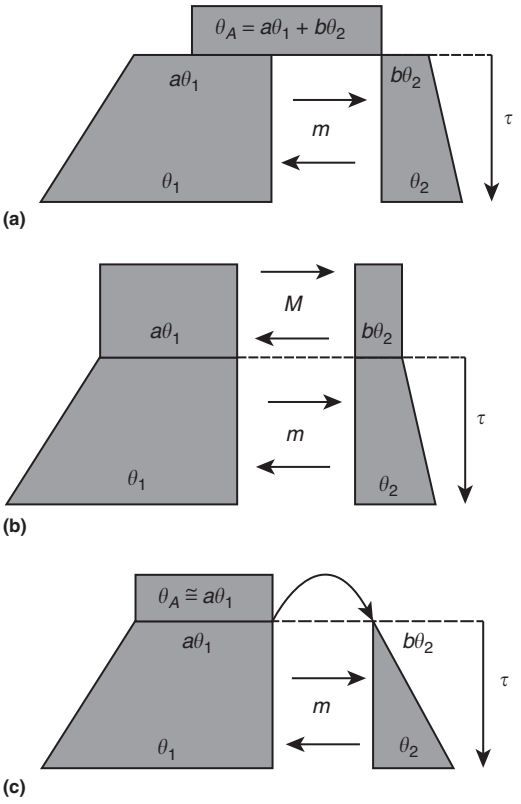

Figure 1 Models of classical vicariance (a), soft vicariance (b), and colonization (c). In (a), an ancestral population is split into two descendent sister population at time τ before the present. The vicariance time or colonization time is τ whereas $\theta = 4\,N\mu$ with N being the effective population size and μ being the per gene per generation mutation rate. The relative effective population sizes of the two sister populations is scaled by a and b at different time periods. In (b), M, the number of ancestral migrants per generation connects the two ancestral sister populations until it becomes reduced to m at time τ in the past. In (c), a small number of individuals from the ancestral population colonizes the descendent population at time τ and grows exponentially until the present ($\tau = 0$).

multispecies hierarchical Bayesian model (Hickerson *et al.*, 2006). In this case, the relative frequency of models of founder colonization versus cyclical isolation and admixture can be quantified across a whole assemblage of co-distributed sister species pairs by estimating hyperparameters that describe the magnitude of demographic expansion that is associated with colonization across species under the hierarchical model that allows species-specific parameters to independently vary across taxa (Hickerson and Meyer, 2008; Ilves *et al.*, 2010).

Although this type of inferential framework can be complex, it has so far been achieved using the simulation-based approximate Bayesian computation (Sunnåker *et al.*, 2013) such that probabilities of various complex models of island and continental colonization can be compared while allowing for the variance associated with the coalescent (Patiño *et al.*, 2015). When deployed on a large regional scale, this approach can elucidate the relative importance of colonization and vicariance in the speciation process across more than hundred avian taxon-pairs that span the Andes mountains, and several large river drainages. In the former case, mountain building appears

to have largely split preexisting ancestral populations consistent with vicariance, whereas in the latter case the inference suggests dispersal over preexisting river barriers followed by isolation and divergence across the rivers (Smith *et al.*, 2014).

Similar comparative studies looking at large numbers of taxon-pairs have also focused on the classic great American biotic interchange that joined North and South America before the Pleistocene (Webb, 2006). While such comparative inferences can illuminate the process of community assembly and colonization of terrestrial taxa from one continent to another (Cody *et al.*, 2010; Diamond, 1975), they can also shed light on processes underlying vicariance with respect to marine taxa that were split by the rising land bridge (Lessios, 2008). This classic biogeographic setting raising the paradox that estimating the timing of events rely on assumptions of DNA mutation rates, which are in turn obtained from estimates that are made by oftentimes controversial geologic dating such as the rise of the Central American Isthmus (Ho *et al.*, 2015). Moreover such large-scale comparative studies estimating DNA mutation rates (Knowlton and Weigt, 1998), or timing of events related to the great American biotic interchange (Bacon *et al.*, 2015) can suffer from substantial bias if a coalescent model is not used (Marko *et al.*, 2015; Hickerson *et al.*, 2003; Charlesworth, 2010), and if there are biases in data inclusion (Lessios, 2015). Moreover, many such comparative studies are provisional because they rely on the easily obtained single-locus mitochondrial DNA in animals and chloroplast DNA in plants. Encouragingly, multispecies coalescent models for sub-genomic scale data are starting to be developed for co-expansion models (Xue and Hickerson, 2015) that could be used to detect the magnitude of divergence through colonization and it is likely that they could be extended to different geographic models of speciation and divergence while accommodating the surge in partial genome data now obtainable from a wide array of nonmodel taxa (Ekblom and Galindo, 2011).

Inference of Biogeographic Histories with Spatial Population Genetic Models

The success in the aforementioned population genetic approaches to estimate historical demographic parameters relevant to biogeographic history such as colonization times and population expansion magnitude and timing all ignore information about geographic space. Although there is a long history of a set of methods referred to as *landscape genetics* that spatially explicitly relate geographical features such as altitude, topography, and ground cover, on patterns of genetic variation and structure (Manel *et al.*, 2003), these typically ignore landscape history and population history with respect to patterns of genetic variation, and thereby ignore the stochasticity and time-dependency of drift, mutation, and selection with respect to the landscape. However, of these, nonmodel-based methods have still proven to be useful for making inferences of geographical history of species. For example, one of the most common of such methods is to calculate the heterozygosity over space and make inferences of geographical origins of range expansion based on this value's expected negative correlation with the age of populations (Pierce *et al.*, 2014; Deshpande *et al.*, 2009). Alternatively, patterns in the comparisons between geographic and genetic distance are also common ways in which spatial and genetic information is used to infer the geographic origins of population expansions (Ramachandran *et al.*, 2005).

On the other hand, there is a set of emerging methods that are both spatial and incorporate explicit genetic historical models. One such commonly used method does not parameterize historical demographic history, yet uses diffusion models to infer the history of a sampled gene genealogy over both space and time (Lemey *et al.*, 2010). This versatile method uses a Bayesian statistical approach to infer continuous diffusion of the sampled genealogy using random walk models while simultaneously reconstructing the evolutionary history in time from a genetic sample. This results in the ability to trace the geographic origins of the sampled gene genealogy itself, which can be especially useful for tracking the historical spread of viruses (Kühnert *et al.*, 2011; Kuzmina *et al.*, 2013), plant genera (Terra-Araujo *et al.*, 2015), origins of biological invasions (Pfenninger *et al.*, 2014; Eme *et al.*, 2013), and even the geographic spread of language families (Bouckaert *et al.*, 2012). While this method is normally deployed on single gene datasets, which are typically most useful for the geographic inference of infectious diseases (Pybus *et al.*, 2012), they can be extended to make inferences based on a multilocus species tree (Nylinder *et al.*, 2014).

However, these methods typically make inference without parameterizing the geographic distribution of populations or population densities in continuous space. Although the standard way to model genetic data retrospectively using coalescent theory is routine under a set of discrete demes (Beerli and Felsenstein, 2001; Wakeley, 2001), modeling this process in a spatially continuous context has been quite challenging due to several technical problems (Felsenstein, 1975; Barton *et al.*, 2010, 2013). One solution has been to discretize continuous space into a two dimensional grid in order to implement simulation-based methods to reconstruct the spatial and temporal dynamics of population history. One commonly used framework is to spatially explicitly couple SDMs (Elith and Leathwick, 2009), demographic and population genetic models in order to capture the spatiotemporal and geographic dynamic of species divergence. The general idea is to first combine geo-references observations of species occurrence with environmental estimates to *predict* the expected range of a species at the present as well as at different times in the past (Waltari and Hickerson, 2012; Gavin *et al.*, 2014; Alvarado-Serrano and Knowles, 2014). These models are then converted into demographic models with deme-specific carrying capacities and migration potentials that are then used to define spatiotemporal demographic histories that generate patterns of genetic variation under coalescent models. Such integrated frameworks can then use patterns of observed genetic variation to test competing spatiotemporal hypotheses of colonization and divergence histories (Brown and Knowles, 2012). This general approach has been used to understand the relative likelihood of colonization versus vicariance driven divergence in at the timescale of the late Pleistocene in both plants and animals (Cornille *et al.*, 2013; Hung *et al.*, 2013) as well as understanding the spatial dynamics of invasive colonization at recent timescales (Estoup *et al.*, 2010). Although simulation-based ABC inference uses genetic summary statistics for

comparison between simulated and observed data to approximate a series of model probabilities, these metrics typically contain no spatial or geographical information. Encouragingly, new summary statistics that jointly use genetic and geographical information have recently been developed and have been shown to potentially improve the ability to discriminate among different complex geological histories of species divergence and colonization (Alvarado-Serrano and Hickerson, 2015), especially when aided by *Random Forest* discrimination algorithms (Pudlo *et al.*, 2015), a technique retooled from machine learning.

Discretized spatial models have been helpful, yet there are computational inefficiencies that lead to only enabling spatial inference across relatively shallow historical times. Fortunately, these shallower historical times can be relevant with regards to understanding the geographical history of recent species divergences, and there are new techniques for inferring the geographic origins of colonization and range expansion that do not require extensive simulation experiments (Peter and Slatkin, 2015).

Furthermore, there has been steadily growing theoretical work in spatially explicit coalescent models in one (Wilkins and Wakeley, 2002) and two dimensions (Limic and Sturm, 2006; Barton *et al.*, 2002). While these set of models do not discretize space into a grid, they often make various simplifying assumptions. One strategy that looks promising is to allow for local extinction and recolonization dynamics to obtain a spatially explicit model that may converge to fit real datasets even while implementing a torus on the edges of the geographical space (Barton *et al.*, 2010, 2013). On the scale of the geographic history within a species, genetic data can now be simulated efficiently under this spatially explicit model (Kelleher *et al.*, 2013, 2014), such that an approximate Bayesian computation framework could be built to use these simulation techniques for parameter estimation.

As the number of geographic models from which to better understand the spatial history of new species formation grows in number and complexity which can then better inform how the spatial histories of species can be shaped by future climate and landscape changes (Yannic *et al.*, 2013). By improving biogeographic investigations of species histories by integrating genetic data with independent information, researchers will better understand how the interacting processes of geographic isolation and long-distance dispersal drive regional patterns of biodiversity. Furthermore, greater availability of not only fossils but fossil DNA will continue to shed light on how isolation and the ability to disperse have both shaped regional patterns of speciation and extinction (Mann *et al.*, 2015).

See also: Biogeography, Ecological Theories in. Biogeography of Islands, Lakes, and Mountaintops; Evolutionary. Phylogeography

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Biogeography, History of

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Glossary

Biogeography The study of geographic variation in all characteristics of life – from genetic, anatomical, physiological and demographic differences among geographic populations, to the diversity, distinctiveness, and differences in composition, and key processes of biological communities, ecosystems, and entire, regional biotas.

Buffon's Law Different regions of the globe, even those with similar environmental characteristics, are inhabited by distinct assemblages of plants and animals.

Continental drift The movement of the portions of the Earth's crust that comprise the continents.

First Law of Geography The very general tendency for the similarity among characteristics of particular areas to decline as the distance between those areas increase (synonymous with spatial auto-correlation).

Plate tectonics The general theory, inclusive of continental drift, of the origin, movement, and destructions of Earth's continental and oceanic plates.

Introduction

Put in its simplest terms, *biogeography* is the geography of life. Traditionally it was defined in more limited terms as 'the study of geographic distributions of organisms.' A thorough review of the historical development of this field, and its descendant disciplines – evolution and ecology, however, provides a more accurate understanding of biogeography as one of the most holistic and foundational of the biological sciences (Lomolino *et al.*, 2016; see also resources of the International Biogeography Society www.biogeography.org).

As we shall see, the historical development of biogeography has roots deep within the development of human civilizations and, indeed, may reach far into the early, evolutionary history of our own species when its aboriginal populations adapted to and simultaneously were modified by variation in environmental conditions as they migrated across this planet. Throughout its long history, biogeography like most sciences has progressed – not as an orderly, sequential accumulation of facts and advances in our understanding of the natural world, but more like the complex geological development of the Earth and the evolutionary development of its species. That is, biogeography developed from a complex interplay between empiricism and theory in fits and starts – a reticulating phylogeny of pattern description and conceptual advances marked by periods of discovery and innovations, and diversification and division among sub disciplines, alternating with periods of synthesis and reintegration (see Lomolino and Brown, 2009 for a review of the 'reticulating phylogeny' of island biogeography theory). Given this long and complex, albeit genuinely fascinating and singularly instructive history, a comprehensive account of the development of biogeography is far beyond the limited scope of any one article. Instead, my purpose here is to provide an overview of the key empirical discoveries and conceptual advances of the discipline, with special emphasis on the antiquity of some of its central ideas from the local knowledge of early civilizations to the globalization of that knowledge, and from the early articulations of its fundamental principles during the seventeenth through nineteenth centuries to the theoretical revolutions of the twentieth century.

Aboriginal Knowledge

Given the seemingly meteoric rise of humanity from its modest origins some 200 000 years ago to ultimately become the Earth's dominant species and ecosystem engineer, the idea that we too have been molded by a variety of environments across the planet is often met with much skepticism. Yet archaeologists and anthropologists continue to provide incontrovertible evidence that the physical and physiological characteristics of our species has borne and, to a real albeit less pronounced degree, continues to bear the imprint of the regular variation in environments and pervasive pressures of natural selection. Survival in the face of competition, predation, parasitism, and other struggles for existence not only altered the shape and stature of individuals, but also put a premium on accumulating knowledge on patterns in variation in local environments: locations of food plants and game, of water and natural shelters, and trends in variation in temperature and other climatic conditions as early humans migrated across their then limited ranges. Those living in areas of sharp environmental gradients, in particular populations inhabiting coastal/shoreline environments or those of montane regions with their tightly packed elevational gradients of diverse habitats, are likely to have been especially aware of and keen to adapt to the geography of nature.

Survival of these aboriginal populations may also have required exploration – a means of hedging bets against the frequent waning and collapse in local resources (whether due to natural causes or to overexploitation by their own populations). The overall impact of these and other forces were the expansion, drifting, and migration of the ranges of these local populations to other regions, climatic zones, and ecological realms (likely including many transitions from a terrestrial to

coastal and then to oceanic existence). As each population expanded its range, their collective knowledge continued to expand along with it, now to the point that their knowledge of the geography of nature expanded from the level of local habitats to the variation across a range of habitats within their region.

By 100 000 years ago, these forces of man and of nature drove the expansions of modern humans out of Africa, and by 50 000 years ago they had established populations across the coastal regions of Arabia and southern Asia, through Indonesia and onto Australia. By 30 000 years ago, human populations had colonized most of Europe and central Asia, had reached eastern Siberia, and were perched on the Beringian land bridge prior to their rapid advance into the New World. Yet, while these expanding branches of the human species across the globe demonstrated remarkable capacities for adapting to local and regional habitats and climates, their knowledge base likely remained regional – i.e., at the scales of environments they encountered and as independent and often isolated populations. It was likely that they identified the best local sites for game or fish, that the stature of plants varied with elevation, that temperatures cooled as they climbed local mountain ranges or dove deeper below the ocean's surface; that the numbers and diversity of food plants or game or fish varied along these same gradients and was higher in larger habitats or larger pools of water. Thus, some of the principal patterns and fundamental principles of biogeography – that place matters; that environments and life forms varied in a regular manner across local geographic gradients (here, the gradients of elevation, depth, and size or area of the habitat) was ancient knowledge, essential to the survival and geographic expansion of Earth's dominant species (for an interesting discussion of ancient knowledge and the origin of myths and legends, see Vitaliano, 2007). Establishment of the foundations of biogeography as a genuine science, however, would require much more than local knowledge of many hundreds if not thousands of independent populations of our species. It would require a fundamental expansion in the scale of this knowledge – a globalization of observations on the natural world that could only come from world explorations and integration of their many marvelous discoveries to establish the central patterns and fundamental, unifying principles of biogeography.

The Geography of Nature from Regional to Global Scales

By 10 000 years ago, human populations had colonized nearly all reaches of the globe save for the most isolated oceanic islands and the frozen lands of Antarctica. Globalization of knowledge on the geography of the natural world, however, would await the Age of Explorations by Europeans, which began some 500 years ago. Although the principal mission of these voyages across the seas and to distant lands were not scientific, they often included a naturalist whose specimens and narrative accounts of exotic plants and animals became grist for those seeking a broader understanding of how life varied across the globe. Many of these early naturalists were driven by a quest to serve the Creator – to understand the marvelous menagerie of life he placed across the globe, each local fauna and flora being perfectly adapted to the local conditions and surviving since the time of Creation until their discovery by the faithful explorers (Linnaeus, 1781; see also Browne, 1983; Lomolino *et al.*, 2016, Chapter 2).

By the eighteenth century, naturalists and museums had amassed a burgeoning collection of accounts and specimens from distant lands; but how to make sense of this massive and seemingly disarticulated collection? That mission – to develop a systematic description of life, was the life's work of many naturalists of those days, but none more accomplished than Carolus Linnaeus (1707–1778), who developed the system of binomial nomenclature that we continue to use today as a tool for ordering the ever-expanding lists of life forms inhabiting the planet. But making sense of this massive accumulation of knowledge on what we now call 'biological diversity' required far more than a system for cataloging species: it required a set of principles on how to assemble such knowledge into a coherent theory on the distributions, diversity, and distinctiveness of life across the planet.

Fundamental Principles of Modern Biogeography

Principles fundamental to biogeography are, of course, also fundamental to geography. As restated by Waldo Tobler, *The First Law of Geography* holds that "*everything is related to everything else, but near things are more related than distant things*" (Tobler, 1970). This principle of spatial auto-correlation is, in essence, a codification and generalization of earlier observations of aboriginal peoples, discussed above. A related, and at least as fundamental a pattern in the geography of nature is the other aboriginal knowledge of the distinctiveness of place – that environmental conditions vary in a regular manner from place to place – along the geographic gradients referred to above (distance from coastline and elevation above or depth below sea surface). This highly nonrandom spatial variation in environments forms the geographic template for natural selection and other forces that shape the geography of life across the planet.

That the distinctiveness of place applied to the world's biotas – i.e., at regional to global scales, however, was not established until the revelations of the Age of European Explorations. Thus, during the eighteenth century, European naturalists advanced what would become biogeography's most fundamental pattern, *Buffon's Law* (Buffon and Comte de, 1761). This principle was named in homage to Linnaeus' contemporary – Georges Louis Leclerc Comte de Buffon (1701–1788), whose studies of both extant and fossil animals (in particular mammals of the New and Old World tropics) revealed that different regions, even those with similar environmental conditions, are inhabited by different species.

Subsequent generations of explorer naturalists during the eighteenth and nineteenth centuries would establish the generality of Buffon's Law to plants and other animal taxa beside mammals, and to other climates and environments outside of the tropics. These confirmations of Buffon's Law simultaneously challenged Naturalists of Europe and elsewhere to explain how a world whose climates, land forms and ocean basins, and species were assumed to be fixed and

immutable could have achieved their current distributions and diversity. Buffon's explanation was one that challenged the concept of world stasis espoused by Linnaeus and dictated by established doctrine of the eighteenth century. Instead, Buffon asserted that life forms became established in a region of the higher latitudes of the Northern Hemisphere during an ancient period of global warming, and they then migrated southward into successive regions of the New and Old Worlds, becoming modified (in Buffon's terms, 'advanced' or 'degenerated') during these migrations. By advancing a theory that allowed both Earth's climates and its species to change, Buffon explained the distinctiveness and the similarities among regional to global biotas.

Thus, the geography of life provided insights that proved foundational for revolutions in science – revolutions that would establish, not only biogeography, but evolutionary biology as well. During the nineteenth century, Charles Darwin (1809–1882) and Alfred Russel Wallace (1823–1813) would be so struck by spatial variation and geographic distinctiveness of biotas (in particular, that of the Galapagos

Figure 1 Alexander von Humboldt's *Tableau physique des Andes et pays voisins*, published first in 1807, continues to serve as a classic exemplar in visualizing the geography of nature as a means of exploring the forces influencing the distributions and diversification of life across the planet (see von Humboldt and Bonpland, 2009).

Islands, and those of Indonesia, respectively) that they independently advanced the theory of evolution by natural selection – their theory explaining not only the distinctiveness of each assemblage of species, but trends in similarity among assemblages as well. They had each read the narratives of Alexander von Humboldt (1852) and were fascinated by his accounts of discovery in the Canary Islands and across the New World tropics of Amazonia, and by his essays on plant geography (Humboldt and Bonpland, 2009) where he described in meticulous but inspiring detail the geographic variation of plants and the suite of environmental factors that affect their distributions (e.g., see Humboldt's classic, schematic 'map' of biotic and environmental variation along the slopes of Mount Chimborazo, Ecuador – **Figure 1**; see also Humboldt and Bonpland, 2009). Darwin's and Wallace's explanations for the patterns in the geography of life across archipelagoes and across the planet (see **Figure 2**) were based on the mutability of species and the pervasive force of natural selection, but also on the assumption that – because the Earth and its land forms and ocean basins were fixed, species must colonize distant regions by dispersal. Darwin was one of the strongest proponents of long-distance dispersal, but not without opposition. Joseph Dalton Hooker (1817–1911) was a contemporary, frequent correspondent and admirer of Darwin, but nonetheless one of his greatest challengers on the question of long-distance dispersal. Instead, Hooker (1866/1867) proposed that distributions of, and the similarity among distant biotas (in particular those of the southern hemisphere) can be attributed to the effects of great climatic and oceanic upheavals that broke up the once continuous flora that had spread across continuous tracts of land at an earlier period. Rather than suggesting that entire continents could raft across the globe (a hypothesis that at that time must have seemed fanciful science fiction), Hooker (1844–1860, 1859, 1866/1867) and his supporters (referred to as 'extentionists') suggested that the relative elevations of land and sea had shifted – at one time, sea levels dropped to expose trans-oceanic land bridges that allowed dispersal of plants and animals among the now isolated southern landmasses.

Removal of the final underpinnings of the doctrine of the fixity of the Earth – its climate, species, and land forms and ocean basins, would require more than a century of research and integration of insight from an expanding variety of disciplines including biogeography, paleontology, ecology, oceanography, and geology (in particular, marine geology). During the latter decades of the nineteenth century and early twentieth century, scientists – most notably Antonio Snider-Pelligrini (1802–1885) (Snider-Pelligrini, 1859) and Alfred Lothar Wegener (1880–1930), had commented on the apparent fit of the continents across the Atlantic Ocean, inferring from this that the continents may have once been connected at an earlier time in Earth's history and the evolutionary history of its species. Wegener (1912), in particular, developed a comprehensive theory of *continental drift* which included a reconstruction of earlier positions and patterns of subsequent separation of the continents, along with the potential forces that could drive the movement of entire landmasses. Wegener's theory also included speculations on the impacts of all this on the world's biotas, both past and present.

Wegener's theory was soundly rejected by the established scientific community, and it wasn't until some two to three decades after his death that the geological data – especially that on bathymetry and its relationship to tectonic activity – provided incontrovertible evidence for the reality of continental

Figure 2 Alfred Russel Wallace developed the first, global-scale visualization of the distinctiveness and differentiation among the world's regional biotas, each region and subregion developing their distinct assemblage of species by evolutionary divergence, while the similarity in biotas among regions reflects the influence of biotic mixing via long-distance dispersal across existing barriers or ancient, but now submerged land bridges (Wallace, 1876).

drift, eventually leading to the more comprehensive theory of *plate tectonics* was generally accepted. While dispersal, natural selection, and evolution accounted for many patterns of distribution and diversity of life, many other patterns could not be explained without considering patterns in the origin, movement, and destruction of the Earth's tectonic plates. Although evidence for Hooker's proposed mechanism for connecting now isolated biotas – trans-oceanic land bridges – never surfaced, the more general premise of his theory of the geographic dynamics of southern biotas is now recognized as the foundations of vicariance biogeography.

During the latter decades of the twentieth century, scientists from an ever-widening breadth of disciplines would continue to advance our understanding of the dynamics in the Earth – its land forms and ocean basins, its climatic and environmental conditions, and the forces of dispersal, vicariance, and natural selection and other processes that have influenced evolutionary divergence among regional populations. The myriad of marvelous patterns in the distributions and distinctiveness of life – the mysteries of what we now call biodiversity throughout the geological record, can now be explained by applying the principles and tools of the perhaps singularly holistic and integrative body of theory that now comprises modern biogeography.

See also: Biogeography, Human. Biogeography, Patterns in. Paleobiological Revolution, History of

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Biogeography, Human

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African Origins

The earliest *Homo sapiens* bones so far found came from sediments dated to a little under two hundred thousand years old in Ethiopia (McDougall *et al.*, 2005). Indeed most of the hominin line (humans and our ancestors excluding the apes), originated in Africa (Klein, 2009). For instance, all *Australopithecus* species, the immediate ancestor of *Homo*, are African.

That 'so far' in the first sentence is crucial. Anthropologists, archeologists, and paleontologists are always discovering earlier evidence. At the end of the nineteenth century, the earliest find of something that looked roughly human happened to be in Java; and so, at the time, scientists argued that the human line originated in Asia – with 'Java Man', eventually named *Homo erectus*. In fact, Erectus was perhaps the first hominin to expand its range outside of Africa – nearly two million years ago.

The evolutionary tree of human ancestry in **Figure 1** is one of several possible, because considerable debate surrounds the details. For instance, was *H. erectus* only Eurasian, or was it just the Eurasian form of *Homo. ergaster* of Africa? Indeed, are *H. ergaster* and *H. erectus* different species? The leader of the famous Dmanisi cave excavations in Georgia, David Lordkipanidze, thinks not. He argues that the substantial variation among the five *H. erectus* crania of approximately the same age found at this one site indicates that one variable species existed across Africa and Eurasia about two million years ago, not the two or even three often suggested (Lordkipanidze *et al.*, 2013).

The figure shows six species of the genus *Homo*. Some writers use the word 'human' to mean any of these species – *Homo habilis*, *H.ergaster*, *H.erectus*, and so on. Here, the term 'human' is used to mean only *Homo sapiens*.

Out of Africa

The human species initially left Africa about one hundred and twenty-five thousand years ago, as indicated by bones and stone tools of that date in southeast Arabia (Armitage *et al.*, 2011). However, the next oldest well-established indications of humans outside Africa are from about sixty thousand years ago, again in the Middle East. The first human diaspora failed. But the second was a roaring success (Oppenheimer, 2003).

Claims exist of diasporas earlier than sixty thousand years ago. One is based on seventy-thousand-year-old stone tools in southern India (Petraglia *et al.*, 2010). The problem is that nobody has yet found bones associated with the tools. That means that we cannot be sure who made the tools. Neanderthal is the obvious candidate, given the age and nature of the tools. But nobody has yet found Neanderthal remains closer than Turkmenistan, two thousand kilometers from southern India. Another claim is based on teeth from maybe more than 80 000 years ago in southern China (Liu *et al.*, 2015). This latter exciting find is too recent, though, yet to be widely accepted. Clearly, many puzzles in human evolution and hence historical biogeography remain to be solved.

By forty-five to forty thousand years ago, humans had reached Australia. Again, claims exist of an earlier arrival at about 60 000 years ago, as judged by dating of stone artifacts there, which could have been made by only humans. A major problem with that date though, is that it comes not from the artifacts themselves, but from the sediments in which the stone artifacts were found, but without clear signs that the artifacts did not in fact sink down from younger into older layers of sediment (O'Connell and Allen, 2004).

By the time early humans were in Australia, they were also in eastern Asia and much of Europe. Human presence over Eurasia is hardly surprising, given the lack of sea barriers to movement on the continent. However, to get to Australia humans would have had to cross a minimum of one hundred kilometers of open sea from either New Guinea or East Timor. That barrier might explain why Australia had to wait another forty thousand years for the next arrivals – Asians, who brought the dingo with them.

Some writers describe our spread across the Old World as rapid. But even if humans left Africa fifty thousand years ago, five thousand years to cover the twelve thousand kilometers to Australia is two to three kilometers a year, or a bit over five meters a day. That sort of speed indicates that we need to think of human movement across the world not as a migration, but as a population expanding and moving ameba-like across the world (**Figure 2**).

Humans left Africa as the northern hemisphere was heading toward the peak of the last ice age. The European ice cap finally extended down to the Midlands in Britain and to northern Germany in now mainland Europe. Not surprisingly, humans had to wait until the end of the ice age about ten thousand years ago and for the retreat of the ice cap, to enter Scandinavia.

However, humans were in north central Siberia a little over thirty thousand years ago, which is almost at the peak of the last ice age. We know this from the famous Yana Rhinoceros Horn site on the Arctic coast of central northern Siberia (Pitulko *et al.*, 2004). How could humans have lived there then if they could not get into Scandinavia until twenty thousand years later? It turns out that the Eurasian ice cap extended from Britain to only about half way across northern Russia. Farther east, the climate was too dry, it seems, for massive amounts of ice to form.

There humans stayed for fifteen thousand years or so, blocked from movement east by the massive North American ice sheet. In western North America this barrier extended from southern Alaska down to northern Washington. In the east it reached south as far as New York. Central Park there still carries signs of the three hundred meters of ice that once covered it. It is full of boulders dropped by the ice sheet, and the Park's bedrock still show the north-south scars from the boulder and stone laden ice scraping over it.

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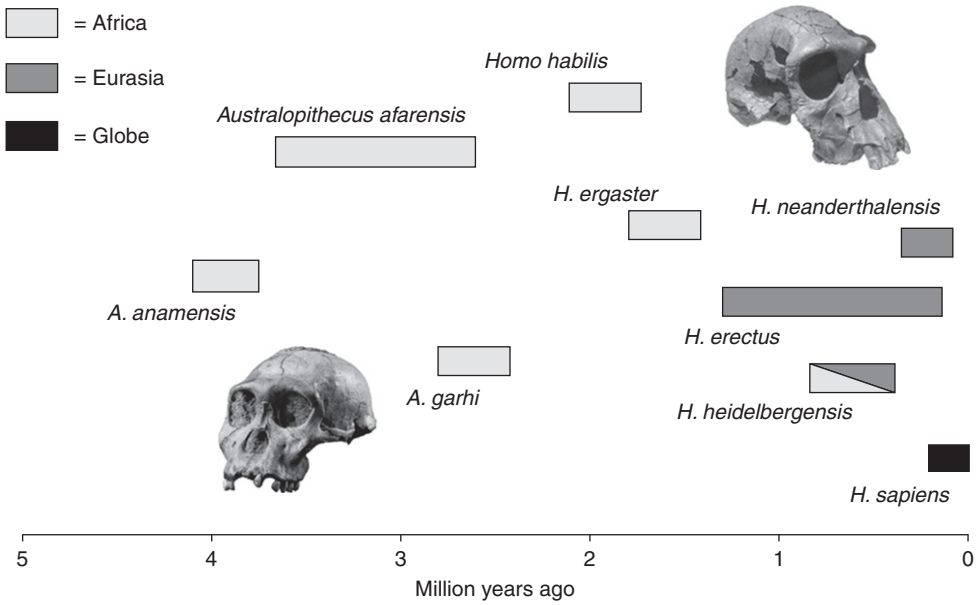

Figure 1 A current estimate of human ancestors' timeline and geographic distribution. Credit: John Darwent.

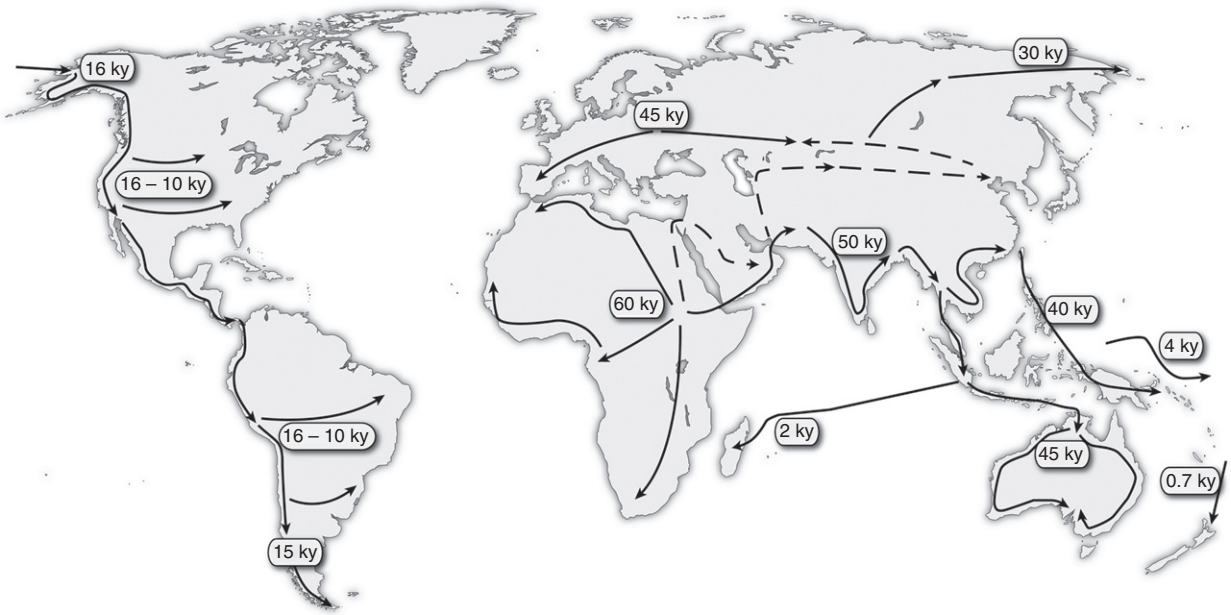

Figure 2 Main routes of the Out of Africa diaspora. Credit: John Darwent.

The last ice age reached its peak about twenty thousand years ago. Ice ages end rapidly, and by sixteen thousand years ago, the North American ice sheet had retreated enough for humans to start moving east from Siberia into the Americas. What is now the Bering Strait was then dry land. In effect, the water that made up the northern ice sheets came from the sea, which twenty thousand years ago was one hundred and twenty meters lower than it is now. The Bering Strait then was seventy meters above sea level.

Once again, humans appear to have moved fast. Just five hundred years after leaving Siberia – maybe sixteen thousand years ago – humans were in central Texas (Waters *et al.*, 2011). Less than a thousand years after that, those early migrants had reached southern Chile. We know that from the famous Monte Verde site (Dillehay *et al.*, 2008). The site is famous because it provides the earliest evidence of humans in South America. It is also notorious because of the sometimes harshly expressed disagreements with the finding that it was older than the oldest so-called Clovis sites in North America.

Archeologists recognize the Clovis culture by its elegantly crafted stone spear and arrow blades. They particularly characterize it by the smooth groove at the base of the blades,

presumably for slotting the blade into a shaft. The Clovis culture lasted for a few hundred years around thirteen thousand years ago. Archeologists used to consider it to be the culture of the first humans into the Americas. However, the firm dating in the 1980s of Monte Verde at nearly fifteen thousand years old, in other words nearly two thousand years older than any Clovis artifacts means that for thirty years now, we know that Clovis cannot have been the first culture in the Americas.

Routes Out of Africa

Central Texas, site of the earliest evidence yet of humans in the Americas, is half way across North America. The presence of humans here and at Monte Verde raises the issue of the routes followed by the earliest arriving humans in any part of the world. Did the immediate ancestors of the two-hundred-thousand-year-old first human in Ethiopia live in Ethiopia? Or did they move there from somewhere else in Africa? Did the emigrants from Africa who eventually peopled the rest of the world go across the south end of the Red Sea (properly the Reed Sea), or the north end? Were the first Americans immigrants from Siberia, or did they come across the Atlantic from western Europe – the 'Solutrean hypothesis' for the peopling of the Americas, refuted by the most recent genetic evidence (Rasmussen *et al.*, 2014). Did the Monte Verdeans arrive in southern Chile after crossing the southern end of the Andes from Argentina, as some argue, or did they come all the way down the coast from Alaska?

Early humans could well have taken a mostly coastal route in their expansion across the world. Along all the seashores of the world – Africa, Asia, the Americas, Australia – one can find immense mounds of shellfish, often mostly oyster shells, one hundred meters or more long, tens of meters wide, and up to five meters high. Trees grow on some of the mounds. The advantage of shellfish is not only that they are highly nutritious but also that they are easy to harvest. Even children can, with little effort collect a full meal of them.

A coastal expansion across the world means that modern-day archeologists interested in the details of the route need to be trained scuba divers, especially if their interest is the Old World. That is because at the time, sea levels were already tens of meters lower than now, if not yet at the one hundred and twenty meters lower than the ice age's peak.

Central Texas is of course not on the coast. It is, though, south of the so-called 'ice-free corridor' that appeared maybe fourteen thousand years ago as the ice sheet covering North America melted. The corridor was just east of the Rocky Mountains in roughly today's Alberta and Montana. It separated the Cordilleran ice sheet to the west from the far larger Laurentide ice sheet that extended all the way to Greenland. The corridor raises the question of whether the early Texans, and other early Americans, came down the coast to south of the ice sheets and then moved inland, or whether they expanded through the ice-free corridor into the center of North America and spread out from there?

The very earliest Americans must have moved down the coast, because before the corridor opened they had arrived in both Texas in southern North America, and in Monte Verde in southern South America. For maybe hundreds of years after the corridor opened, a coastal route for all the early North American immigrants, maybe even the Clovis culture, seems likely. The edges of retreating ice sheets are for several kilometers out a sodden, flooded terrain of boulders and rubble. Every now and again, so-called katabatic winds reaching a hundred kilometers or more an hour sweep off the ice. The ice-free corridor would have been one and a half thousand kilometers of appallingly cold, barren wasteland, and as yet no good evidence exists to indicate that the earliest Americans used the corridor.

The Americas were the last of the major continents to be peopled. But not the last continent, and not the last land. The last places were oceanic islands, especially the Pacific islands (Kirch, 2000). Humans reached the western Pacific islands, the Solomons east of New Guinea for example, maybe thirty thousand years ago. And there the human expansion across the Pacific got stuck for the next twenty-five thousand years. Substantial ocean-going boats were the requirement for further conquest of the Pacific, and those the islanders did not design and build until maybe five thousand years ago.

A particular form of pottery, Lapita pottery, shows that humans reached the mid-Pacific islands, Fiji for example, maybe four thousand years ago. Two thousand years later, humans had reached most of the Pacific islands. These voyages of settlement are extraordinary. Hundreds, even thousands, of kilometers of ocean crossed. Days, weeks, months maybe, with no sight of land. New Zealand was the last major landmass that humans reached – just seven hundred years ago.

Many boats must have foundered during the spread across the Pacific; so vast are the distances. As just one example, the Maoris of New Zealand came originally not from the closest landmass, Australia, but from the Cook Islands, over two thousand kilometers away. Genetic studies indicate that the entire present-day Maori population might have descended from just seventy female ancestors (Murray-McIntosh *et al.*, 1998).

Madagascar's first immigrants too might have come not from the closest continent, Africa, just four hundred kilometers away, but from Indonesia, specifically southeast Borneo (Razafindrazaka *et al.*, 2010). In a straight line, Borneo is three thousand kilometers from Madagascar. Both genetics and linguistics confirm this extraordinary migration. Maybe at about the same time, two thousand years ago, or maybe five hundred years later, Africans immigrated. One reason to think that the Borneans got to Madagascar before the Africans is that Malagasy, the language of Madagascar, is in effect a Bornean language. Five hundred years ago, the French arrived. And the island continent now is a mix of Indonesian, African, and French cultural influences.

How Do We Know?

Disparate scientific disciplines contribute to the discipline of human biogeography – genetics, archeology, and linguistics.

Because genetic differences accumulate over time, the level of genetic diversity in a population or region can indicate age; and as already indicated, Africa has the greatest diversity (Cann *et al.*, 1987, Cavalli-Sforza, 2000). Some studies indicate twice

as much genetic diversity there as in any other major world region. The concept of an African 'race' is clearly biologically untenable.

Immigration can also augment the genetic diversity of a population. Nowadays, big cities are immensely variable genetically because of immigration. However, immigration is not why Africa is so genetically varied. People have certainly immigrated into Africa. But Africa has more of its own genes than has any other continent. Indeed, all other continents' genetic profiles are essentially subsets of the African genetic profile. Any human population lacks many of the genes of other continents, but no regional population lacks African genes. No other way exists for such a genetic pattern than an origin in sub-Saharan Africa. Another reason for the drop in diversity away from Africa is that as humans dispersed, only a subset of each population moved on (Amos and Hoffman, 2010). 'Genetic bottleneck' is the jargon term for such a process.

The drop in genetic diversity from the source population is very evident with the peopling of the Americas from Siberia. Among Siberians, one can find all of the three major blood groups. But among Native Americans of North and especially South America, the O blood group is in the vast majority. Over eighty percent of Native North Americans are O. Ninety-nine percent of native South Americans, Amerindians, are O. Not only that, but native Americans and Beringians have a form of gene that nobody else in the world has (Schroeder *et al.*, 2007). Only one way exists for such a pattern. The vast majority of native Americans descended from a small number of Siberian immigrants.

And where did the Siberians come from? Genes indicate certainly eastern Asia, but also central Russia, and also western Europe. That last origin comes from a surprise recent find (Raghavan *et al.*, 2014). So if humans expanded along coasts in their first movement out of Africa, we clearly subsequently migrated along inland routes.

Where We are Influences What We are

Skin color is an obvious example, if a divisive one, of people in different environments differing anatomically, indeed physiologically, because individuals with certain traits do better in certain environment than do individuals without those traits. Oddly enough, even Darwin was wrong on skin color. He could not see how it matched the environment (Darwin, 1871, p. 174). But it does. People from the tropics tend to be darker than people whose ancestors came from outside the tropics.

The reason is that tropical peoples' skin has in its surface layers more melanocytes. By blocking and scattering the passage of light, the melanocytes help prevent sunburn, in other words, damage to the surface layer of skin cells. More crucially, the melanocytes filter out ultraviolet light by both absorbing and scattering it. That prevents the rays from penetrating deeper into the skin, and so prevents the ultraviolet from destroying vitamin B9 in any blood circulating near the skin (Jablonski and Chaplin, 2002). Vitamin B9, also known as folate or folic acid, has a variety of vital jobs in the human body. One is in the production of red blood cells. Another crucial part it plays is in the development of the fetus' spinal cord and brain.

So why do we not all have dark skin and why do females not have darker skin than do males? The answer is that UV light stimulates the production in our skin of vitamin D, crucial for healthy bones via its effect on the body's metabolism of calcium, among other elements. Too little sun, and a diet without vitamin D supplements, and children develop rickets. Strictly dressed Moslem women in northern latitudes can also suffer.

Yet the palest people are not those the farthest north. Arctic people are darker than western Europeans. It turns out that the Arctic peoples' traditional diet, heavy in fish and meat, is high in vitamin D. Until recently that was not true of western Europeans. Their diet was largely cereals, and cereals are unusually low in vitamin D (Khan, 2010). A main reason we do not see rickets in western European populations nowadays is that the major producers of bread and cereals add large amounts of supplemental vitamin D.

Many other aspects of our physiology and anatomy differ between regions (Harcourt, 2012, 2015). The lengths of our limbs and the shape of our bodies differ. For instance, Africans tend to be longer and thinner than Europeans and certainly Arctic people. A likely reason is that long, thin bodies lose heat more easily than do short, squat ones. Africans are better than Europeans at retaining salt when they sweat. Why? Probably because salt is in shorter supply in Africa than it is in Europe, so weathered are the rocks and soils of Africa.

But all these anatomical and physiological differences between people from different regions are in many textbooks of biological anthropology. A topic rarely found in any of the textbooks is the question of why in biogeographical terms, the tropics are more culturally diverse than are higher latitudes.

Geography and Cultural Diversity

The latitudinal gradient in diversity of plant and animal taxa is well known. Human cultures show a similar pattern, with a far greater density of cultures in tropical latitudes than outside them (Harcourt, 2012, 2015). Take Ecuador and Britain: travelers could hear twenty-three indigenous languages as they voyaged across Ecuador; they would hear no more than twelve in Britain. South America is two-thirds the area of North America, but one can hear fifty percent more languages in South America than in North America, nearly five hundred as against a little over three hundred (Lewis, 2009; **Figure 3**). The author terms these latitudinal contrasts in diversity (whether biological or cultural) the 'Forster effect,' after Johann Forster, the biologist on James Cook's second voyage around the world, who was one of the earliest biologists to remark on it (Harcourt, 2012, 2015).

Current winning arguments to explain the Forster effect in non-human species include the idea that rates of taxonomic diversification are higher in lower latitudes (Brown, 2014), and that life has had more time to diversify in large areas of the lower latitudes (Fine, 2015). After all, just twenty thousand years ago, much of northern Europe and America looked like Greenland currently does.

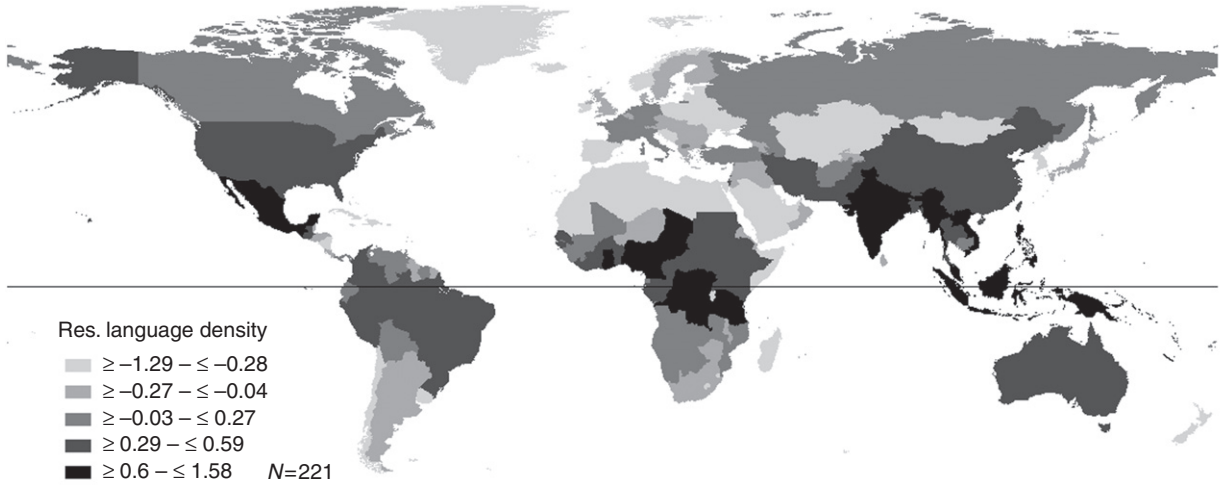

Figure 3 Density of languages per country in relation to the number expected given the area of the country. Map from www.ethnologue.com.

These ideas do not seem to work so well for human cultures. That is because humans arrived at roughly the same time in both tropical and temperate regions in first the Old World outside of Africa, and subsequently in the New World (Harcourt, 2012, Ch. 2). Indeed, as mentioned previously, one of the current earliest reported dates of arrival outside of Africa, a minimum of 80 000 years ago, is not in the tropics, but in temperate southeastern China (Liu *et al.*, 2015). And yet temperate China does not have more cultures than do tropical regions to the south. Nor does it have more than does the Amazon, where humans did not arrive until about 15 000 years ago. Add the likelihood that in the last ice age, Africa's and Asia's forests, which are large regions of high linguistic diversity now (Harcourt, 2012, Ch. 5), were reduced then to remnants (Jablonski, 1998; Hamilton and Taylor, 1991; Harcourt, 2012, Ch. 2), and there too human cultures might not have had much longer to diversify than have American cultures. Nevertheless, lack of time to diversify could partly explain New Zealand's paucity of native cultures. The original Maoris arrived less than a thousand years ago.

Of the many explanations offered for the Forster effect (Lomolino *et al.*, 2010, Ch. 15), one seems nicely to explain the distribution of cultures (Harcourt, 2012, 2015). Part of the explanation is based on the fact that the average size of a tropical culture's geographic range is smaller than that of a culture from higher latitudes. The same is true for many plant and animal species. To give an example for human cultures, the Guato and Bororo people of Brazil each range over less than seven thousand square kilometers. Compare that to the seventy thousand square kilometers of the MacKenzie Inuit of the Canadian Northwest Territory. In brief, more cultures pack into an area of a given size in the tropics than they do at higher latitudes.

An explanation for the fact of small geographic ranges in the tropics is the high productivity of the tropics. That high productivity means that a culture with a large enough population to survive over millennia can exist in a smaller area in the tropics than outside the tropics. Warmth and water produce an abundance of plants and animals, in other words an abundance of food. And crucially, where there is water, that abundance is available year-round in the tropics. That is why so many temperate bird species go to the tropics in the temperate winter. If cultures can survive in small geographic ranges, then more cultures can pack into a region.

The same productivity argument could explain the high diversity of Native American cultures on the Gulf Coast compared to farther north, and the west coast of the United States compared to inland. In both cases, the coastal environment is richer than the inland one. Compare, for example, California's highly productive Central Valley with neighboring Nevada's near-desert.

High tropical productivity alone cannot explain New Guinea's extraordinary density of cultures, the highest in the world. To understand this, we must turn to topography. Eastern New Guinea is in essence a mountain chain cut by deep ravines. Almost every major ridge has its own culture. Difficulty of movement, then, can prevent amalgamation of cultures, and promote diversity of them. Hence, we see a high diversity of cultures in the lower reaches of the Himalayas and the Karakorams.

New Guinea cultures are notoriously warlike and xenophobic. Does mere xenophobia, then, prevent mixing and hence cause diversity? Maybe, but if so, we need to ask which came first, the cultural diversity (resulting from geographic barriers), or the xenophobia.

In Europe, twenty-two of thirty-three boundaries between genetically distinct populations are obvious geographic barriers. A sea separates the French and English. A mountain range, the Alps, separates France from Italy. It looks as if geography might be separating cultures. However, thousands of ships and airplanes cross the English Channel every year, and have done for decades. Thousands of cars, buses, trains pass through Alpine tunnels every year. Soldiers have criss-crossed Europe for centuries, and soldiers are not famously celibate.

On the other hand, either side of these geographic boundaries and in nine of the remaining eleven cases where no geographic barrier exists, the genetically different populations speak a different language or dialect (Barbujani and Sokal, 1990). In other words, it looks as if indeed language

could be a barrier to cohabitation as is, in the case of animals, inability to understand or be stimulated by another species' courting behavior.

To conclude, the great cultural diversity of humankind seems to have been shaped in part by some of the same biogeographical influences as have shaped the diversity of other animals and also plants. Biogeographically, humans are in some important ways just another species.

See also: Directed Evolution, History of. Dispersal Biogeography. *Homo*, Diversification of

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Biogeography, Marine

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Glossary

Allopatry Occurring in nonoverlapping geographic areas.
Biodiversity feedback A process by which species arising in areas of high biodiversity colonize areas on the periphery. The peripheral colonizers subsequently spawn new species that recolonize the original area and further increase diversity in the centers of origin.
Endemic Native species that is confined to a certain geographic region, often a biogeographic province.
Peripatric Occurring in a peripheral geographic area.
Phylogeography The study of the geographic distribution of individuals and their associated gene genealogies to infer historical processes responsible for contemporary distributions.
Relictual A remnant species that inhabits only a fraction of its historical geographic range, often because of environmental change.
Sympatry Occurring within the same geographic area such that frequent encounters are likely.

Introduction

Marine biogeographic provinces, based on distinct floras and faunas, have been recognized for over 150 years (Forbes, 1859). These provinces represent parts of the world that host unique biotas, both areas of recent evolutionary innovation and refuges of ancient lineages that persist today. Although impenetrable barriers are relatively uncommon in the sea, boundaries between these provinces are frequently associated with continents, sharp ecological gradients, or vast expanses of open ocean. Three observations by Forbes (1859) still guide the field of marine biogeography today: (1) each zoogeographic province is an area where new lineages arise and tend to mix with emigrants from other provinces; (2) each species is created only once and individuals tend to expand their range from their place of origin; and (3) to be understood, provinces, like species, must be traced back to their origin in the past.

The first global characterization of marine biogeographic provinces was compiled in the pioneering volume *Tiergeographie des Meeres* (Ekman, 1935), later updated and translated into *Zoogeography of the Sea* (Ekman, 1953). Therein, Sven Ekman described a series of large regions and subregions, including the continental shelf, tropical, temperate, and polar waters, their separation by zoogeographic barriers, and their endemism. Briggs (1974) divided the continental shelves into a series of large biogeographic regions that, in turn, contained smaller biogeographic provinces, each defined on the basis of endemism. This work established the now-accepted practice of defining biogeographic provinces on the basis of 10% endemism at the species level within published species inventories, most frequently fishes or well-known invertebrate groups such as mollusks. A central theme was that the greater the proportion of endemic biota, the greater the evolutionary significance of the province (Briggs, 1974).

Marine biogeography has seen a recent reevaluation in the face of considerable research over the past few decades (reviewed by Briggs and Bowen, 2012). Although there are debates about the definitions and boundaries, here we follow previous work and define the biogeographic provinces as shallow (<200 m) marine regions with at least 10% endemism (Briggs, 1974). In many provinces, data on endemism remain unavailable, or incomplete with only the most widely studied taxa (typically fishes) defining the boundaries. Recent works, including new studies from remote locations, and particularly phylogeographic approaches, have revealed genetic subdivisions within and between species that modify or redefine the various divisions (Avise, 2000). Notably, detailed phylogenetic comparisons of warm temperate and tropical biota are erasing the earlier artificial separation of these regions into different zoogeographic regions. As a result, marine biogeographic provinces have been realigned recently (Briggs and Bowen, 2012), and we follow this treatment below in defining marine biogeographic provinces.

Marine Biogeographic Barriers

The world's oceans are all connected, and have been for a very long time. While a few vertebrates have achieved circumglobal distributions (Gaither *et al.*, 2015), most marine species are constrained to a much smaller range, often defined by the edges of biogeographic provinces. In a continuous transglobal medium, what constitutes these boundaries? The most obvious barriers are geographic. Africa moved north into Eurasia about 11–18 million years ago (MYA), closing the Tethys Sea and creating an impenetrable barrier between the Indo-Pacific and Atlantic-Mediterranean systems (**Figure 1**). Occasionally tropical (or warm-temperate) marine species are able to colonize between the Indo-Pacific and Atlantic by going around southern Africa. This is especially possible at the end of each glacial epoch, when the cold water (Benguela) upwelling attenuates and a warm water corridor opens around southern Africa (Peeters *et al.*, 2004).

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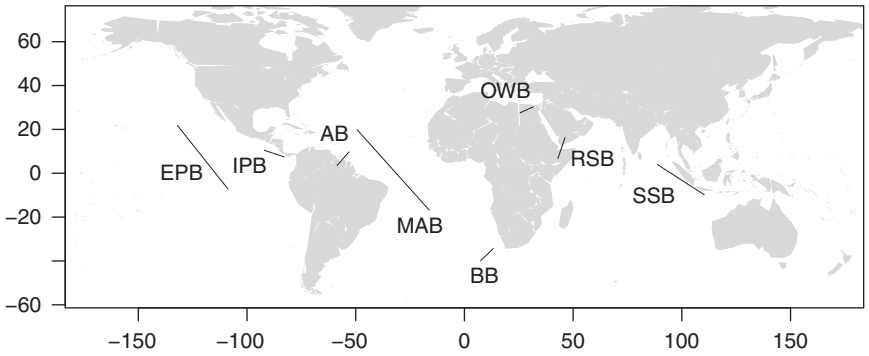

Figure 1 Map depicting the eight major marine biogeographical barriers: the East Pacific Barrier (EPB), the Isthmus of Panama Barrier (IPB), the Amazon Barrier (AB), the Mid-Atlantic Barrier (MAB), the Benguela Barrier (BB), the Old World Barrier (OWB), the Red Sea Barrier (RSB), and the Sunda Shelf Barrier (SSB) (Rocha *et al.*, 2007).

Another major geographic barrier is the Isthmus of Panama. An equatorial current flowed from the Pacific into the proto-Caribbean until about 6 MYA, followed by an impenetrable land barrier about 3 MYA. Many evolutionary studies have benefitted from this natural laboratory, with sister species on each side of Central America (Lessios, 2008). Notably, mankind has cut canals through both of these barriers, with few colonists crossing the (mostly freshwater) Panama Canal, but many species moving from the Red Sea into the Mediterranean Sea through the Suez Canal (Golani *et al.*, 2007).

An especially interesting land barrier is the Sunda Shelf (Indo-Pacific) barrier between the Indian and Pacific Oceans (**Figure 1**). This barrier only exists during glacial periods, when sea level drops over 100 m and exposes a nearly complete land bridge between Asia and Australia. The presence of an intermittent barrier, on ~100 000 year cycle, has allowed some species to diverge into sister species, while in other cases the Pacific and Indian Ocean cohorts reconnect and interbreed or hyrbidize (Gaither and Rocha, 2013; Hobbs *et al.*, 2009).

Open ocean is another major barrier, with the largest being the East Pacific Barrier between central Pacific islands (Hawaii and Polynesia) and the eastern Pacific, about 4000 km. Darwin (1872) labeled this an impenetrable barrier to nearshore organisms, and that view is supported by much taxonomy. However, recent phylogeographic studies have demonstrated that some organisms can make this crossing with sufficient regularity to maintain species integrity (Lessios and Robertson, 2006).

Freshwater outflows can also be barriers in the marine realm, with the Amazon River being the best-studied example (Floeter *et al.*, 2008). This enormous outflow cleaves the tropical West Atlantic into Caribbean and Brazilian Provinces, and many sister species have formed as a result (Briggs and Bowen, 2013). One interesting facet of this barrier is that it applies primarily to reef organisms. The nutrient-rich waters of the Amazon feed vast sponge beds under the freshwater Amazon plume, so for species that occupy sponge habitat, no barrier exists (Rocha *et al.*, 2002).

Marine Biogeography and Evolution

For many decades, evolutionary biologists have debated whether speciation is different in the sea. Most marine fauna have a pelagic larval stage that can stay in the water for days to months. This makes possible extensive dispersal and gene flow across thousands of kilometers, preventing allopatric speciation except on the largest scale between ocean basins (e.g., Iacchei *et al.*, 2015). However, shallow marine habitats can be very heterogeneous, offering many habitats and niches that provide opportunities for specialization and speciation along ecological boundaries (Bird *et al.*, 2012). Hawaiian limpets, for example, have evolved into three species in sympatry, after a rare colonization event from Japan about 5 MYA (Bird *et al.*, 2011). These limpets faced different selection pressures in the high, middle, and low intertidal, and adaptive divergence has produced three species in response to these differing ecological conditions (Bird, 2011). The emerging consensus is that speciation in the sea follows the same processes as terrestrial and freshwater biota, but on different scales of isolation, dispersal, and ecological divergence (Bowen *et al.*, 2013).

A central debate in marine evolution is about the role of biodiversity hotspots, such as the Caribbean Sea and the Coral Triangle (between Indonesia, New Guinea, and the Philippines). Do these hotspots serve as evolutionary incubators (centers of speciation) that export new species, or do they acquire species that arose elsewhere (centers of accumulation)? Recent evidence indicates that the hotspots are centers of origin (Briggs, 2003; Cowman and Bellwood, 2013). However, isolated peripheral provinces, including Hawaiian and the Red Sea, are centers of endemism and may export species as well (Eble *et al.*, 2015). Hence both areas of high endemism and areas of high biodiversity are important for evolutionary processes, with peripheral provinces producing new species primarily through allopatry, and hotspots producing more species in sympatry, with divergences along ecological lines (Briggs, 2005).

Plate Tectonics and Marine Evolution

The surface of Earth is divided into submerged oceanic plates and the thicker continental plates, all in continuous motion driven by flow in the underlying mantle. At several points in Earth's recent history (Cenozoic Era, 66 MY), tectonic changes have prompted revolutions in the distribution of marine life.

Here we provide three examples of how geology has altered the marine biogeographic history.

1. *The movement of a biodiversity hotspot*. For much of the Cenozoic Era, the Tethys Sea provided a continuous belt of warm water around the globe. This global connectivity ceased as Africa moved north into Eurasia about 18 MYA. As the connection between Atlantic-Mediterranean and Indian Ocean biospheres was severed, the center of highest tropical diversity moved east, from the Mediterranean-Indian region to the current hotspot in the Coral Triangle (Bellwood and Wainwright, 2002). About 5 MYA the Coral Triangle began exporting biodiversity (center of origin) that now accounts for an estimated 60% of global diversity in some fish groups (Briggs, 2003; Cowman and Bellwood, 2013).
2. *The trans-Arctic interchange*. Up until about 3.5 MYA, the Bering Strait and Arctic ice cap provided an impenetrable barrier between the North Pacific and North Atlantic oceans. With the opening of this barrier by warming and rising sea levels, a massive exchange of marine biota occurred, primarily from the high diversity North Pacific into the depauperate North Atlantic. Vermeij (1991) documented 265 mollusks invading the Atlantic, with only 24 moving in the opposite direction. Important fish groups such a salmon (family Salmonidae) and sculpins (family Cottidae) colonized into the Atlantic, while the cods (family Gadidae) may have moved the other way. This trans-Arctic passage closed with the return of Pleistocene sea ice, but Vermeij and Roopnarine (2008) warn that the Atlantic-Pacific interchange could resume by year 2050 under current climate predictions.
3. *The Isthmus of Panama*. The last vestige of the circumtropical Tethys Sea was eliminated about 3 MYA with the rise of the Isthmus of Panama. As noted above, this land bridge effectively divided a continuous tropical marine fauna into Caribbean and East Pacific cohorts (Lessios, 2008).

Marine Biogeographic Provinces of the World

Biogeographic provinces provide a broad overview of marine biodiversity, but of course this vast array of life is subdivided into smaller ecosystems and communities. For example, Spalding *et al.* (2007) divide coastal biodiversity into 232 ecoregions as a suitable framework for marine conservation measures. Likewise, within provinces there can be concordant genetic breaks among many species (Avise, 1992; Carpenter *et al.*, 2011; Toonen *et al.*, 2011) that indicate shared limits to dispersal among divergent taxa at a scale finer than the biogeographic provinces on which we focus here. Finally, there are a variety of unique ecosystems with communities that may exceed the 10% endemism criterion, but are beyond the scope of this article. Here we provide three examples of such unique ecosystems before moving into our survey of the shallow coastal marine biogeographic provinces of the world.

1. *Marine Lakes*. Marine lakes form when inland depressions in fractured karst (limestone) become flooded by rising sea level. There are roughly 200 marine lakes known, with most located in Vietnam, Palau, and Indonesia. Although these lakes remain connected to the sea through fissures or small tunnels, they typically have greatly reduced tidal influence, high rainfall and other freshwater inputs, and shelter from coastal winds. Combined, these factors reduce mixing and increase vertical stratification (Hamner and Hamner, 1998), and in turn, result in marine lakes being isolated and different enough from nearby ocean environments that they develop a suite of unique species separated from coastal congeners for many thousands of years (Dawson and Jacobs, 2001; Dawson, 2005).
2. *Hydrothermal vents*. These fissures in the Earth's crust, occurring at mid-ocean ridges and other volcanic hotspots, produce chemical-rich seawater that can exceed 400 °C. In the lightless environment below 1000 m, hydrothermal vents support chemosynthetic microbes that are the basis for one of the strangest ecosystems on earth, with highly specialized shrimps, clams, snails, and giant tube worms. Thermal vents are probably as old as the oceans (~4000 MY; Elkins-Tanton, 2011), and have been proposed as the origin of life on Earth (Wächterhäuser, 1990). However, phylogenetic studies show that the fauna are much more recent (<100 MYA) and some are derived from shallow marine species <20 MYA (Van Dover *et al.*, 2002). Six biogeographic provinces are recognized for thermal vent fauna, occurring on the scale of ocean basins: Western Pacific, Northeast Pacific, East Pacific Rise (30° N to 30° S), Azores, Mid-Atlantic Ridge, and Central Indian Ocean (Van Dover *et al.*, 2002). Further exploration may reveal additional faunal provinces. A biogeography of the deep sea has recently been proposed (Watling *et al.*, 2013), and we refer interested readers there for additional information.
3. *Whale Falls*. Carcasses of dead cetaceans (whales, dolphins, and porpoises) fall to the deep seafloor to create a highly localized ecosystem fueled by decomposition (Goffredi *et al.*, 2004). Such communities, called whale falls, are typically found at depths greater than 1000 m and can persist for decades, during which they provide a hard surface for invertebrate colonization as well as a source of sulfides from the microbial decay of organic compounds contained in the whale bones (Bennett *et al.*, 1994). These whale fall communities can provide stepping stones for the dispersal of deep sea hydrothermal vent species (Feldman *et al.*, 1998).

Our focus here is on shallow coastal seas, and following Briggs and Bowen (2012), we define the following biogeographic provinces for shallow (<200 m) marine habitats across the globe (**Figures 2** and **3**).

Warm Regions (Tropical and Warm-Temperate Waters)

Eastern Atlantic Region

Lusitania

This province encompasses the warm-temperate waters from the southern end of the English Channel down to southern Morocco and eastward to include the Mediterranean

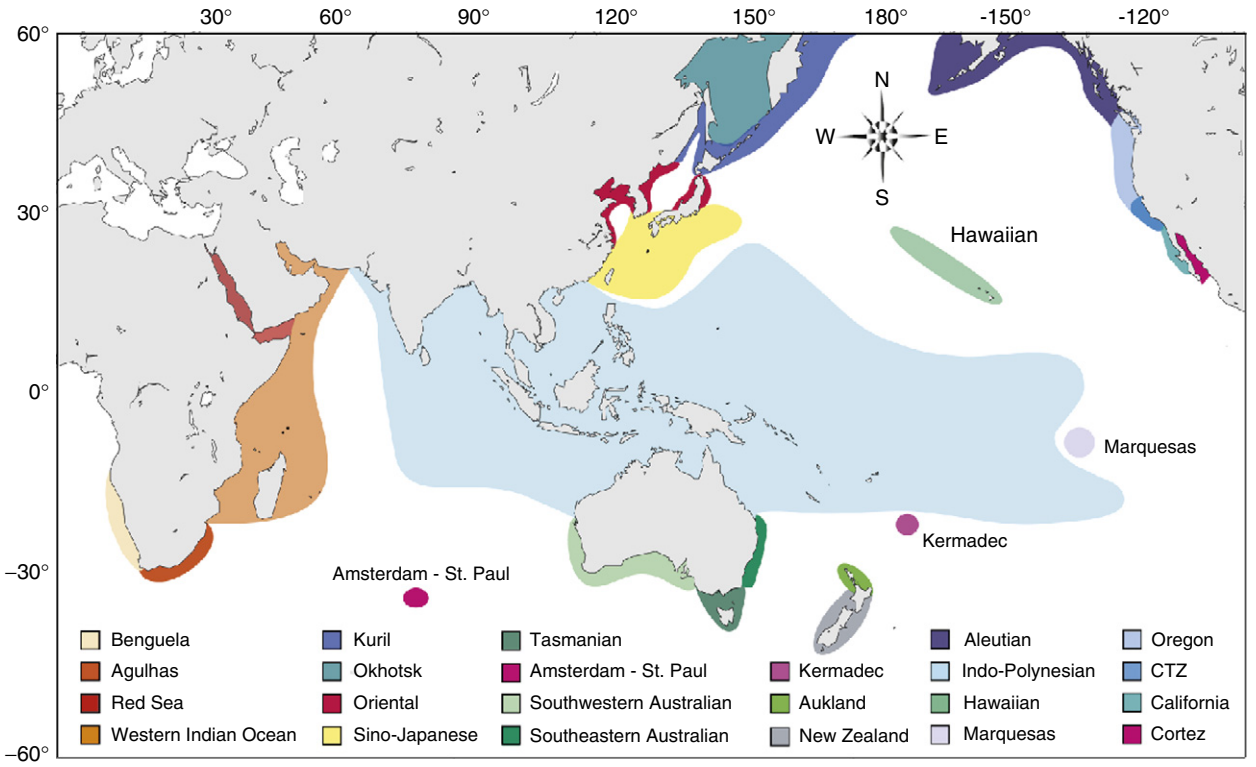

Figure 2 Map of the Indo-West Pacific Provinces. Each province is identified by a distinct color as shown in the key below the map. In general, the key lists provinces from West to East and from North to South. One province from the subantarctic region (Macquarie) and one of the New Zealand–Australian region provinces (Antipodes) are outside of the range of this map and are not shown.

(with 28% endemism) as well as the Azores, Madeira, and the Canary Islands. Although Briggs and Bowen (2012) place this province with the warm-temperate provinces on the basis of a prevalence of warm water fishes, Almada *et al.* (2013) have subsequently argued that the Macaronesian archipelagos are highly distinct from the mainland coasts of the Lusitanian Province, and these should not be grouped together.

Black Sea

This subtropical sea has a Mediterranean climate but is located in the temperate zone. It contains a mixture of relictual species mixed with more recent Mediterranean and freshwater invaders as well as a suite of alien species.

Caspian Sea

This is the largest enclosed body of water in the world, sometimes classified as the world's largest lake. Indigenous brackish water species (and genera) form the basis of the high endemism with the remainder of species drawn largely from the Mediterranean, freshwater, or Arctic biota.

Aral Sea

This 5.5 MY old sea is technically an endorheic lake (lacking an outflow), lying between Kazakhstan and Uzbekistan. Formerly the fourth largest lake on the planet, Soviet irrigation projects diverted inputs in the 1960s, and by 2007 only 10% of the 68 000 km^2 remained (Filippov and Riedel, 2009). The catastrophic decline in area has led some to label this as 'one of the planet's worst environmental disasters' (Daily Telegraph, 2010). The Aral Sea has been subject to rapid environmental changes throughout its entire history, leading some researchers to question the estimates of endemicity for this disappearing province (Filippov and Riedel, 2009).

Tropical Eastern Atlantic

Extending from southern Morocco south to Angola, and including the offshore islands of the Cape Verdes, São Tomé, and Principe, the Tropical Eastern Atlantic is characterized by a narrow continental shelf and seasonally strong upwelling. The waters are typically stratified with warm, turbid, lower salinity water (due to high river inputs) above cool nutrient-rich water, separated by a strong thermocline at about 100 ft (30 m). Although diversity is lower in comparison to the Tropical Western Atlantic, endemism is about 30% and this region is highly productive, ranking as one of the most important fishing zones in the world (Spalding *et al.*, 2007).

Benguela

Along the western coasts of Namibia and South Africa, this warm-temperate province is strongly influenced by the intense upwelling of cold nutrient-rich waters from ~200–300 m. The Benguela current results in a general equatorward flow of cooler water into the South Atlantic gyre. This nutrient-rich water is ~30–65 times more productive per unit area than the average global ocean. There remains some debate about whether the Namib, Namaqua, and Southwestern Cape bioregions each represent discrete biogeographic provinces or subregions (Lombard *et al.*, 2004), but with the boundaries

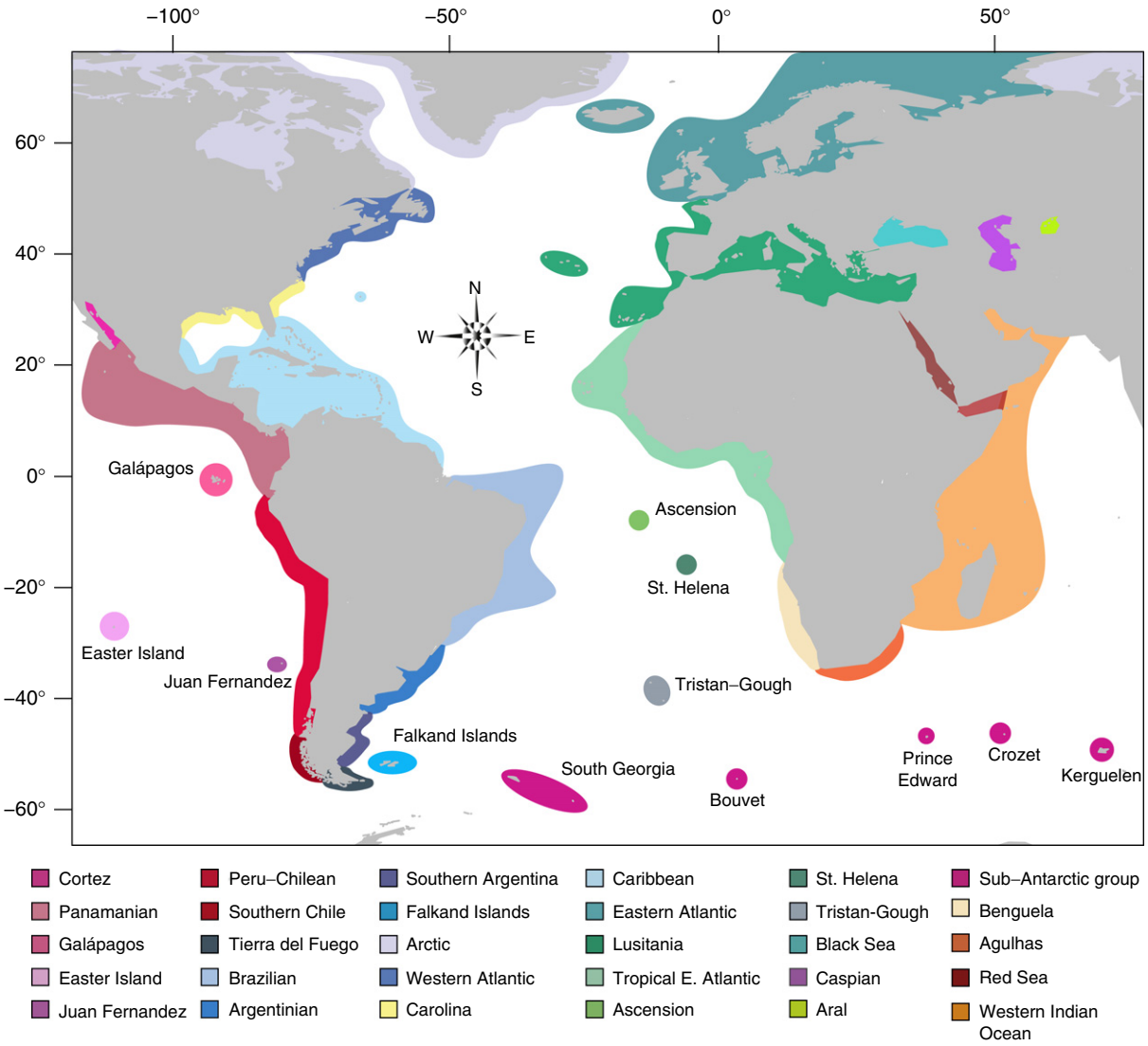

Figure 3 Map of the Atlantic and East Pacific Provinces. Each province is identified by a distinct color as shown in the key below the map. In general, the key lists provinces from West to East and from North to South. The Antarctic Province is outside the range of the map and is not shown here.

reported in Briggs and Bowen (2012) this province contains about 12% endemic species. The Cape of Good Hope forms the dividing line between the warm-temperate Benguela Province and the Agulhas Province (below) in the Indo-Pacific.

St. Helena

Located 4000 km east of Brazil, and 2000 km west of Africa, this is one of the most remote islands in the world, in the middle of a large expanse of deep ocean. St. Helena was an important haven for ships transiting from Asia to Europe during the age of sail (1500–1800s). With more than 400 species of endemic birds and plants, the terrestrial biodiversity is matched by high endemism of marine fishes and invertebrates (13%). St. Helena has nearly equal taxonomic affinities with the marine fauna of Brazil (16%) and the Tropical Eastern Atlantic (15%) (Floeter *et al.*, 2008).

Ascension

Located 1300 km northwest of St. Helena, Ascension Island lies 1600 km from the coast of Africa and 2250 km from the coast of South America. In contrast to the faunal affiliations of St. Helena, Ascension is more similar to the Brazilian province (29% shared species) than the Tropical Eastern Atlantic (6% shared species), with 11% endemic species.

Tristan–Gough

Located 2400 km from southern Africa, and 3400 km from South American, the Tristan–Gough volcanic hotspot includes four small islands, the largest of which are Tristan da Cunha and Gough. Lying more than 1800 km from the nearest land (Bouvet Island), and 2000 km from the nearest inhabited island (St. Helena) the archipelago of Tristan da Cunha is recognized as the most remote inhabited location on the planet. Formally designated as a UNESCO World Heritage Site, Gough

Island is considered one of the least disrupted ecosystems on Earth, and is home to a suite of endemic and endangered terrestrial and marine species.

Western Atlantic Region

Carolina

Located along the southeast United States and adjacent Mexico, this province includes two distinct regions isolated by the Florida Peninsula: one in the northern Gulf of Mexico, and the other along the Atlantic coast of North America. This region is sometimes regarded as a transition zone between the temperate Virginian province and tropical Caribbean province (e.g., Freeman *et al.*, 2007). Opisthobranchs (sea slugs and their relatives) show high endemism in the Atlantic section of this province (García and Bertsch, 2009), and numerous phylogeographic studies highlight genetic partitions between the two sections of this province (Avise, 1992).

Caribbean

Encompassing all the Western Atlantic tropics north of the Amazon River, this province extends from Bermuda and Cape Canaveral, Florida to northern Brazil. The Caribbean hosts the most extensive coral reefs in the Atlantic and is the hotspot for tropical Atlantic biodiversity. Endemism is high in reef fishes (33%, Floeter *et al.*, 2008), decapod crustaceans (32%, Boschi, 2000), and corals (37%, Veron, 2000). Much of the tropical fauna elsewhere in the Atlantic is derived from this province (Briggs and Bowen, 2013).

Brazilian

Extending from the Amazon River to Santa Catarina in southern Brazil, this province includes the offshore islands of Atol das Rocas, Fernando de Noronha, St. Paul's Rocks, and Trindade (Floeter *et al.*, 2008). Between the Brazilian and Caribbean provinces, the northeastern coast of South America from the Orinoco and Amazon rivers is characterized by soft bottoms and turbid waters, the Amazon barrier to dispersal of reef biota (Rocha, 2003). Despite this barrier, the Brazilian fauna is mostly derived from the Caribbean fauna, with 10.5% endemism in fishes (Floeter *et al.*, 2008), 12.5% in decapod crustaceans (Coelho *et al.*, 2008), and 25% in corals (Veron, 2000).

Argentinian

This province extends from Santa Catarina, Brazil to the Valdez Peninsula, Argentina. The northern boundary marks the transition from tropical reef fauna to warm-temperate fauna and sandy beaches, with rocky formations largely restricted to Mar del Plata and Valdes Peninsula. Invertebrate groups including mollusks and crustaceans show high diversity in this region that has yet to be properly documented. Although creation of a regional Ocean Biogeographic Information System (OBIS) node in the area has increased knowledge, this region is still identified as a major knowledge gap in marine biodiversity (Miloslavich *et al.*, 2011).

Western Pacific Region

Sino-Japanese

This warm-temperate province runs from the tip of the Korean Peninsula more-or-less directly across to the port city of Hamada, Japan and then on the oceanic side of Japan from Cape Inubō in the Chiba Prefecture, south to just before the start of the Ryukyu Archipelago. Along the coast of China the exact boundaries are unknown because the distribution patterns of marine species are not fully described (at least in Western literature); however, based on sea surface temperature, this province probably starts around Shanghai and extends south to Hong Kong. Much of the Island of Taiwan shows strong affinity to this warm-temperate region of the coast, although the southeastern portion is entirely tropical due to the influence of the Kuroshio Current.

Kermadec

The Kermadecs are a subtropical volcanic island arc only $\sim 33\ km^2$ in total area, located halfway between New Zealand's North Island and Tonga. Recognition of the distinct terrestrial and marine biota resulted in New Zealand declaring the islands a nature reserve in 1937. Although not many endemic fishes have been described, rates of endemism among invertebrates are exceedingly high, with gastropods, bivalves and bryozoans all in the area of 70% (Griffiths *et al.*, 2009).

Auckland

Circling the northern tip of North Island, New Zealand, this province shares a history and many species with the next two provinces (Waters *et al.*, 2007). There is believed to be a considerable endemic component to this region, but no published estimates currently exist for marine fishes or invertebrates. In the absence of detailed surveys, we follow Briggs (1995) and recognize this province, but highlight the need for systematic surveys of endemism for this region.

Southeastern Australian

Located on the eastern coast of Australia from Sandy Cape, north of Brisbane down to around Green Cape, roughly halfway between Sydney and Melbourne, this warm province is limited to the south by cold water in the Bass Strait between Victoria and Tasmania. The combination of cold water and warm water currents in this region limits dispersal of species and appears to have contributed to rapid and repeated speciation (Ayre *et al.*, 2009; Puritz *et al.*, 2012). With extremely high endemism of about 85% in shore fishes and over 60% in the benthic community, this region is also noteworthy as having the highest levels of endemism on the planet for macroalgae (Phillips, 2001).

Southwestern Australian

Extending around the southern coast of Australia from Shark Bay, Western Australia to Cape Jaffa, roughly halfway between Adelaide and Melbourne, this large and diverse province is home to extensive fringing reefs and offshore islands, with biodiversity that exceeds the Great Barrier Reef. Estimates of endemism range as high as 85% for shore fishes, 95% for mollusks, and 90% for echinoderms. The southern extent of

this province is likewise limited by the cooler waters around Victoria.

Agulhas

The Agulhas province wraps around the tip of southern Africa from the Cape of Good Hope northward to roughly Cape Vidal, South Africa. The mixing of the warm Agulhas and the cold Benguela currents dominates the oceanographic regime. The input of upwelling nutrients increases the productivity of this region and, combined with the influence of the two opposing current systems, creates a unique environment with a suite of endemic fishes, soft corals, and other invertebrates estimated at about 33% (Griffiths *et al.*, 2010).

Tropical Indo-West Pacific Region

Western Indian Ocean

From roughly the border of South Africa, ranging north to Arabian Sea and Persian Gulf, and including Madagascar, and the offshore island groups of the Seychelles and Comoros, this region contains about 14% endemic fishes. Although the Indian Ocean has around 25% endemic fishes, the separation of the Western Indian Ocean and Red Sea Provinces leaves too little endemism for the remainder of the Indian Ocean to be distinguished from the Western Pacific. For this reason, most of the Indian Ocean is affiliated with the central-West Pacific in the Indo-Polynesian Province (below).

Amsterdam–St. Paul

Île Amsterdam and the neighboring Île Saint-Paul are small, volcanic islands in the central southern Indian Ocean, roughly equidistant from Australia, South Africa, and Antarctica. This highly isolated habitat serves as an important breeding and nursery grounds for Subantarctic Fur Seals, Southern Elephant Seals, and Rockhopper Penguins.

Red Sea

The Red Sea is connected to the Gulf of Aden (northwestern Indian Ocean) through the narrow ($\sim$18 km) and shallow (137 m) Strait of Bab al Mandab. It is the world's northernmost tropical sea, lying between two strips of arid (desert and semi-desert) land, and is consistently the most saline (range 35–41 ppt) and warmest (range 21–34 °C) of tropical seas. Estimates of endemism for the Red Sea (including adjacent Gulf of Aden) are about 14% for fishes, 10% for crustaceans, and 7% for mollusks (DiBattista *et al.*, 2015a). Characterization of regional biodiversity is complicated by a lack of surveys along the African coast from Eritrea through Somalia, a situation that is unlikely to change in the foreseeable future. The unique communities of the Red Sea appear to be a result of the combined effects of turbulent geologic history, unusual environmental conditions, and physical isolation (DiBattista *et al.*, 2015b).

Indo-Polynesian

The largest biogeographic province in the world extends across half the planet, from the Arabian Gulf to the Tuamotu Archipelago in the central South Pacific. Latitudinally, the province begins at the northern tip of the Ryukyu Archipelago (Japan), extends south to Sandy Cape on the east coast and Shark Bay on the west coast of Australia. Genetic surveys of dispersive reef organisms are consistent with the boundaries of this enormous province, likely because dispersal between Australia and the Tuamotus requires no deep water passage further than $\sim$800 km (Schultz *et al.*, 2008). This continuity of shallow habitat is considered the primary factor shaping the cohesiveness of the Indo-Polynesian Province (Briggs and Bowen, 2012). However hierarchical clustering of reef fishes, based on presence/absence data, indicates additional subdivisions within this broad biogeographic region (Kulbicki *et al.*, 2013).

Hawaiian

Despite the cohesion of the Indo-Polynesian province across nearly half the globe, there are three isolated provinces along the eastern edge with high rates of endemism; Hawaii, Marquesas, and Easter Island. The volcanic Hawaiian Islands rise steeply from the deep seafloor of the central North Pacific to provide hundreds of square kilometers of shallow-water reef habitat in the middle of a vast expanse of deep ocean. The Hawaiian biota is primarily derived from Indo-Pacific ancestors (Kay and Palumbi, 1987), and recent work demonstrates that this province both imports and exports species through a process called biodiversity feedback (Bowen *et al.*, 2013). Endemism is uniformly high: about 15% of seaweeds, 18% of stony corals, 24% of annelids, 20% of echinoderms, 26% of mollusks, 38% of crustaceans, and 25% of reef fishes are endemic to the Hawaiian Archipelago (Kay and Palumbi, 1987; Randall, 2007). The reef fauna of Hawaii illustrates the impact of larval dispersal on community composition (Selkoe and Toonen, 2011). Many reef fishes with shorter pelagic larval durations (PLDs) of 10–40 days are absent or poorly represented here. There are no native shallow-water snappers (genus *Lutjanus*), only one native grouper (family Serranidae), and no clownfishes (genus *Amphiprion*). In contrast, the moray eels (family Muraenidae) with PLDs $>$80 days, and surgeonfishes (family Acanthuridae) with PLDs $>$50 days, are well-represented in Hawaii (Randall, 2007). Adjacent Johnston Atoll (865 km southwest) is included in this province due to strong taxonomic affinities, and it is believed to be both an outpost of Hawaiian fauna and a biogeographic gateway into the archipelago (Gosline, 1955; Timmers *et al.*, 2011; Skillings *et al.*, 2014).

Marquesas

Located in French Polynesia (South Pacific), these volcanic islands lie 1370 km northeast of Tahiti and 4500 km west of South America. This area is characterized by strong upwelling of nutrient-rich water that results in cooler temperatures, higher productivity, and less coral cover than typical South Pacific islands. The Southern Equatorial Current flowing westerly past the Marquesas may diminish colonization or gene flow from the Indo-Polynesian province, a finding reinforced by phylogeographic studies (Gaither *et al.*, 2010; Szabo *et al.*, 2014; Fernandez-Silva *et al.*, 2015). This unusual environment results in a relatively high endemism for reef fishes (11.6%).

Easter Island

Located 3700 km from the coast of Chile and 1900 km from Pitcairn Island, this is another isolated volcanic island on the outskirts of the vast Indo-Polynesian Province. Easter Island lacks protective barrier reefs, but hosts diverse coral reefs and 22% endemic reef fishes (Randall and Cea, 2010). Interestingly, Isla Salas y Gómez, 500 km east of Easter Island (~3200 km west of the Chilean mainland) has a more typical Indo-Pacific fish community and is considered an isolated outpost of the broad Indo-Polynesian Province rather than grouping with adjacent Easter Island (Parin, 1994).

Eastern Pacific Region

California

Located between Los Angeles, California and Magdalena Bay, Mexico, this province was recently revised due to the recognition of a broad transition zone in faunal composition from Point Conception south to Los Angeles (Burton, 1998; Dawson, 2001). Endemism was estimated previously at about 33% for fishes and 21% for mollusks, but these may be overestimates given that the boundary is moved and the province now covers less area.

Cortez

The Gulf of California, also known as the Sea of Cortez, is the body of water that separates the Baja California Peninsula from the Mexican mainland. The Gulf has existed for about 5 MY and has a rich diversity of island, coastal, and estuarine habitats. Although endemism is just over 10% for fishes, some have argued this province may be among the most diverse on the planet for micro-invertebrates and parasites (Campos *et al.*, 2009) which are not currently considered in these endemism counts.

Panamanian

This province extends along the coastal margin from the mouth of the Gulf of California south to the Gulf of Guayaquil, on the border between Ecuador and Peru. Endemism is about 49% for fishes (Robertson and Cramer, 2009) and 38% for decapods (Boschi, 2000). Offshore islands (with the exception of the Galápagos below) generally show affinity to the broader Panamanian province among the shore fishes, including the Revillagigedo Islands with 8.0% endemism, Clipperton with 5.8%, Malpelo with 2.5%, and Cocos with 4.6% (Robertson and Cramer, 2009). Thus, these offshore islands are all considered outposts of the Panamanian Province.

Galápagos

The only offshore archipelago in the East Pacific with sufficient endemism to be recognized as a distinct biogeographic province, the Galápagos Islands have 13.6% endemism for shore fishes (McCosker and Rosenblatt, 2010) and 16% for decapods (Boschi, 2000). This province is famed for the endemic species that inspired Darwin's (1872) theory of evolution by natural selection. Although the Galápagos straddle the equator, the Humboldt Current brings cold waters and frequent rains to the islands, except during El Niño events every 3–7 years.

Peru–Chilean

Including most of the western coast of South America, this warm-temperate province extends from the Gulf of Guayaquil to near the Taitao Peninsula (Chile) with roughly 13% endemism. Spanning over 35° latitude, there are few generalizations to be made about the climate and habitat throughout this range, other than the influence of the cold Humboldt Current along this coastline. This consistent current coupled with nearly continuous habitat along the coast results in a homogenous fish fauna along the western coast of South America. The Humboldt Current System is the most productive fishing area on the planet, with roughly 20% of the global catch coming from this region. During El Niño events, warm water moves east across the Pacific and suppresses upwelling, which leads to fish stock crashes and cascading ecological and economic impacts.

Juan Fernández

This province consists of three volcanic islands 650 km west of Valparaiso, Chile. Despite being over 2500 km from the nearest Pacific Island (Isla Salas y Gómez), both shore fishes and decapods show a stronger affiliation to the southwest Pacific than to the closer coast of Chile (Pequeño and Sáez, 2000), and endemism is estimated at about 25%.

Cool Regions (Cold-Temperate and Polar Waters): Cold-Temperate and Polar Northern Hemisphere

Eastern North Pacific Region

Aleutian

The 1900 km Aleutian Islands form the boundary between the Bering Sea and the North Pacific. This province stretches from the Pribilof Islands north of Aleutians (representing the southern limit of winter pack ice) to the northern tip of Vancouver Island (Horn *et al.*, 2006). Summer daylight can reach up to 20 h, leading to extensive algal blooms, while winter daylight can be restricted to 5 h with extremely limited productivity. Although fish endemism is sufficient for recognition of the province, endemism of the invertebrate fauna is particularly high, with 24% reported for decapods (Valentine, 1967) and 23% for mollusks (Boschi, 2000).

Oregon

This province extends from Vancouver Island south to a transition zone with the California Province. Recent surveys of the Californian fishes and invertebrates demonstrate that geographic endpoints of their ranges are variable and many more species cross Pt. Conception than end at this long-recognized biogeographic boundary (Burton, 1998; Dawson, 2001). If the Province were to terminate at Monterey Bay, endemism would be only about 2% in the decapods and shore fishes. In contrast, if the cold water California Transition Zone biota is included, the endemism level reaches the 10% cutoff for recognition as a distinct province. These data justify the formal recognition of the California Transition Zone, within the Oregon Province, from Monterey Bay south to Los Angeles, where the warm-temperate Californian Province begins.

Western North Pacific Region

Oriental

This province is discontinuous and includes three segments: the nearshore waters of the Yellow Sea, the southwestern portion of the mainland coast of the Sea of Japan, and shallow waters around northern Honshu Island, Japan. The first two segments are broken by the tip of the Korean Peninsula, which falls within the Sino-Japanese Province (defined above). This region is characterized by turbid, cool, highly productive waters, and patterns of endemism in this area are probably the result of a complex geological history with periodic isolation during glacial stages and subsequent recolonization as outlined below.

Kuril

A faunal break at the Tsugaru Strait, between the islands of Honshu and Hokkaido, marks the beginning of the Kuril Province, both along the outer coast of Japan, and within the northern Sea of Japan. This narrow province excludes the Okhotsk Sea (see below) but extends along the volcanic Kuril Island chain, and the eastern coast of the Kamchatka Peninsula ending at the northern end of Olyutorsky Bay in the Bering Sea. The two southern-most islands are of disputed ownership between Japan and Russia, in part because the region is among the most productive in the North Pacific. With expansive kelp forests, and their associated fish and invertebrate communities, exploitation by both marine mammals and humans is extensive.

Okhotsk

Confined to the Sea of Okhotsk, this province stands apart from the adjacent waters despite the lack of apparent barriers today. Although now continuous with the North Pacific through the Kuril Islands, and with the Sea of Japan around Sakhalin Island, phylogeographic studies conclude that the Sea of Okhotsk was isolated during periods of low sea level (Liu *et al.*, 2007). There are no recent taxonomic evaluations of this province, but high endemism in ascidians, pycnogonids (sea spiders), and fishes has been reported (Briggs, 1974).

Western Atlantic Region

There are conflicting opinions about the biogeographic provinces in this region. The region extends from the Strait of Belle Isle (or Labrador Straits) in Eastern Canada south to Cape Hatteras, North Carolina, with disagreement primarily focused on the biota found between Cape Hatteras, and Cape Cod, Massachusetts, often called the 'Middle Atlantic Seaboard.' Large numbers of tropical and warm-temperate species colonize during the summer months, resulting in many authors aligning this region with the Carolina Province to the south. These recruits typically disappear during winter, and the predominance of boreal species, combined with very low endemism led Briggs (1974) to argue that the Middle Atlantic Seaboard clearly belongs to the Boreal region. We follow that designation here, but recognize this remains a topic of debate. Using these boundaries gives estimates of 19% endemism among shore fishes, and 21% for opisthobranch gastropods.

Eastern Atlantic Region

This region runs from the Norwegian archipelago of Svalbard (formerly Spitsbergen) and Novaya Zemlya ringing the Barents Sea to the southern entrance of the English Channel. Iceland, the volcanic Faroe Islands and the Baltic Sea fall within this province. As the world's largest estuarine area, the Baltic Sea has been recognized previously as a distinct province (Golikov *et al.*, 1990), but this designation is not justified on the basis of endemism. Likewise, the unusual biotic mixture of Iceland has been a subject of debate, but with the exception of a few recognized subspecies, the island lacks endemics, and so falls within the Eastern Atlantic Boreal region. Based on these boundaries, the region contains about 20%–25% endemism for fish and invertebrate taxa. The richest biota occurs here on the eastern side of the North Atlantic, with more than 50% of transatlantic species originating from the North Pacific within the past 3.5 MY (Vermeij, 2005).

Arctic Region

The present glacial regime of the Arctic region dates to about 2.9–2.4 MYA (Mudelsee and Raymo, 2005). This relatively recent origin results in about 25% endemism overall, but very few endemics at higher taxonomic levels, particularly in comparison to the much older Antarctic. A notable exception is the narwhal, *Monodon monoceros*, which has a relictual Arctic distribution (Jefferson *et al.*, 1993). Phylogeographic analyses of Arctic charr (*Salvelinus alpinus*) indicate a recent recolonization following the last glacial retreat ($\sim$20 000 years ago, Brunner *et al.*, 2001). Although the cold Arctic region has traditionally been divided into a number of separate provinces, recent works find an essentially homogeneous biota lacking the regional endemism or phylogeographic structure to support such divisions. The polar cod (*Boreogadus saida*) is a regional indicator species based on the studies of Cohen *et al.* (1990) because it extends to all parts of this region but not into any of the cold-temperate regions.

Cool Regions (Cold-Temperate and Polar Waters): Cold-Temperate and Polar Southern Hemisphere

South American Region

This region was previously united in a single Magellan Province spread from Chiloé Island (Isla Grande de Chiloé) in southern Chile on the Pacific side to Río de la Plata at the Argentina/Uruguay border on the Atlantic side (Briggs, 1974). This designation was based on the shore fish fauna of the region, which show no indication of provincial endemism (Sielfeld and Vargas, 1999). Recent surveys, however, find very high endemism rates for invertebrates in southern Chile, Tierra del Fuego, southern Argentina, and the Falkland Islands that justify the designation of four distinct provinces within the South American region (Griffiths *et al.*, 2009).

Southern Chile

This province now begins at Península de Taitao in the Aysén region of Chile. Although previous surveys report no evidence

of provincial endemism, 109 marine shore fish species occur only within the waters of Chile according to FishBase (2004), and four additional marine endemic species have been described since then for a current total of ~113 fish species (Living National Treasures, 2015). Likewise, the molluskan fauna of the Chilean coast show a sharp increase in diversity above 42° S latitude (around Ancud), and most of these species do not penetrate the Atlantic side of South America, resulting in sufficient endemism to recognize a distinct Southern Chile Province.

Tierra del Fuego

At the southern tip of the South America, this rocky outcrop results from the formation of the Andes combined with the repeated Pleistocene glaciation. Known for some of the finest trout fishing in the world, with sea-run brown trout often exceeding 9 kg, this productive cold province is isolated from the Antarctic fauna, and distinct from the cold-temperate provinces to either side.

Southern Argentina

Beginning at the Valdez Peninsula, Argentina, this region is characterized by cool, moderately productive waters but does not extend up as far as the original Magellan Province. Unlike the shore fish fauna of Chile, the waters of Argentina are home to only four endemic species (Living National Treasures, 2015; Sielfeld and Vargas, 1999). In contrast, there is very little overlap between the Pacific and Atlantic polychaete and anemone faunas (Haussermann and Forsterra, 2005). Boschi and Gavio (2005) found ~35% endemism among decapod crustaceans on the Pacific side and ~18% on the Atlantic.

Falkland Islands (Islas Malvinas)

Located about 300 miles off the southern Patagonian coast, this archipelago lies essentially on the boundary of the subantarctic oceanic and tundra climate zones. With strong ties to the Patagonian biota, the islands are home to numerous endemic freshwater fish of the genus *Galaxias*. There are only two marine endemic fishes, but the endemism rate of invertebrates is sufficient to merit provincial status (Griffiths *et al.*, 2009).

New Zealand–Australian Region

Tasmania

An island state of the Commonwealth of Australia, Tasmania is located 240 km south of the Australian continent. Located in the infamous 'Roaring Forties' westerly winds that encircle the southern hemisphere, the island is surrounded by the Indian and Pacific Oceans and separated from mainland Australia by the Bass Strait. Geographically and genetically isolated by the combination of distance, strong currents and mixed climates, the island is well-known for unique terrestrial and marine biota. The boundaries of this province are still the subject of some debate, however. Briggs (1995) argues that it extends to the Victoria coast of Australia, but Griffiths *et al.* (2009) report endemism rates for only the island of Tasmania. Regardless, even if percent endemism were reduced by half, this province would still be distinct and recognized for its unique biota.

New Zealand

Located 1500 km east of Australia and 1000 km south of New Caledonia, Fiji and Tonga, islands in this group have been isolated for some 80 MY, leading to the highly distinct and endemic biota. The cool and productive waters are home to nearly half of the world's cetaceans, and large numbers of fur seals, sea birds, and penguins. Like Tasmania, New Zealand has very high rates of endemism for all marine invertebrate classes, possibly as high as 85% (MacDiarmid and Patuawa, 2010). There remains some question about whether the warm-temperate zone along the north coast between Auckland and East Cape contains sufficient endemism for recognition of a separate province (Briggs and Bowen, 2012). Judging simply by number of endemic species, New Zealand is the hotspot of the Southern Ocean, closely followed by Tasmania (although this order may well change with the proposed revision of boundaries).

Antipodes

A series of volcanic islands in the subantarctic waters south of New Zealand, this province consists of the Auckland, Antipodes, Campbell, and Bounty Islands. Characterized by steep rocky cliffs and sharp drop-offs, the fish fauna of these islands is not well known, but the molluskan fauna appears to be of sufficient endemism for designation as a province (Griffiths *et al.*, 2009). Dell (1962) reported molluskan endemism rates of 16% for the Aucklands, 23% for the Bounties, and 23% for the Snares, and asked if all these island groups should be separate provinces, a question which has yet to be answered.

Subantarctic Region

The biota of this region are highly distinctive owing to four historical factors: (1) persistence of a small ancestral group of Mesozoic and early Cenozoic taxa, (2) geographical isolation produced by the opening of Drake Passage, (3) extinction of many early Tertiary warm-temperate species, and (4) invasions of the region by cold-temperate species from the North Pacific. The result is six distinct provinces (Briggs and Bowen, 2012).

South Georgia

South Georgia and the South Sandwich Isles rise steeply from the sea bed roughly 1400 km to the southeast of the Falkland Islands. At the far southern latitude of 55° S, the area receives only about 1000 h of sunlight per year, which limits photosynthetic productivity, but not biodiversity. Hogg *et al.* (2011) conclude that South Georgia Island hosts greater biodiversity than the Galápagos Islands. Although seawater temperatures typically range from 0 to 4 °C throughout the year, the islands are far enough from the pole that the waters typically remain ice-free, with the exception of icebergs. The marine invertebrate classes surveyed to date have high endemism, and up to 34% of the shore fishes may be endemic (Briggs, 1974).

Bouvet

Bouvet Island (Bouvetøya) is a subantarctic volcanic territory administered by Norway. Lying 2200 km southwest of South Africa and 1700 km north of Queen Maud Land, Antarctica, this uninhabited island is more than 90% glacier and has been

a nature reserve since 1971. Although not as well surveyed as the South Georgia Province, the Bouvet Province has a very distinct fauna with over 50% endemism in gastropods (Griffiths *et al.*, 2009).

Crozet

The Crozet Islands (Archipel Crozet) are a French Overseas Territory consisting of a subantarctic volcanic archipelago in the southern Indian Ocean that formed about 50 MYA. With high precipitation (2000–3000 mm per year) and winds in excess of 100 km h^{-1} for ~100 days each year, these islands are uninhabited except for the research station Alfred Faure on Île de la Possession. A nature reserve since 1938, this archipelago was previously considered part of the Kerguelen Province, but now merits assignment to a separate province based on high endemism among marine invertebrates (Griffiths *et al.*, 2009).

Prince Edward

The Prince Edward Islands (Prince Edward and Marion) are located 1750 km southeast of the South African mainland. Declared a Special Nature Reserve by South Africa in 2003, they are uninhabited except for a South African research station on Marion Island. This province is recognized on the basis of high endemism among marine invertebrates surveyed to date, but detailed biotic surveys are lacking.

Kerguelen

The Kerguelen Islands (Archipel des Kerguelen), also known as the Desolation Islands, are part of the French Southern and Antarctic Lands with some 300 small islands spread across a few hundred kilometers. This volcanic archipelago emerged from the ocean ~35 MYA, although no eruption has been recorded since discovery of the islands in 1772. Home to a permanent research station, endemism of this biota is among the best known of the region, with roughly 60%–70% endemism among shore fishes, mollusks, and ascidians (Primo and Vázquez, 2007). This province probably should include the nearby Heard Island and McDonald Islands, but detailed biodiversity surveys are lacking.

Macquarie

Technically part of Tasmania, and a Tasmanian State Reserve since 1978, Macquarie Island lies roughly halfway between New Zealand and Antarctica. The island is located where the Australian tectonic plate meets the Pacific plate, and is the only place in the Pacific Ocean where rocks from the Earth's mantle are actively exposed at sea level. With taxonomic affinities to New Zealand, provincial status is based on over 60% molluskan endemism (Dell, 1964).

Antarctic Region

By comparison to the Arctic biota, the fauna of the Antarctic region is quite old. Contemporary conditions began about 24 MYA with the formation of the major ice sheet. This province includes all of the waters and island groups surrounding the continent out to the 1 °C February isotherm. Levels of endemism among mollusks, decapods, ascidians, sponges, polychaetes, and shore fishes range from about 42% to 88%, but there seems no support for provincial endemism within the region (Griffiths *et al.*, 2009). This degree of endemism is not surprising given over 20 MY of isolation, but it is noteworthy that there is also an extremely high rate of generic endemism in the region (75% of continental shelf and upper slope fishes; Eastman, 2005). Phylogeographic studies are consistent with lack of provincial subdivision, with little to no structure in a half dozen species surveyed across this vast region to date.

Conclusions

Considerable diversity, endemism, and new biogeographic provinces have been revealed by the suite of studies conducted since Briggs (1995), and more remain to be discovered. These provinces can serve conservation and management goals by highlighting the areas of unique biodiversity across the globe. We argue that in setting conservation priorities for these coastal areas of the globe, it seems reasonable to first consider those locations that are contributing species and augmenting diversity (i.e., the centers of origin). From these centers, new lineages spread and bring diversity and biological innovation (Vermeij, 2005). Newly colonized areas can then spawn new species and further increase diversity in the centers of origin through a process termed *biodiversity feedback* (Bowen *et al.*, 2013). These biodiversity centers include the Coral Triangle in the Indo-Pacific, the Caribbean Province in the Western Atlantic, the North Pacific Region, and the Antarctic Region including the surrounding halo of small island provinces. Endemism in the cold Southern Ocean tends to be greater than similarly sized provinces in the Northern Hemisphere. Efforts to conserve biodiversity hotspots have largely focused on the tropics (Krug *et al.*, 2009), but it is not the tropics as a whole that merit prioritization. Many temperate and cold water hotspots offer equally rich biological treasures for conservation that have been undervalued to date (Hogg *et al.*, 2011).

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See also: Dispersal Biogeography. Phylogeography

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Biogeography, Microbial

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Glossary

Assemblage A collection of species that exist in a given habitat.
Biogeographic pattern A distribution of biotic diversity that is nonrandom with respect to space and/or environmental factors.
Community See Assemblage.
Composition The identity and relative abundance of taxa in a sample, assemblage, or community.
Dispersal The distance and rate at which an organism can travel, either passively or actively.
Diversity The richness and abundance of taxa.
Microorganisms/microbes Organisms with sizes <0.5 mm and masses $<10^{-5}$ g including Archaea, Bacteria, viruses, fungi, and other small Eukaryotes.
Population All individuals of a single taxon within an area.
Richness The number of taxa in a sample, assemblage, or community.
Selection The process allowing the proliferation of organisms that are relatively better adapted to external environmental conditions.
Taxon A group into which related organisms are classified based on a relatedness cutoff.
Taxon resolution The level of genetic variation of the taxa considered.
Trait A quantitative or qualitative characteristic of an organism.

Introduction

What is Microbial Biogeography?

Microbial biogeography is the study of the distribution of microorganisms across space and time. Microbes are generally defined as those organisms with sizes <0.5 mm and masses $<10^{-5}$ g foremost consisting of all members of the domains Archaea and Bacteria, but also including some fungi and other small Eukaryotes, as well as viruses. Since microbes constitute the vast majority of biodiversity on Earth and are critical components of ecosystem processes such as decomposition, nutrient and mineral cycling, and parasitism, there is much interest in understanding how this diversity is organized in nature. Recent advances in molecular genetic methodologies have allowed the culture-independent identification of organisms that cannot be differentiated visually, thus enabling the large-scale characterization of microbial diversity directly from the environment. Microbial biogeography reveals: where microorganisms live, why they live there, and in what abundance. It also aims to provide insight into the underlying ecological and evolutionary mechanisms that generate, maintain, and hinder microbial biodiversity, thereby facilitating predictions of where certain microorganisms can survive and how they will respond to changing environmental conditions.

Microbes are increasingly recognized as exhibiting distribution patterns similar to those of macroorganisms (Section Evidence for Microbial Biogeography). For example, microbes generally follow well-established biogeographic patterns such as the distance–decay relationship (Section Distance–decay) and taxa–area relationship (Section Temporal Changes). This was initially unexpected given some of the general disparities between microorganisms and macroorganisms, such as their size (μm vs. m), time between generations (min vs. years), population size (millions vs. hundreds of individuals), and hypothesized dispersibility (global vs. local). These similar distribution patterns instead support the concept of some 'universal' processes influencing all biodiversity.

However, important differences between biogeographic patterns for microorganisms and macroorganisms do exist, and likely result from differences in the relative importance of underlying ecological and evolutionary processes (Section Ecological and Evolutionary Mechanisms). For example, dispersal (i.e., the movement of an organism across space and time) is a key process responsible for the distribution and diversity of both microbes and larger organisms. Microbes can presumably disperse across much greater distances and at much greater rates than macroorganisms due to their larger population sizes and ability to withstand adverse conditions as well as travel in a variety of ways (e.g., through the atmosphere, via water currents, and on or in other organisms). This may explain why some microbial species exist at complementary latitudes in both hemispheres (e.g., marine bacteria; Amend *et al.*, 2013) while larger animals like polar bears and penguins are often restricted to just one pole (although there are notable exceptions, such as humpback whales and certain seabirds such as terns, which can be found at both poles).

The ability to study biogeography for microbes in the same way as larger organisms is complicated by the fact that species concepts are unclear for most microorganisms and can differ substantially from those for animals and plants. The primary reason for this is that the majority of microorganisms reproduce asexually (e.g., all members of the domains Bacteria and Archaea), with genetic diversity originating via mutations and direct genetic exchange mechanisms among sometimes distantly related organisms (e.g., horizontal gene transfer). Boundaries between species-level taxa and mechanisms of speciation are therefore obscured and cannot be based on the

degree of interbreeding (nor on morphology or other phenotypic traits due to size limitations) in the same way as classic species concepts. Even for microbes that are capable of sexual reproduction (exclusively fungi and other small Eukaryotes), species definitions and taxonomy can be problematic (e.g., due to a lack of resolution in morphological identification techniques; Foissner, 2006). Moreover, with the exception of cyanobacteria in stromatolites and some fossilized eukaryotic microbes, the vast majority of Bacteria and Archaea have no fossil record. Therefore, the extent to which current distributions are influenced by evolutionary history, geological history, and/or speciation (i.e., the 'historical' processes that broadly account for some biogeographic variation in some animals and plants) is particularly contentious and impractical for microbes. Despite these challenges, microbial biogeography is an active area of research, encouraging simultaneous advancements in microbial species concepts and evolution (e.g., Fraser *et al.*, 2009).

The Baas-Becking Hypothesis: The Birth of Microbial Biogeography

An important foundation of microbial biogeography is the Baas-Becking hypothesis, which states that for microorganisms, "everything is everywhere, [but] the environment selects" (Baas-Becking, 1934). The assertion that 'everything is everywhere' implies that the dispersibility and abundance of microorganisms allows them to maneuver on a global scale despite adverse geologic and environmental features. The claim that 'the environment selects' implies that current environmental conditions are solely responsible for distinct microbial populations or communities at local scales. In other words, as long as the environment is ecologically appropriate for a microorganism's growth, geological barriers and past events are irrelevant (O'Malley, 2007).

The Baas-Becking hypothesis elicits two predictions: (1) similar environments in different locations should harbor similar microbial communities and (2) all microbial taxa should be found everywhere, albeit at varying abundances. It has not been possible to test these predictions until relatively recently due to the lack of suitable methodologies for collecting and identifying the vast diversity of microbial taxa present in environmental samples. As a result, the empirical evidence and conceptual theory for microbial biogeography significantly lags behind that of macroorganisms.

Molecular Genetics: Enabling Microbial Biogeography

Recent advances in molecular genetic methods have enabled global studies of microbial diversity necessary for testing the Baas-Becking hypothesis. Before these molecular genetic methods existed, microbial species were onerously identified using cultivation techniques and morphological classifications, which miss the vast majority of microbial taxa. Now cost-effective, high-throughput DNA sequencing allows genetic analysis of environmental samples directly without the need for cultivation. These methods have been an important development for microbial biogeography because they allow a much greater subset of microbial diversity to be classified at a higher taxonomic resolution compared to traditional observational techniques. In fact, results from these new genetic methods have challenged the findings from earlier morphological studies. Initially, morphological studies supported the second assertion of the Baas-Becking hypothesis by finding virtually all microbial species detected in diverse and widespread habitats, suggesting that microbes were indeed globally distributed (e.g., Finlay, 2002). However, findings over the past decade using molecular genetic methods have challenged this result (e.g., Foissner, 2006), on the grounds that morphological classifications are not adequate representations of genetically differentiated taxa.

With these modern molecular techniques, taxonomic classification of microbes from environmental samples is now typically defined by nucleotide sequence similarity in one or more genomic regions. The most commonly used taxonomic marker genes are the evolutionarily conserved rRNA genes: 16S for Bacteria and Archaea; 18S for fungi and other Eukaryotes. Taxa, or Operational Taxonomic Units (OTUs) are conventionally defined at a $\geq$97% similarity cutoff in the rRNA gene, and are operationally analogous to species or genus. In this way, the most abundant taxa in a single sample are identified, and microbial biogeography studies often compare the composition of these identified microbial taxa among multiple samples. It is important to note several caveats to this approach. First, because the majority of microbial taxa are rare, it is nearly impossible to exhaustively sample all microbial diversity in every sample (Sogin *et al.*, 2006). Additionally, conventional OTU definitions are somewhat arbitrary and investigations of sequences at higher resolutions (either with a higher cutoff or with different genes) allow identification of a greater amount of microbial diversity (Section Taxonomic resolution). Although the sequence similarity approach is imperfect (Fraser *et al.*, 2009), it provides a convenient and standardized working definition necessary for investigating microbial diversity despite challenges associated with a lack of accepted species concepts for microbes (Section What is Microbial Biogeography?).

Evidence for Microbial Biogeography

Due in part to the Baas-Becking assumption that 'everything is everywhere,' debate remains regarding the extent to which microbes display biogeographic patterns. This section reviews the current evidence for microbial biogeographic patterns, with a focus on free-living microbes. While the biogeography of viruses and host-associated microbes is also an active line of research, their survival and therefore biogeography in many cases depends on the biology and ecology of nonmicrobial host organisms, which are beyond the scope of this article (e.g., of the biogeography of host-associated microbes, see Costello *et al.*, 2009; Hoberg and Daniel, 2008; Mikheyev *et al.*, 2010).

Endemism

Endemism occurs when a species is restricted to a unique geographic location; it is one of the simplest demonstrations

of a biogeographic pattern. However, identifying microbial endemics is complicated by the high diversity of natural assemblages and the fact that this diversity is composed of thousands of extremely rare taxa (Sogin *et al.*, 2006). Although continually improving sequencing methods may provide more complete samplings of microbial biodiversity, it is still nearly impossible to not only detect all taxa present in a given sample, but also prove the absence of any given species, explaining why demonstrations of microbial endemism are relatively rare. Instead, significant genetic divergence among taxa in different geographical locations, or restriction of particular microbial clades to specific regions of the world, is used as evidence for microbial endemism. For example, Whitaker *et al.* (2003) demonstrated genetic differentiation among hyperthermophilic Archaea from geothermal hot springs on different continents. It is important to note that endemism is evidence that microbial communities can be isolated from each other due to dispersal limitation (e.g., Talbot *et al.*, 2014), thus contradicting the Baas-Becking hypothesis.

Distance–Decay

Biotic communities tend to be more similar the closer they are in space, producing a negative relationship between similarity in community composition and geographic distance (Nekola and White, 1999). This 'distance–decay relationship' is commonly observed for macroorganisms and can be due to multiple factors including dispersal limitation and spatial correlation of selective environmental variables (e.g., ecoclines). Distance–decay has been consistently observed for microbes across a range of taxonomic and spatial scales (e.g., Cho and Tiedje, 2000; Whitaker *et al.*, 2003; Green *et al.*, 2004; Horner-Devine *et al.*, 2004; Martiny *et al.*, 2011), making it some of the strongest evidence that microbes exhibit spatially structured distributions akin to larger organisms.

Taxa–Area

The 'taxa–area relationship' is the observed increase in species richness with increasing size of the area sampled (**Figure 1**). This relationship has been observed repeatedly in larger animals and plants and is attributed to larger sampling areas simply containing more types of habitats and microenvironments than smaller ones (Rosenzweig, 1995). Demonstrating this relationship for microbes is challenging due to the difficulty in exhaustively sampling microbial diversity in a given sample, let alone across increasingly larger areas. However, microbial communities appear to also show taxa–area relationships, especially in terrestrial environments (Green *et al.*, 2004; Horner-Devine *et al.*, 2004).

Temporal Changes

Nonrandom changes in microbial community composition and diversity over time – for example, seasonally or even daily – are increasingly recognized as an important biogeographic pattern for microbes. For example, microbial community composition in the English Channel exhibits robust and predicable seasonal dynamics (Caporaso *et al.*, 2012). This is facilitated by dormancy, a feature not seen in larger animals but thought to be a common strategy in some microbes, which allows taxa that are not adapted to current environmental conditions to persist in low abundance in a nonactive state. The composition of the active microbial community therefore fluctuates over time in response to changing conditions. This is known as the 'seed-bank hypothesis' (Lennon and Jones, 2011) and has implications for our understanding of ecological resilience and thresholds to change.

Moreover, some studies have found a positive relationship between the number of species and time (analogous to the taxa–area relationship; Section Taxa–Area), such that the number of species observed increases with the time span of observation. This is likely due to immigration as well as the inclusion of more varied conditions and has been observed in a range of taxonomic groups and ecosystems across many years (White *et al.*, 2006). In certain environments, such as the Mid-Atlantic Bight, time rather than space or environment is the primary driver of microbial community composition (Nelson *et al.*, 2008).

Latitudinal Gradients

It is well established that larger animals exhibit latitudinal gradients in species richness such that the greatest biodiversity exists in the tropics and decreases toward the poles. Many

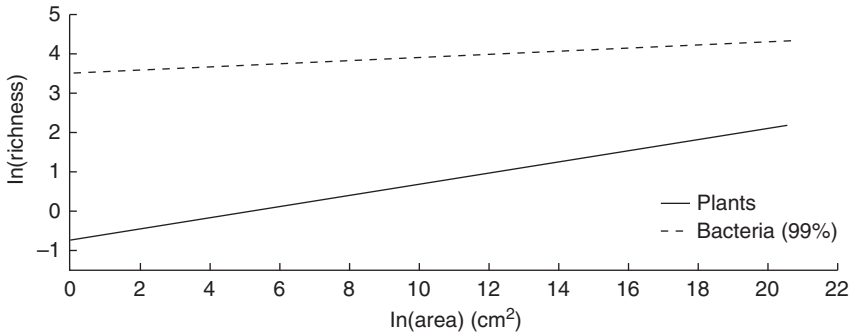

Figure 1 The taxa–area relationship for salt marsh organisms. Plants and bacteria both exhibited significant taxa–area relationships, but the *z*-value for plants ($z=0.103$) was significantly greater than that of bacteria ($z=0.040$). This may be due to factors such as lower taxonomic resolution, lower habitat specificity, and horizontal transfer of ecologically relevant genes for bacteria. Reproduced from Horner-Devine, M.C., Lage, M., Hughes, J.B., Bohannan, B.J.M., 2004. A taxa–area relationship for bacteria. Nature 432, 750–753.

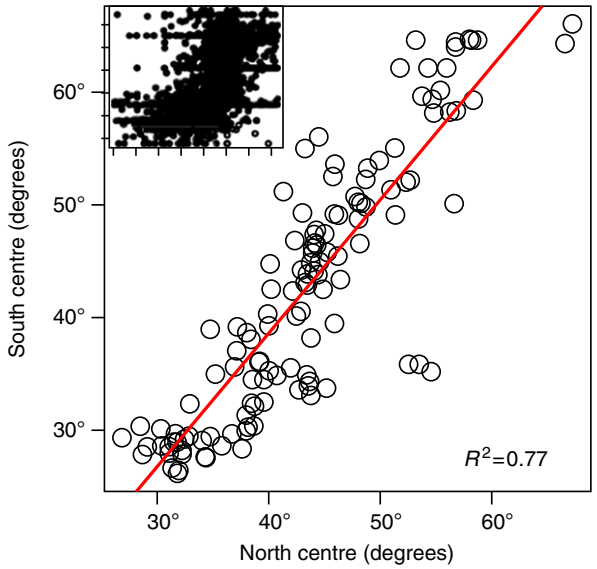

Figure 2 The northern OTU center location versus the southern OTU center location of marine bacteria, showing that marine bacterial distributions are complementary at opposite hemispheres. The correlation is strongest when considering only the wide-ranging OTUs (upper 99th percentile of the OTUs with the greatest latitudinal extent; $R^2=0.77$, $P<0.0001$). The inset figure shows all near-surface marine bacteria, illustrating how latitudinal complementarity diminishes as more narrowly distributed taxa are added ($R^2=0.33$, $P<0.0001$). Reproduced from Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., *et al.*, 2013. Macroecological patterns of marine bacteria on a global scale. Journal of Biogeography 40, 800–811.

hypotheses have been invoked to explain this general pattern (Rosenzweig, 1995; Hillebrand, 2004; Wiens *et al.*, 2010). However, it remains unresolved whether such a latitudinal pattern holds for microorganisms. Although gradients tend to weaken with decreasing animal size, implying that microbes should lack latitudinal richness gradients (Hillebrand, 2004), marine bacteria do show some evidence of a classic latitudinal gradient (Pommier *et al.*, 2007; Fuhrman *et al.*, 2008) and may also follow Rapoport's rule, as shown in **Figure 2** (Amend *et al.*, 2013). However, soil bacteria seem to have no relationship with latitude but instead a strong dependence on soil pH, which can vary greatly among soil samples (Fierer and Jackson, 2006). Thus, some environmental factors (e.g., soil acidity) may be strong enough to overwhelm latitudinal effects. Certain microbial populations have also been found at complementary latitudes in opposite hemispheres; for example, a study on marine surface bacteria showed not only a latitudinal gradient but also complementary distributions with similar populations at both poles (Amend *et al.*, 2013). These 'bipolar' (or 'anti-tropical') distributions are much more rare in macroorganisms as their dispersion on this large scale is limited by many factors, such as their larger body sizes (although there are exceptions, e.g., the humpback whale).

Elevation and Depth Gradients

Elevation gradients in composition are well established for macroorganisms, as dramatic changes in climate and therefore biota can occur with elevation over relatively short geographic distances. The gradients tend to exhibit either hump-shaped or monotonically decreasing relationships with elevation. In contrast, no consistent elevation patterns have emerged for microbes. For example, bacterial richness in the Rocky Mountains decreases from low to high elevations (Bryant *et al.*, 2008), while in the Andes there was no relation with elevation (Fierer *et al.*, 2011). Like elevation gradients, depth gradients in aquatic systems may arise from physical and chemical stratification. It is well recognized that microbial communities exhibit much more distinct variations with depth (see reviews by Martiny *et al.*, 2006; Lindström and Langenheder, 2011).

Ecological and Evolutionary Mechanisms

Microbial biogeographic studies to date have established that microbial communities exhibit nonrandom variability in space and time; moreover, these microbial distribution patterns are sometimes similar to those of larger organisms (Section Evidence for Microbial Biogeography). In this section, the possible underlying ecological and evolutionary processes producing these patterns are discussed. Existing evidence for their relative importance in driving microbial biogeography is also reviewed.

Framework

The Baas-Becking hypothesis (Section The Baas-Becking Hypothesis: The Birth of Microbial Biogeography) provided an important starting point for modern microbiologists to consider the driving forces of microbial distributions, namely 'dispersal' and 'selection.' However, a more comprehensive framework delineating all the possible mechanisms influencing microbial diversity in both time and space is currently being adopted (Martiny *et al.*, 2006; Hanson *et al.*, 2012). This framework takes into account biogeographic and evolutionary theory, dividing the processes influencing microbial biodiversity at all levels of taxonomic resolution into four main categories: selection, dispersal, drift, and mutation (**Figure 3**).

Selection includes all aspects of the contemporary environment such as abiotic variables (e.g., temperature, pH, nutrient availability) as well as the interactive effects of other organisms (e.g., competition, predation). Microbial taxa or genotypes that are best adapted to such external factors will survive and proliferate. This is the same concept as that implied by the 'environment selects' portion of the Baas-Becking statement. Dispersal refers to the distance and rate at which a microbe can travel, either passively or actively. Drift refers to stochastic changes in the composition of genes or communities that are facilitated by random demographic births and deaths. Finally, mutation covers the genetic basis of diversity and speciation in microbes, including horizontal gene transfer mechanisms. An earlier but related version of the framework (Martiny *et al.*, 2006) considers dispersal, drift, and mutation as part of the broader-level category of 'historical processes,' a term borrowed from traditional biogeography as typically applied to larger organisms.

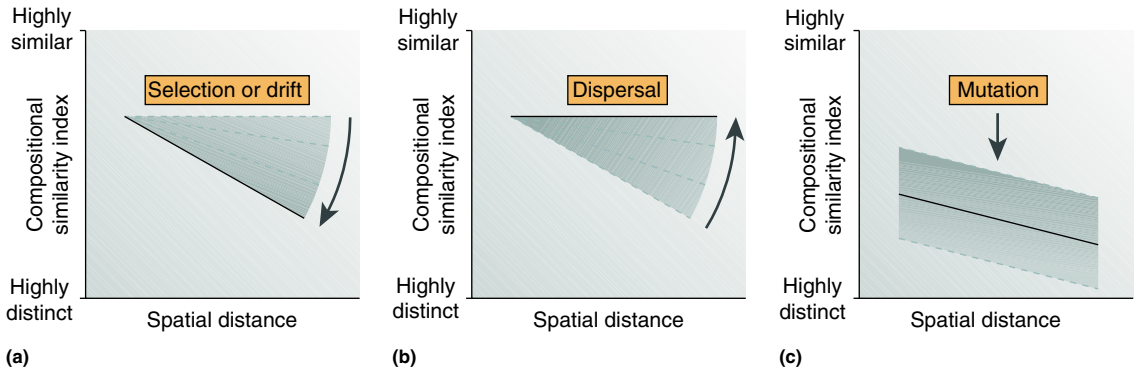

Figure 3 The effect of the four processes on the relationship between community similarity and spatial distance. (a) Selection and drift increase the strength of the distance–decay relationship (i.e., they steepen the slope). (b) Dispersal weakens the distance–decay relationship (i.e., it flattens the slope). (c) Mutation decreases the similarity between locations, regardless of the distance between them.

Relative Importance of Processes

Studies have confirmed that the process of selection (i.e., local environmental factors) greatly influences the composition of microbial communities in both space and time (reviewed in Hanson *et al.*, 2012). For example, numerous studies have linked spatial variation in microbial communities to variation in pH or salinity (e.g., Fierer and Jackson, 2006). Temporal variation in microbial communities also tends to be significantly correlated with seasonal variation in environmental variables such as temperature.

But what other mechanisms besides environmental selection can explain biogeographic patterns in microbial communities? The answer likely depends on determining the relative role of dispersal. Dispersal has the effect of mixing diversity across time and space, thus canceling out the influences of other processes (drift, mutation, and even selection) and weakening biogeographic variation. As for larger organisms, biogeographic variation may arise because not all taxa can disperse to or establish in all locations equally, an effect known as 'dispersal limitation.' The existence of dispersal limitation, even for some microbes, would partially falsify Baas-Becking's hypothesis.

However, measuring or tracking dispersal directly is difficult for most organisms, let alone those that are invisible to the human eye. For this reason, dispersal limitation for microbes is often indirectly inferred from the observed correlation between microbial community composition and geographic distance, after removing the influence of measured environmental factors (i.e., removing the effect of selection). When studies use this approach, many do find a correlation between microbial composition and geographic distance (Cho and Tiedje, 2000; Whitaker *et al.*, 2003; Martiny *et al.*, 2011), suggesting that microbial communities are not equally mixed by dispersal and are therefore dispersal limited. At the same time, other studies find no evidence for dispersal limitation in microorganisms (e.g., Finlay, 2002, and reviewed in Hanson *et al.*, 2012) and thus the debate over whether 'everything is everywhere' persists.

Consequently, a current line of research is to determine when and where dispersal limitation occurs for microorganisms. If dispersal limitation does exist for microbes, a subsequent area of research will be on whether additional processes (e.g., historical factors such as drift, local extinction, or past environmental legacy) play a role in microbial distributions and in what capacity.

Limitations and Outlook

Limitations

Spatial scales and temporal variations

The strength and existence of microbial biogeographic patterns are greatly dependent on spatial scales (Hanson *et al.*, 2012). This is likely because the ability of a microbe to disperse from one location to another decreases as spatial distance between those locations increases (Nekola and White, 1999). Therefore, the relative importance of the underlying processes (Section Relative Importance of Processes) varies with spatial scale. Additionally, dispersal limitation on very small spatial scales can cause significant community dissimilarities within certain habitats (e.g., biofilms and sediments; Martiny *et al.*, 2011), but with no effect on larger scales (i.e., their 16S rRNA genes are still globally distributed). Thus, characterizing biogeographic patterns at different spatial scales may inform the influence of microbial dispersal and other mechanisms controlling microbial distributions.

Temporal variations must also be considered when studying microbial biogeography. Recent studies have shown that microbial communities are highly dynamic in time and that seasonality is an important driver of microbial distributions (Section Temporal Changes). Moreover, biogeographic processes can have an inherent time component (e.g., dispersal rate) and thus the influence of these processes can be time dependent (Bell, 2010). Studies intending to characterize variation in microbial communities over space should therefore measure, control for, or at the very least, be interpreted in light of possible temporal variations.

Taxonomic resolution

The amount of variation detected within microbial communities is highly dependent on the level of taxonomic resolution used to define taxa (Hanson *et al.*, 2012). This is

determined by the sensitivity of the identification method, however for modern sequencing techniques (Section Molecular Genetics: Enabling Microbial Biogeography), taxonomic resolution can be arbitrarily defined by changing the sequence similarity cutoff value, for example. Low-resolution taxonomic definitions can combine taxa with different traits and evolutionary histories, blurring any variation in distribution as a result of those differences. Because broad-level taxonomic groups (e.g., genus or family level) tend to have wider distributions than narrower ones (e.g., species or subspecies/strain level), using broad taxonomic definitions may dilute biogeographic patterns and their underlying processes, for both microbes and macroorganisms (Martiny *et al.*, 2006; Hanson *et al.*, 2012).

Inferring process from pattern

Much evidence now exists demonstrating that microbes display a range of biogeographic patterns not unlike larger organisms (Section Evidence for Microbial Biogeography). However, using observational patterns to reveal the underlying ecological and evolutionary processes driving them (Section Ecological and Evolutionary Mechanisms) is problematic and prone to misinterpretation. This difficulty arises foremost because multiple mechanisms can combine to create the same pattern, and secondarily because instantaneous observations are insufficient for capturing this combination of processes occurring along a continuum of space and time. Mechanistic experiments to manipulate community assembly and dispersal under controlled conditions are needed to directly test the influence of each of the four hypothesized processes (Section Framework; Hanson *et al.*, 2012).

Applications and Significance

The motivation for studying microbial biogeography extends beyond deciphering the distribution of microbial biodiversity across the globe. Because microbes exist in every known habitat on Earth and are key players in ecosystem dynamics, including nutrient cycling and parasitism, studies of their diversity in space and time are finding an increasingly wide range of applications, some of which are described below.

Anticipating responses to environmental change

Under changing conditions microbes can adapt, move, die, or go dormant. How resilient are microbial communities to these scenarios? Microbial biogeography can help address this question by elucidating the factors controlling microbial distributions and abundances. For example, defining the range limits and dispersal abilities of different microbes can enable prediction of if and how microorganisms will adapt to changing environments. Likewise, identifying the factors correlated with microbial community composition and the 'microbial seed bank' (Section Temporal Changes) are first-steps toward understanding how whole microbial communities, and their activities, may shift under environmental change.

Improving human well-being and sustainability

Microbes are not only key components in the functioning of all ecosystems, but are also intimately involved in human health in a multitude of ways. Indeed, we think of microbes as pathogens and parasites, but a rapidly expanding field of research has discovered the importance of 'friendly' bacteria living in and on larger organisms, including humans. Applications of microbial biogeography to the study of the human 'microbiome' (e.g., Costello *et al.*, 2009) have been integral in understanding how these microbial communities protect against pathogens as well as how they are involved in many aspects of human health from obesity to autism to gastrointestinal disorders.

Microbial biogeography, as a framework for understanding microbial diversity generally, is also valuable for understanding the role of microbes in nearly every aspect of modern human society where microorganisms are involved. This includes agriculture, food production and spoilage, bioremediation, bioterrorism, wastewater treatment, and alternative energy production. For example, biogeography has been applied to the bioremediation of oil spills by oil-degrading microbes (Valentine *et al.*, 2012). It has even been applied to wine grape cultivation. Bokulich *et al.* (2014) discovered biogeographic patterns in grape-associated bacteria according to grape variety and regional climatic factors across viticulture zones, demonstrating how microbial biogeography of agricultural commodities may be used to enhance quality characteristics and economic value.

Underexplored extreme environments

Microorganisms are known for their capacity to exist and thrive in extreme conditions considered to be inhospitable to larger organisms. This includes high salinity, pressure, and temperature environments; low-energy systems; and toxic, acidic, or radioactive conditions. 'Extremophiles' grow directly in these conditions, while other microbes may simply evade these stressors by forming spores or entering states of low-metabolic dormancy (Lennon and Jones, 2011). Nonetheless, extreme microbial habitats are interesting targets for studying the limits and origins of life, and are also valuable models for understanding fundamental controls over microbial diversity and biogeography (e.g., Whitaker *et al.*, 2003).

Microbial biogeography may also be useful for providing insight into biodiversity in underexplored and difficult-to-access extreme environments, such as the deep biosphere and outer space. For example, panspermia (i.e., the distribution of life throughout outer space via comets, asteroids, and meteoroids) relies on microbes being able to travel and survive through the harsh space environment (e.g., vacuum conditions, extreme temperatures). Microbial biogeography on Earth can provide clues as to candidate microbes for panspermia and life on other planets. For instance, microbes can exhibit latitudinal dependencies such as the Rapoport effect (Section Latitudinal Gradients), which dictates that organisms living near the equator are found within smaller latitude ranges than those living near the poles. Thus more widespread, higher-latitude microorganisms can likely tolerate a wider range of climates, and therefore may also be capable of inhabiting otherwise lifeless Earth-like planets with uncertain environmental conditions.

See also: Biogeography, Ecological Theories in. Biogeography, Evolutionary Theories in. Biogeography, History of. Biogeography, Patterns in. Dispersal Biogeography

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Biogeography of Arthropods

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Biogeography, the study of the distribution of organisms in space and time, frequently focuses on circumstances leading to these distributions, and consequently on ecological and/or historical factors that have played a role. The discipline integrates information across fields of evolutionary biology, ecology, geology, and geography: Selection within a given environment, in concert with isolation that might be achieved through geological or ecological events imposed on the organism, or the activities of the organism itself, have all acted at some level to mold ancient and recent distributions. The complexities of these interactions in leading to patterns of biotic distribution have been of interest to biologists since the time of Wallace and Darwin and before. However, it was only toward the end of the twentieth century that understanding of plate tectonics, and the development of cladistic approaches, transformed biogeography from being largely descriptive, to become a rigorous science. Over the last few decades and into the twenty-first century, biogeography has progressed significantly as a science alongside developments in the associated disciplines mentioned above. The goal of this article is to underscore the role and interplay of different factors in shaping biogeographic studies in arthropods. The author chose specific examples to highlight each of these factors; these are examples only and no attempt is made to be comprehensive – the literature is massive.

Age of Arthropods – Insights From Fossils

Arthropods are an ancient group, dating back far before the origin of many birds and mammals (Giribet and Edgecombe, 2013; **Figure 1**). While much of what we infer regarding biogeography is – necessarily – based on extant distributions, fossils have provided some intriguing insights about the biogeographic origins of certain lineages (Grimaldi and Engel, 2005); amber deposits, which date back to 230 million year old (a nematoceran fly and two mites), provide particularly detailed information on the morphology of arthropods, and sometimes even carry ecological information (Schmidt *et al.*, 2012). Cambrian marine communities were dominated by arthropods, including trilobites, with much diversity illustrated by the Cambrian Burgess Shale of British Columbia (Briggs, 2015). Using multiple molecular datasets with fossil calibrations, current data suggest a Cambro–Ordovician colonization of land of different arthropod lineages ($\sim$510–471 mya) that occurred at about the same time as land plants (Rota-Stabelli *et al.*, 2013). Further diversification is thought to have started in the Silurian–Devonian, and has been linked to the rise of the angiosperms, for example, in phytophagous beetles (McKenna and Farrell, 2006). Likewise in ants, which have existed on Earth for $\geq$100 million years, specimens became common in the fossil record in the Eocene (LaPolla *et al.*, 2013). Here again, the diversification has been linked to the rise of angiosperm-dominated forests and their correspondingly more complex leaf-litter layers (Moreau *et al.*, 2006; Wilson and Hölldobler, 2005). However, at least in beetles, it has recently been suggested that the rapid increases in diversity through certain periods might rather be due to little extinction (Smith and Marcot, 2015). In any case, arthropods are ancient, and their history has been shaped by geological and climatological processes from ancient times to the present.

Geological Movements in Arthropod Biogeography

In terms of biogeography, some of the most interesting ancient patterns are associated with the break-up of Gondwana, which imposed isolation on previously connected biotas. The separation began at the beginning of the Jurassic (about 184 Mya), with the eastern part (Antarctica, Madagascar, India, and Australia) separating from Africa and the western part (South America) moving away from Africa as the South Atlantic Ocean opened, beginning about 130 Mya. Subsequently, the section that included what is now India–Madagascar–Seychelles broke off, and Australia (and later New Zealand) began to separate from Antarctica about 80 Mya. The Indian plate collided with Asia about 45 Mya, and South America separated from Antarctica about 30 Mya.

North America and Europe were connected until the Atlantic Ocean opened about 70 mya. Close phylogenetic relations between various insect groups in eastern North America and Europe are evidence of the past unity. Thus, a number of representatives of Collembola, Hemiptera, Homoptera, Coleoptera, Diptera, and Hymenoptera show this pattern (Noonan, 1988). Among spiders, the mygalomorph spider genera *Antrodiaetus* and *Atypoides* (Antrodiaetidae) show a disjunct Holarctic distribution in which trans-Beringian and trans-Atlantic routes appear to account for the present-day distribution in Japan and North America (Hendrixson and Bond, 2007).

In the same way, the stepwise break-up of Gondwana has led to disjunctions between organisms from Andean South America, Australia, New Zealand, and Madagascar. For example, relationships among the Plecoptera suborders – Antarctoperlaria and Arctoperlaria – has been linked to the break-up of Pangaea into Laurasia and Gondwanaland; then when Gondwana broke up, the ranges of the suborder Antarctoperlaria and its families became disjunct, with distinct representatives on each of the distant landmasses (Zwick, 2009). Relationships among basal lineages of scarab beetles can be explained by the break-up of Gondwana (Kim and Farrell, 2015; **Figure 2**). Gondwanaland Moths (Lepidoptera: Palaephatidae) are limited to Chile, Argentina and Australia. Diversification of insects across the Gondwana landscape has frequently been associated with plant associations. Thus, among bugs, members of the family Peloridiidae (Hemiptera) show a classic Gondwana distribution: Australia, New Zealand, and southern South America. They are associated

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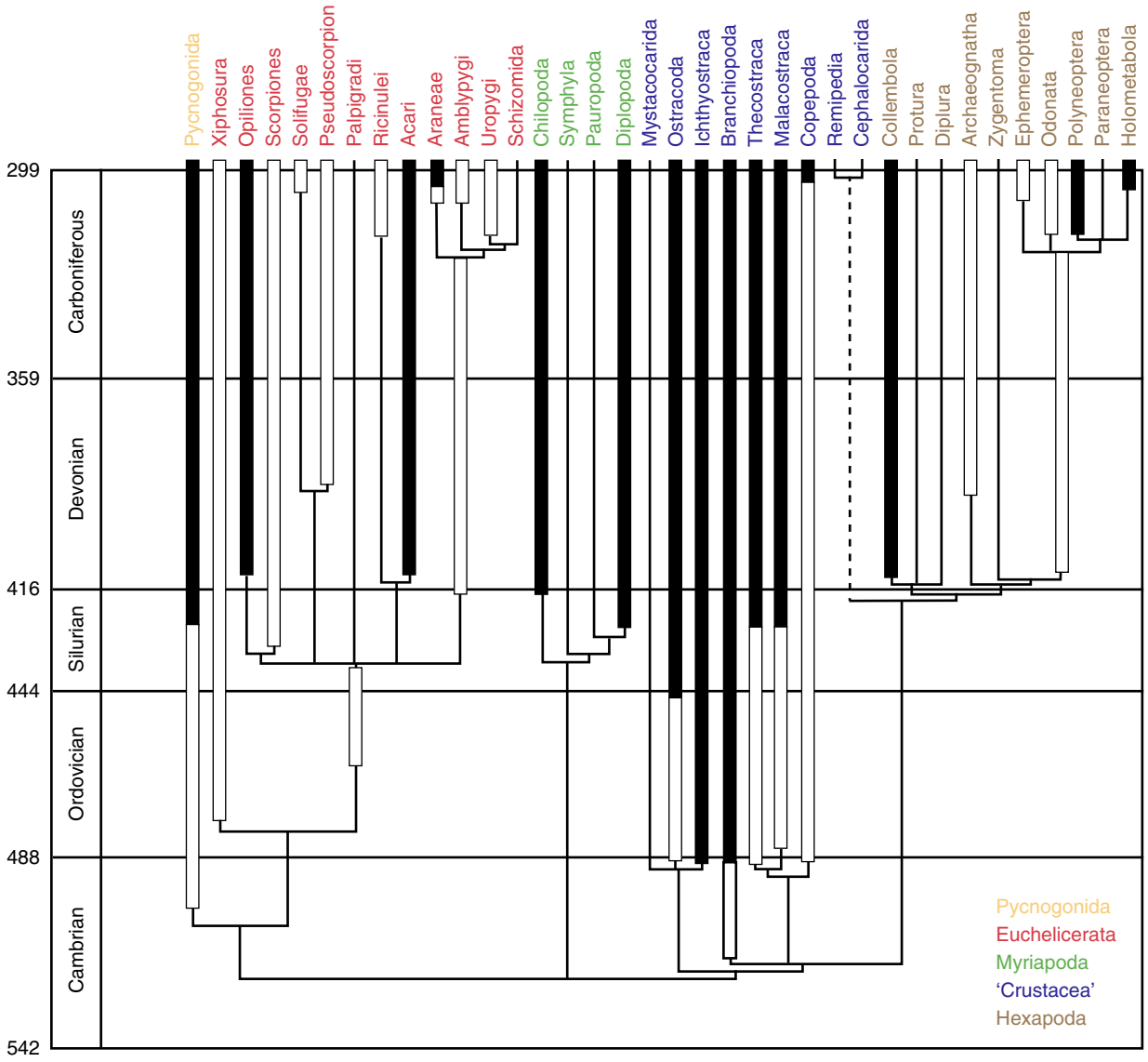

Figure 1 Relationships between living arthropod lineages with paleontological calibration (Giribet and Edgecombe, 2013). Molecular dating and paleontological inference have been used to place the origin of the Hexapoda in the Cambian. Solid bars indicate unambiguous fossils assigned to the crown group; open bars indicate the presence of fossils assigned to the stem group.

with moss in *Nothofagus* (southern beech) forests and probably feed on the moss (Burckhardt, 2009). *Araucaria* beetle herbivores also share Gondwanan distributions with their host: Divergence between the Australian and the South American *Araucaria*-feeding taxa occurred around the Cretaceous/Paleocene (Sequeira and Farrell, 2001). A number of spider lineages have also been shown to reflect the geologic history of continental movement, in particular several mygalomorphs (Ayoub *et al.*, 2007) and an ancient lineage of pelican spiders (Wood *et al.*, 2013). Gondwanan affinities are also found in non-spider arachnids. For example, opilionids show a relationship between Sri Lanka–Australia–New Zealand (Giribet *et al.*, 2012).

There are also examples from other parts of the world where insects and spiders are clearly associated with the geological history of the region, for example, in southeast Asia, where the complex history of the region has played a major role in fostering diversification (Lohman *et al.*, 2011).

On a somewhat more recent timescale, North America has proven particularly intriguing for examining biogeographic hypotheses. The Baja California peninsula has a complex geological history having undergone latitudinal and longitudinal displacements along the northwest to southeast peninsula starting approximately 15 million years ago with more recent events associated with the formation of islands and seaways (Dolby *et al.*, 2015). Deep divergence has been found between the spider *Homalonychus selenopoides* on the east side of the Colorado River and its congener *Homalonychus theologus* on the west side of the river including the Baja California Peninsula (Crews and Hedin, 2006). In the same way, the

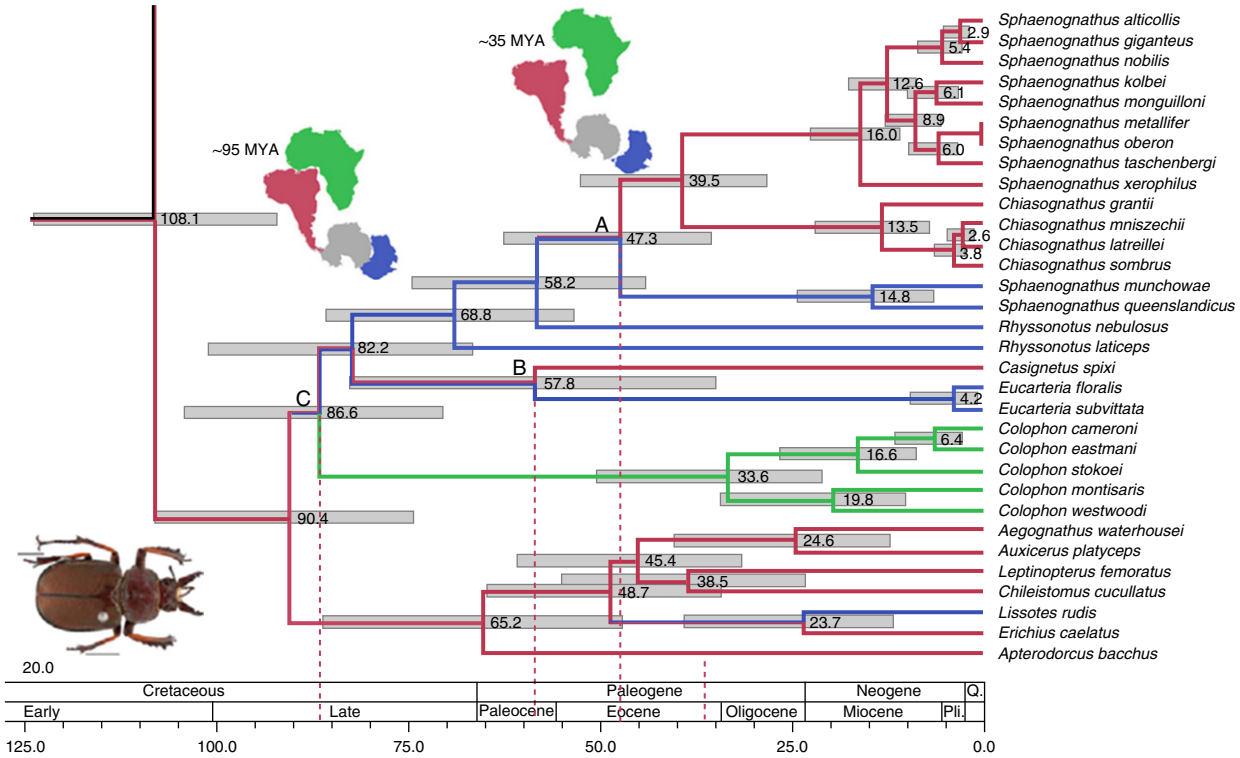

Figure 2 Stag beetles (Lucanidae) are one of the earliest branching scarab lineages. The split between many of the lineages are largely concordant with the pattern of continental break-up of the super-continent Gondwana. Colored branches indicate Neotropical (red), Australasian (blue), and Afrotropical (green). Reproduced from Kim, S.I., Farrell, B.D., 2015. Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a Gondwanan origin of Darwin's stag beetle. Molecular Phylogenetics and Evolution 86, 35–48.

islands of the Western Mediterranean are continental fragments of the eastern Iberian Peninsula dating to about 30 Mya; among many different arthropod groups, including beetles (Condamine *et al.*, 2013) and spiders (Bidegaray-Batista and Arnedo, 2011) molecular data suggests that diversification has been driven by the separation of the landmasses.

Climate can operate as an isolating mechanism in a similar manner to that of geology. Thus, for example, the aridification of Australia has led to relict populations in remnant patches of rainforest in the north east (Bell *et al.*, 2007) and around other isolated patches of the continent (Rix and Harvey, 2012; Figure 3).

Dispersal and Colonization

Besides the role of geology and climate in providing barriers, and imposing isolation through vicariance, dispersal between landmasses has also played a major role in shaping biogeographic patterns among arthropods. Thus, dispersal characteristics of colonists are clearly important, not only in predicting which species colonize an area, but also in the process of diversification. Aerial dispersal occurs primarily through flight in insects, and ballooning on silk threads in spiders. The ability for aerial dispersal, and the loss of that ability, plays a key role in biogeographic patterns in arthropods. Coupled with this, the tendency of some insects, in particular Lepidoptera and Orthoptera, to migrate can facilitate the potential for movement and colonization (Danthanarayana, 2012). Certain insects, such as migratory locusts and some dragonflies, can reach very high densities, which can lead to mass emigration events. However, regular migrations are found in certain Lepidoptera, for example, the painted lady and the red admiral undergo seasonal migration as do monarch butterflies.

Among araneomorph spiders and some caterpillars, dispersal is by ballooning, in which a strand of silk, usually let out by an immature, is caught in the wind and the animal is carried off (Bell *et al.*, 2005); as a result of this behavior, certain spiders have been found to be among the first and most persistent colonists on mangrove islands in the Florida Bay (Simberloff and Wilson, 1969).

However, in terms of biogeographic pattern, much work has focused on loss of dispersal. For arthropods with nondirectional dispersal strategies and that colonize isolated habitats, selection will tend to act against individuals that disperse because their nondirectional dispersal – such as ballooning spiders and Lepidoptera larvae – will result in their loss from the gene pool (Gillespie *et al.*, 2012). Dispersal ability may also be lost if not required and its maintenance demands energy.

Dispersal through vectors, such as on the fur or feathers of larger animals, may play a role in allowing insects to colonize remote areas, and rafts of vegetation or ice, have clearly also been important in transporting arthropods to such sites.

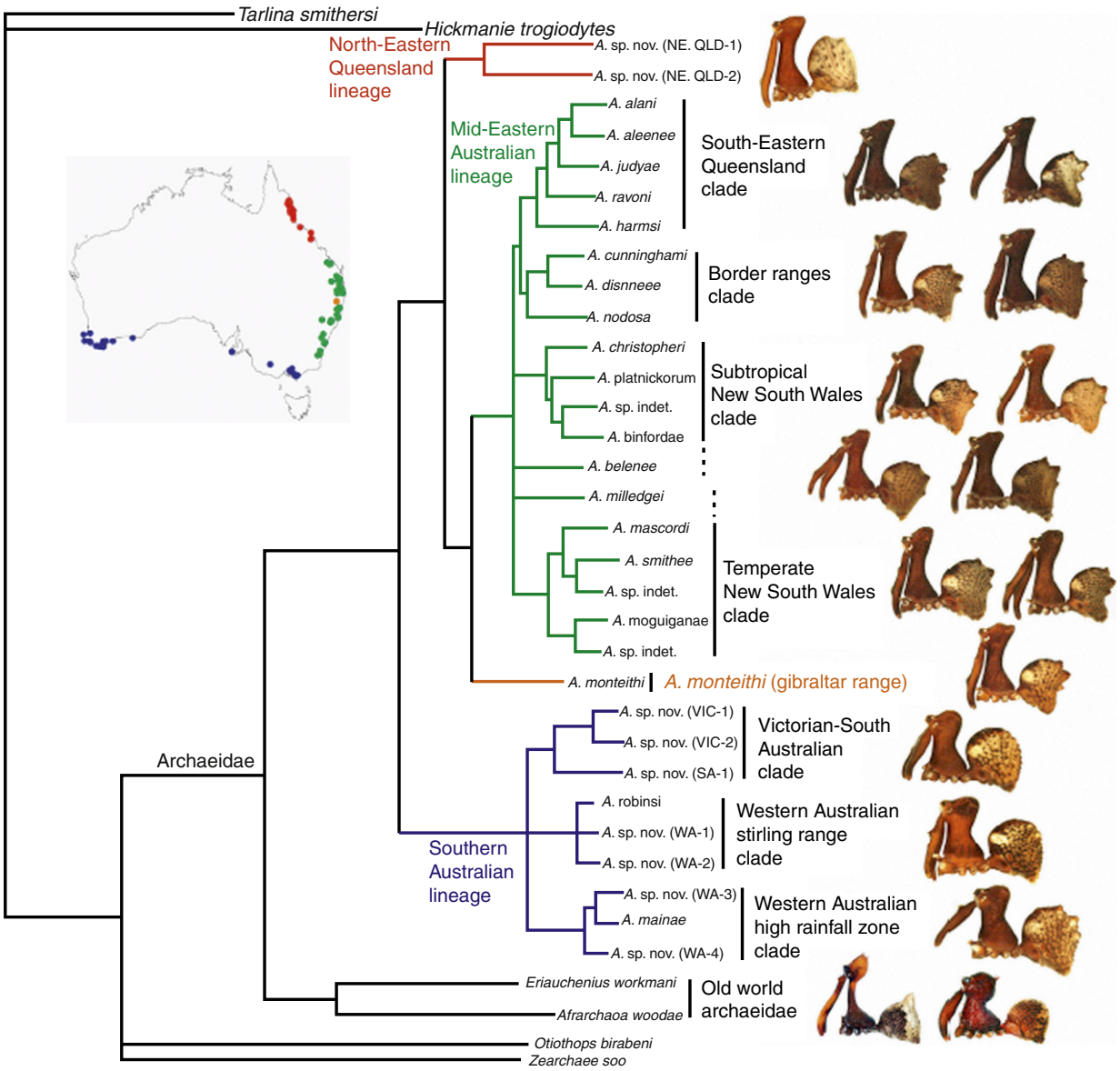

Figure 3 Phylogeny and historical biogeography of assassin spiders (Araneae: Archaeidae) in the Australian mesic zone suggesting Miocene speciation within Tertiary refugia. Major Australian lineages and regional clades are shown according to their distribution as are lateral profile images of representative taxa (Rix and Harvey, 2012).

Biogeography of Interactions

Interactions play a key role in shaping biogeographical patterns, though it is often difficult to infer ecological interactions from fossil data. However, there are exceptions. Evidence of herbivory in arthropods is first shown in the Early Devonian within early detritivore-based terrestrial ecosystems (Labandeira and Currano, 2013). This early herbivory is characterized by two pulses: one during the Early Devonian and a second, more extensive one commencing at the Mississippian–Pennsylvanian boundary. Pollination dates from the late Paleozoic, although the mid-Mesozoic strategy of pollination was replaced in the mid-Cretaceous with current patterns, initially generalist, with subsequent specialization in the Late Cretaceous and Paleogene.

Considering modern lineages, pollinators have long been considered to play a role as selective agents in shaping floral traits, though determining the way in which they might have influenced angiosperm diversification has been more difficult. While studies that combine phylogenies with pollinator data have highlighted the frequency of pollinator-driven diversification, the specific role that pollinators play in potentially driving diversification is still not clear (van der Niet and Johnson, 2012). The fig–fig wasp system is well known for the tight association between the plants and their pollinating wasps (Agaonidae, Chalcidoidea) with which they appear to have approximately codiversified. Moreover, multiple additional groups of non-pollinating chalcids have colonized figs independently with most of the non-pollinators being gall inducers or inquilines; diversification of the wasps has tracked

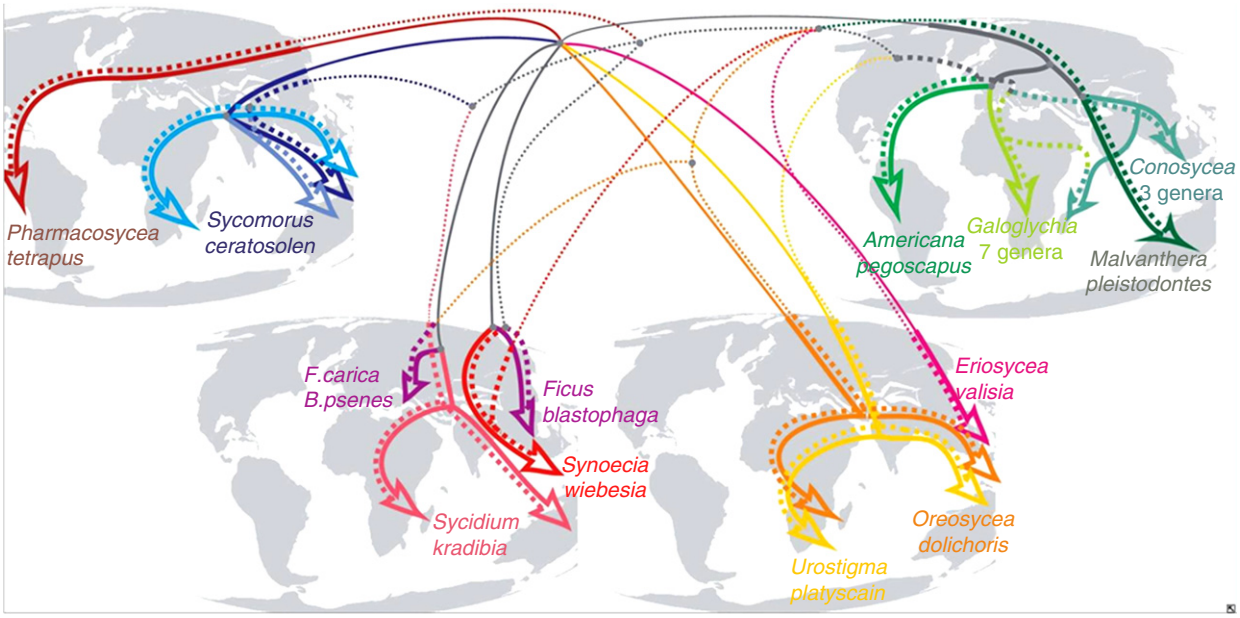

Figure 4 Hypothetical biogeographical scenario of mutualism diversification (presented on 4 different maps for clarity). Solid arrows: figs; dashed arrows: wasps (Cruaud *et al.*, 2012). It appears that the major lineages of both figs and pollinators split during the Tertiary and then spread southward through the evergreen woodland and tropical forest belts of Eurasia.

that of the host plants and associated pollinators, with similar ages and synchronous colonization events between fig trees, pollinator wasps and non-pollinating wasps (Sycophaginae) (Cruaud *et al.*, 2011, 2012; **Figure 4**).

The role of host associations for taxa that have colonized remote islands has been particularly enigmatic. Thus, when a member of a tightly coevolved pair first arrives on a remote island, it is likely that its immediate partner is absent; yet coevolved complexes of pathogens and their vectors, and plants and their pollinators, still exist on these islands, so how is this achieved? Recent work on patterns of codiversification and host association in an obligate pollination mutualism – that between the plant family Phyllanthaceae and the moth genus *Epicephala* on different Pacific archipelagoes, and comparing patterns here with those on the western Pacific Rim (the evolutionary source of both plant and insect) – has shown that not only have the insects and plants reached some of the most remote islands of Oceania, but the mutualism is maintained (Hembry *et al.*, 2013). This raises intriguing questions of how colonization occurs on remote islands and what constrains it, and the extent to which the interaction itself can drive population differentiation.

Island Biogeography

Islands have traditionally been considered to be any relatively small body of land completely surrounded by water. However, their primary biological characteristic, isolation from a source of colonists, is common to many situations on continents where a piece of land is surrounded by dramatically different habitat. As a result, ideas developed for true islands are frequently applicable to similar continental system. One of the best-known effects of insularization is its influence on species diversity, which has been modeled as a balance between immigration and extinction, equilibrium between these depending on the size of the island and the distance from the source of colonists (MacArthur and Wilson, 1967). Empirical support for the model came from a classic study using arthropods on islands of the Florida Keys (Simberloff and Wilson, 1970). Spiders provide one of the classic natural experiments in support of the model: After a 1996 hurricane in the Bahamas, every lizard and web spider originally found on the exposed islands was gone. However, recolonization was rapid and in 1 year, the number of species was the same as before the hurricane struck and equilibrium was recovered quickly, just as it was for Simberloff and Wilson's mangrove arthropods. Interestingly, the same pattern was found again after Hurricane Floyd in l999 in which spiders were eliminated from a more northerly group of islands (Schoener and Spiller, 2006).

Dispersal versus Vicariance: Islands can be divided into two major types – continental and oceanic. Continental islands generally form by breaking off as a fragment from the source. In these, the ecological space will initially be filled as a consequence of connection to the source of colonists prior to insularization (Gillespie and Roderick, 2002). Species numbers will decrease following fragmentation through the process of relaxation. If these islands become more isolated, species will eventually arise through relictualization with the formation of 'paleo-endemics' through vicariance, such as the various Gondwana lineages described above. In contrast, oceanic islands, by definition, have never been in contact with the source of colonists and have abundant 'empty' ecological niche space. On these islands, species numbers will initially increase through immigration, the rate depending on the degree of isolation. If isolation persists, over time species arrive through dispersal and species formation will result in

'neo-endemics.' When isolation is extreme, the ecological space will gradually be filled through speciation (rather than immigration) and adaptive radiation of neo-endemics.

While the geological distinction between these island types is quite clear-cut, changes in island area, topography, and isolation have provided opportunities for subsequent dispersal to continental islands. New Zealand is prime example of a landmass that has been shaped by both ancient events, as revealed through the biogeography of Onychophora (Murienne *et al.*, 2014) and Cyphophthalmi (Boyer and Giribet, 2009; Giribet *et al.*, 2012) and more recent dispersal (Goldberg *et al.*, 2008). Likewise, while the ancient landmass of Madagascar has spider fauna that originates from Gondwana (Wood *et al.*, 2012), there are many colonizers that presumably arrived more recently via Cenozoic dispersal, and mostly from Africa (Yoder and Nowak, 2006).

Adaptive Radiation

Adaptive radiation has now been described for many different arthropod groups, invariably associated with an acceleration in diversification rate and some of the most rapid rates of diversification have been found among adaptive radiations of insects in the Hawaiian Islands (0.8–4.2 Myr^{-1}) (Mendelson and Shaw, 2005). However, recent work on ant-nest beetles (*Paussus*), which are intimately tied to a given ant host from which they exploit food and protection, show tremendous diversity, and some of the highest rates of diversification yet reported (Moore and Robertson, 2014).

In some situations, adaptive radiation is associated with novel hosts, with notable examples of key innovations coming in the form of plant defenses, which in turn can provide the conditions necessary for a herbivore or parasite to develop a trait that is resistant to the defense of the host, and can therefore diversify on to the host. This 'escape and radiate' hypothesis (Ehrlich and Raven, 1964) has now been implicated, at least broadly, in major radiations of herbivorous insects and plants (Winkler and Mitter, 2008). Symbiotic associations may also serve as key innovations, providing a means through which partners can exploit habitats unavailable to either partner alone. For example, microbial mutualists can present ecological opportunity and allow adaptive radiation of their insect hosts (Janson *et al.*, 2008). Moreover, interactions can enhance diversity, as shown in gall midge insects which symbiotically rely on fungus to break down plant material, and are 17 times more diverse than similar lineages without the symbiotic relationship (Joy, 2013).

Diversification within an adaptive radiation can occur as a result of ecological shifts, such as changes in habitat or diet. Recent illustrations of this have been provided by habitat switching in Hawaiian spiders (Gillespie, 2004), host switching by Hawaiian leafhoppers (Bennett and O'Grady, 2012), and gall midges on goldenrods (Stireman *et al.*, 2012), as well as the ant-nest beetles (Moore and Robertson, 2014) mentioned above. Alternatively, ecological associations may be maintained with divergence occurring in allopatry, as has been found in Hawaiian *Drosophila* (Magnacca and Price, 2015). Both elements appear to be involved in a given adaptive radiation, though the prevalence of ecological versus geographical shifts in driving proliferation varies across radiations. For example, across a radiation of hyperdiverse moths in Hawaii (*Hyposmocoma*, Cosmopterigidae), most adaptive differentiation appears to have occurred early in the radiation, with recent proliferation a result of geographical isolation (Haines *et al.*, 2014).

Range Expansion and Invasion

Climatic shifts can have a major effect on the distribution, abundance, and diversity of arthropods. Range expansion of North American *Parnassius* butterflies in the late Pleistocene has been associated with warming climate (Schoville and Roderick, 2009). For insects associated with plants, the climate can lead to expansion or contraction of their resource, with insect diversity being dictated by the availability of plants (Nyman *et al.*, 2012). However, the most dramatic recent examples of range expansion are linked to invasion, often associated with increased movement of humans. Arthropods have become increasingly recognized as important invaders with enormous potential for impact. This topic has been covered in a recent book (Simberloff, 2013).

Target Areas for Biogeographic Study – Oceanic and Continental Islands

Hawaiian Islands and French Polynesia

The Hawaiian archipelago (4000 km from the nearest continent, North America; 3200 km from nearest island group) exhibits extreme disharmony (only 50% of insect orders and 15% of known families represented in the native fauna, (Howarth and Mull, 1992)), coupled with extraordinarily high levels of endemism among all terrestrial arthropods, and numerous cases of adaptive radiation. The accentuation of disharmony through speciation is well illustrated in the moth genus *Eudonia*, which is represented by only one species in central Polynesia and eastern Melanesia (the presumed source), but by 18 species in the Society Islands, 13 in the Marquesas, and over 100 species in Hawaii. The microlepidopteran family Cosmopterigidae has only 180 species in North America (north of Mexico) and 388 in Australia, but has 85 endemic species in the Marquesas and over 350 in Hawaii (Gillespie and Roderick, 2002).

The source of colonists and mechanisms of dispersal have been studied and discussed extensively for many of these lineages, and general patterns are emerging (Gillespie *et al.*, 2012). For the Hawaiian Islands, taxa known for aerial dispersal abilities (birds and spiders in particular) have generally colonized from the east, in agreement with the direction of extreme storms. For other Hawaiian lineages, affinities with the north support the idea that insects feeding within seeds can show a pattern of colonization matching their plant hosts. In addition, some terrestrial taxa have likely reached remote islands through rafting, which appear to be most common for those associated with dead wood, leaf miners, wood borers, and other insects that inhabit plant debris, or those that attach their eggs to vegetation. Stepping-stone colonization may be

the major pattern of colonization for rafters. Thus, *Rhyncogonus* weevils occur on oceanic islands throughout the Pacific, and appear to have colonized archipelagoes in a sequential stepping-stone fashion (Gillespie *et al.*, 2008).

The Hawaiian Islands are characterized by some of the most remarkable examples of diversification in arthropods. Early and ground-breaking studies focused on *Drosophila* (O'Grady and Markow, 2012), and highlighted the importance of sexual selection in fostering the rampant proliferation of species. However, recent work highlights some major new lineages of insects, and the interplay between habitat, host, and geography is shaping diversification, as has been illustrated by leafhoppers (Bennett and O'Grady, 2012), planthoppers (Goodman *et al.*, 2012), cosmet moths (Haines *et al.*, 2014), and bark lice (Bess *et al.*, 2014), as well as *Drosophila* (Magnacca and Price, 2015).

Although a break in gene flow is clearly important for adaptive differentiation, hybridization, and genetic admixture resulting from previously separated populations coming back in contact, have increasingly been implicated in the generation of adaptive variation and functional novelty (Gillespie and Roderick, 2014; **Figure 5**). Though more work is needed, it is clear that genetic mixing and hybridization among recently diverged populations play a key role in fostering adaptive radiation, as has been suggested for spider lineages (Roderick *et al.*, 2012).

Caribbean

The Caribbean region is one of the most biogeographically complex island systems and knowledge of the arthropod diversity is still rather poorly known. Among vertebrate lineages, the islands have served as the setting for adaptive radiation among those with limited dispersal ability (Losos, 2010). Although studies to date have provided insights into how individual lineages have colonized and subsequently diversified within the island system, there are as yet no unifying principles to explain the origin and diversification of Caribbean taxa. Notable controversies include the source of colonists and the means by which they colonized the islands, and biogeographic patterns within lineages. A particular focus of debate has been the role of vicariance versus dispersal in shaping the Caribbean biota and the importance of a hypothesized landbridge ('GAARlandia') between South America and the Greater Antilles during the Eocene Oligocene transition (Iturralde-Vinent and MacPhee, 1999). Arthropods, because they can provide a fine scale resolution of biogeographic

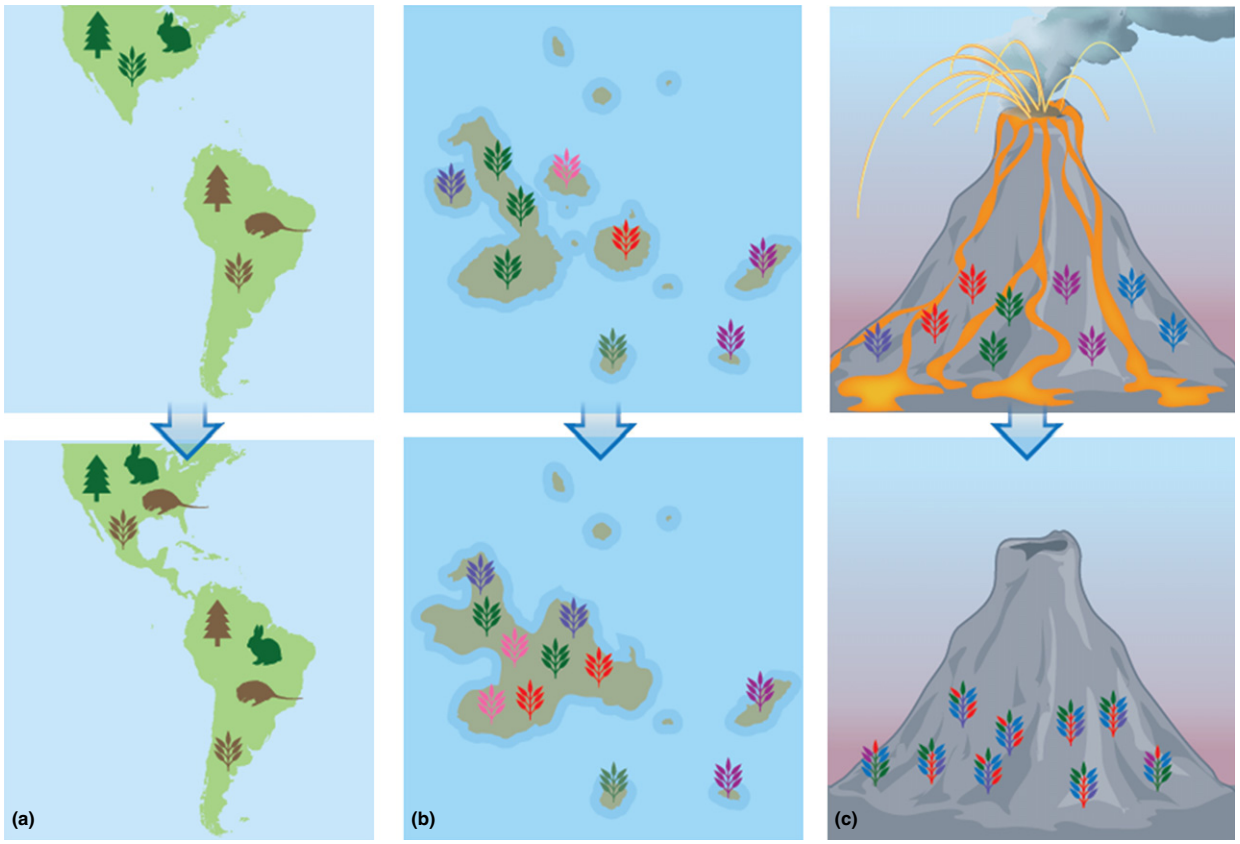

Figure 5 Isolation across scales of space and time. (a) On a multimillion-year timescale, distinct assemblages of biota form in isolation on different continents, and joining of the continents may lead to biotic exchange or species displacement. (b) On a millennial timescale, different species may evolve in isolation on different islands; connections between these islands can then result in a richer biological assemblage as newly formed species come together. (c) On a decadal timescale, populations may be isolated by recurrent events, such as volcanic eruptions; subsequent reconnection of these populations may result in new genetic combinations, a phenomenon that may also occur with invasive species. Reproduced with permission from Gillespie, R.G., Roderick, G.K., 2014. Evolution: Geology and climate drive diversification. Nature 509, 297–298.

patterns, are ideal candidates for elucidating the nature of these relationships. Thus, various arthropod lineages in the Caribbean provided a rich system for testing theories in cladistic biogeography (Liebherr, 1988). More recent studies have examined specific patterns of intra-island diversification and island–island vicariance, as has been demonstrated for *Amphiacusta* crickets (Oneal *et al.*, 2010) and *Drosophila* flies (Brisson *et al.*, 2006).

Colonization of the Caribbean has been examined for several groups, with spiders in the genera *Loxosceles* (Sicariidae) (Binford *et al.*, 2008) and *Selenops* (Selenopidae) (Crews and Gillespie, 2010), as well as butterflies (Matos-Maraví *et al.*, 2014), supporting the GAARlandia hypothesis in the colonization by the lineages of North from South America. In other groups, including *Centruroides* scorpions (Esposito, 2011) and swallowtail butterflies (Lewis *et al.*, 2015), biogeographic analysis could not reject the hypothesis of dispersal being the only route for colonization of the Caribbean.

Macaronesia

The arthropod diversity of the Azores has been studied intensively over recent years, though richness on the islands is rather low and many species are very rare (Terzopoulou *et al.*, 2015). In the Canary Islands, most of the arthropod work has focused on beetles, butterflies, *Drosophila*, cockroaches, bees, spiders, and mites (Juan *et al.*, 2000), with additional work on other lineages, including grasshoppers and mantids. This research has shown that most lineages have originated through dispersal from the nearby continent followed by *in situ* speciation, though the pattern of colonization is more complex than in Hawaii. Over evolutionary time *in situ* speciation comes to dominate over colonization as the source of new species. For example, the spider genus *Dysdera* includes three well-defined lineages; for the lineages that have proliferated, diversity has arisen largely *in situ* (Macias-Hernandez *et al.*, 2008). Molecular data indicates a slowing of the diversification over time, perhaps due to slower speciation or higher extinction rates.

Galapagos

The Galapagos, famed for the remarkable examples of evolution in action provided by Darwin's finches, has mostly been colonized from South American, though evidence from flightless *Galapaganus* weevils suggests a potential role for now sunken seamounts for their early evolution; patterns of diversification in the weevils are linked to volcanic activity together with habitat shifts (Sequeira *et al.*, 2008). A group of grasshoppers in the Galapagos shows affinities with the Old World, and may be a relict of a previously more widespread distribution or the result of long-distance, trans-Atlantic dispersal (Husemann *et al.*, 2015). A fascinating element that is emerging from studies of diversification of arthropods in the Galapagaos is the pattern of repeated evolution of similar ecotypes, as illustrated by wolf spiders (De Busschere *et al.*, 2012) and beetles (Hendrickx *et al.*, 2015) associated with extensive admixture, the latter contributing to diversification.

Target Areas for Biogeographic Study – Terrestrial Islands

Sky Islands

The environments of mountaintops are often quite different from the surrounding slopes, and can include cloud forests, alpine grasslands, herbfields, and paramo; these sites tend to be relictual fragments of previously more widespread habitats, isolated by changing climate (Watson, 2008). Perhaps the best-known sky islands, at least from a biological perspective, are the American Madrean 'sky islands' of southeastern Arizona and New Mexico. Here, striking phenotypic divergence has been found among populations of the jumping spider *Habronattus pugillis* (Masta, 2000), as well as scorpions (Hughes, 2011), and cerambycid (Smith and Farrell, 2005) and carabid (Ober and Connolly, 2015) beetles. Given that these habitats are only ca. 10 000 years old, diversification has been rapid.

Caves

Cave-adapted terrestrial arthropods are particularly common in beetles and spiders, though there are also some species of crickets, pseudoscorpions, amphipods, and isopods (Juan *et al.*, 2010). The two mechanisms proposed to explain speciation in caves are the 'climatic-relict' hypothesis, in which species become restricted to caves after extinction of the surface populations, and the 'adaptive-shift' hypotheses in which cave species are formed through adaptive parapatric divergence in the subterranean environment. In either case, the general pattern is of monophyly within cave populations, with large divergences between populations. However, in some situations, cave species may arise through a predisposition for hypogean living and a preference for dark habitats, as shown for Canary Island dysderid spiders (Arnedo *et al.*, 2008), cave-modified species of phalangodid harvestmen (Opiliones: Laniatores) (Hedin and Thomas, 2010), and Hawaiian *Schrankia* moths (Medeiros *et al.*, 2009), in all of which phylogenetic analysis reveals multiple switches between cave and epigean habitats.

Habitat Fragments

The process of fragmentation has resulted in insularization at many different scales across space and time. Fragmentation of Gondwana has been described above. On more recent time scales, many arthropods are restricted to sand dunes (Van Dam, 2013) or desert salt flats (Crews and Gillespie, 2014). These habitats were historically connected, and various different climatological events have been implicated in shaping the current relationships across the fragmented landscape.

On the young island of Hawaii, ongoing volcanic activity has created a dynamic mosaic of habitats of different age as large areas of forest are destroyed or fragmented when lava flows around them. Fragmentation on scales of hundreds of meters and over several hundred years can be sufficient for genetic differentiation of spider populations (Vandergast *et al.*, 2004), though – because of its transitory nature – this fragmentation is likely to be more important in maintaining

genetic diversity than in separation of populations (Roderick *et al.*, 2012). On larger spatial scales, distinct volcanoes represent geological gradients with independent histories of diversification and community assembly from recent to ancient ecosystems.

The Future of Research in Arthropod Biogeography

The field of biogeography is poised for a revolution as a result of huge amounts of genomic data that are becoming available through rapidly developing technologies that provide a wealth of information on functional genes, as well as an abundance of putative neutral markers. Applying these technologies to arthropods will provide a temporal framework of colonization or fragmentation of a lineage, associated demographic changes involved, relative population sizes, and other aspects that allow inference into the interplay between geologic and climatologic history, and the biology of arthropods.

See also: Dispersal Biogeography. Invasion Biogeography. Vicariance Biogeography

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Relevant Website

http://www.tolweb.org/Arthropoda/2469
Tree of Life: Arthropoda.

Biogeography of Interactions

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Glossary

Antagonism Interactions in which members of one species benefit and members of the other lose fitness; examples include predation and parasitism.

Biotic interchange Event in which the biotas of two regions come in contact and organisms colonize one region from the other.

Coldspot (coevolution) Pair of interacting populations within a geographic mosaic of coevolution, between which coevolution is not occurring.

Commensalism Interaction in which members of one species benefit but the fitness of members of the other species is not affected.

Competition Interactions in which members of both species lose fitness as a result of their interaction. For example, two predator taxa which attack the same species may compete for their shared prey.

Free-living Interactions in which the physical contact between members of interacting species is relatively brief compared to symbiotic interactions.

Geographic mosaic theory of coevolution (GMTC) Theory describing how coevolution between two species varies among their different pairs of interacting populations.

Hotspot (coevolution) Pair of interacting populations within a geographic mosaic of coevolution, between which coevolution is occurring.

Modular Networks broken into weakly connected subnetworks, or modules.

Mutualism Interaction in which the interacting members of both species benefit; may include interactions such as pollination and seed dispersal.

Nested Networks consisting of both a core of generalist species and asymmetric specialist species who interact with these generalists.

Symbiotic Interactions in which members of one species spend much or all of their life cycle in close physical proximity or intimacy to the tissues of a host.

Interactions between different taxa can be studied in a biogeographic context in the same manner as individual taxa can. Ecologists and evolutionary biologists classify species interactions according to the fitness benefit and loss to individuals of the species involved in them. 'Mutualisms' are interactions in which the interacting members of both species benefit; these may include interactions such as pollination and seed dispersal. 'Antagonisms' are interactions in which members of one species benefit and members of the other lose fitness; common examples of antagonisms are predation and parasitism. 'Commensalisms' are interactions in which members of one species benefit but the fitness of members of the other species is not affected. Finally, 'competition' encompasses interactions in which members of both species lose fitness as a result of their interaction. For example, two predator taxa which attack the same species may compete for their shared prey. Mutualisms and antagonisms usually occur between trophic levels; competition occurs between members of the same trophic level, while commensalisms may occur within or between trophic levels.

Interactions are also classified by the degree to which they are 'symbiotic or free-living.' Symbiotic interactions are ones in which members of one species spend much or all of their life cycle in close physical proximity or intimacy to the tissues of a host. Free-living interactions are ones in which the physical contact between members of interacting species are much more brief. Some interactions combine aspects of symbiosis and free-living ecology. For instance, many herbivorous insects are highly symbiotic as larvae which live inside the tissues of their hosts, but their adults are more free-living and live outside the host.

Historical Biogeography of Interactions

Phylogenetic and paleontological studies indicate that some interactions seen in extant biotas can be conserved over macroevolutionary timescales, whereas other interactions no longer occur or are more recent in origin.

Reconstruction of Historical Biogeography of Intimate Associations

The two ways in which the historical biogeography of interactions can be ascertained are via fossils and via phylogeny-based methods. Fossils can indicate changes in interactions that have occurred. For instance, extant hummingbirds are restricted to the Americas, where they are important flower visitors – so much so that many distantly related lineages of plants have evolved to be pollinated by hummingbirds, and shifts to hummingbird pollination are implicated in plant speciation (e.g., Kay *et al.*, 2005). However, fossil hummingbirds are known from the Oligocene of central Europe (Mayr, 2004; Bochenski and Bochenski, 2008), indicating that some Old World plants were likely pollinated by hummingbirds, and may have coevolved with hummingbirds, in the Tertiary, even though no such plants exist today, and these particular interactions can be considered extinct.

For intimate interactions where specialization is very high at the species level, extreme phylogenetic conservatism can allow phylogenies and divergence time estimation to be used to understand historical biogeography. In many cases, the utility of these methods is to narrow down the range of

possible hypotheses for the evolution of the interactions between two lineages.

Phylogenetic studies of bark beetles feeding on *Araucaria* conifers in South America and Australasia (Coleoptera: Curculionidae: Scolytinae) suggest that beetles alive today are relicts of ancient beetle–*Araucaria*, associations. Long basal branches leading to these beetles in the phylogeny (Sequeira *et al.*, 2000) and molecular dating of divergence times (Sequeira and Farrell, 2001) indicate that these associations are very ancient, dating back probably to the late Cretaceous, when *Araucaria* were more widely distributed. The distribution of these associations today in Gondwanan landmasses and the molecular dating do not, however, distinguish between the hypotheses that the distribution of these interactions in South America and Australasia is due to vicariance following the breakup of Gondwana, or is due to dispersal between these continents once they had already separated. However, they do falsify the hypothesis that scolytine–*Araucaria* interactions are much more recent and dispersed among widely separated *Araucaria* populations on distant continents during the mid- or late Tertiary.

In other cases, phylogenetic information can reveal historical biogeographic processes that would otherwise be invisible. For instance, two sister genera of moths, *Parategeticula* and *Tegeticula*, are the only insects which pollinate yucca plants (Agavaceae: *Yucca*) in North America; these moth genera rely on the seeds of yuccas as food for their larvae. Phylogenetic analysis and divergence time estimation of *Tegeticula* and *Yucca* suggest that the former diversified much more recently than the latter, despite the obligate interaction between the two (Althoff *et al.*, 2012). One possible explanation is that *Yucca* initially codiversified with *Parategeticula*, but then *Tegeticula* spread across most *Yucca* species and in doing so displaced most *Parategeticula* populations. The five extant species of *Parategeticula* today are distributed primarily in the southern part of the range of *Yucca*, consistent with the argument that *Tegeticula* displaced the genus throughout much of its ancient range. Although these age estimates are not without error, they do suggest, interestingly, that different pollinator lineages may competitively interact, and possibly competitively exclude each other, over evolutionary timescales, with major implications for understanding coevolution between yuccas and their pollinating moths. Although the examples above come from parasitic interactions between herbivorous insects and their host plants, many other examples exist from animal hosts and their parasites (see Morand and Krasnov, 2010).

Competitive Exclusion/Extinction in the Evolution of Biotas

Competition between different clades, unfolding across a biogeographic template, is thought to be a major factor in the evolution of life, and the biogeography of this competition – which clades end up in which regions of the world, and how they interact when they come into secondary contact through episodes of 'biotic interchange' – has long been recognized as a major component of this (Vermeij, 1991). For instance, for most of the Tertiary, North and South America were isolated by ocean. As a result of the distinct geologic histories of the two continents (North America was frequently connected with Eurasia; South America was initially connected to Antarctica and other Gondwanan landmasses before becoming an island) and their isolation from each other, largely independent and phylogenetically distinct mammal faunas evolved on the two continents. When North and South America were joined by the formation of the Isthmus of Panama in the Pliocene, many mammal lineages migrated into each others' continents, leading to new competitive (and presumably also predatory) interactions. This event is referred to as the Great American Interchange. Like many other episodes of biotic interchange, the invasions were asymmetric: in the Pleistocene, 11% of North American mammal genera invaded the South, while 2% of South American genera invaded the North (Vermeij, 1991); similar asymmetries occurred in bird invasions (Smith and Klicka, 2010). This invasion was found to follow a higher rate of extinction of mammal genera in South as compared to North America during the Pliocene (Vermeij, 1991). In another well-demonstrated example, through the Tertiary, Indian and Pacific ocean marine invertebrate communities have had much higher predation and grazing intensity than do Atlantic ones, leading to regional differences in defensive traits among the two biotas (Vermeij, 1978).

These asymmetries in invasion and interaction intensity are often cited as evidence for competition in the assembly of biotas (Vermeij, 1991). Similarly, competition is often invoked as a factor governing diversification and extinction in adaptive radiation on islands. Different islands within the same archipelago may, by virtue of their age, size, or other characteristics, experience different effects of competition in the diversification and assembly of their biotas (Rabosky and Glor, 2010; Gillespie and Baldwin, 2009).

Biogeography of Interaction Loss, Gain, and Reassembly

A biogeographic approach can also be used to understand factors leading to the loss, gain, and reassembly of interactions. The colonization of a new region by a lineage may lead to the loss of old interactions or the gain of new ones. For example, in colonizing Madagascar from Africa, *Dalechampia* vines lost their associations with specialized resin-collecting bees and evolved to be pollinated by generalist pollen-feeding insects (Armbruster and Baldwin, 1998). The presumed difficulty of co-dispersal by specialized taxa is one reason that interactions on oceanic islands are often argued to be more generalized than their continental equivalents (Kaiser-Bunbury *et al.*, 2010; see below). The gain of new interactions can be difficult to assess via the fossil record or phylogenetic analysis, but it is frequently observed among invasive species. Introduced Japanese white-eyes (*Zosterops japonicus*) act as pollinators of endemic lobeliad plants (*Clermontia parviflora* and *Clermontia montis-loa*) in Hawaii (Aslan *et al.*, 2014). In the Society Islands of French Polynesia, the endemic gray-green fruit dove (*Ptilinopus purpuratus*) feeds on fruits of the invasive tree *Miconia calvescens* and is likely to be an important disperser of its seeds. In the same archipelago, two invasive birds of Old World origin, the red-vented bulbul (*Pycnonotus cafer*) and the silvereye (*Zosterops lateralis*), feed on fruits of, and may serve as important seed dispersers for, a diverse flora of introduced and invasive trees and shrubs from the New World and other areas outside the native range of these invasive birds

(Spotswood *et al.*, 2012). These examples, although recent and not necessarily yet leading to evolutionary changes, indicate the potential for novel interaction gain and loss following the colonization of new regions.

Interactions can also be maintained or reassembled despite colonization of a new region. For example, the only fungus-growing termites (Macrotermitinae) which have been able to colonize Madagascar from Africa are those which have vertical rather than horizontal transmission of their mutualistic fungi (*Termitomyces*; Nobre *et al.*, 2010). In other cases, interactions can be reassembled even when interacting species disperse separately. Leafflower trees (*Phyllanthus* s. l.: *Glochidion*) are found in continental regions of tropical and subtropical Asia and Australasia. They are pollinated by specialized leafflower moths in the genus *Epicephala*, which actively gather pollen from male flowers, transfer it to the stigmas of female flowers, and then lay eggs in the flowers' ovaries so that their larvae may consume a subset of the developing seeds (Kato *et al.*, 2003). Species-specificity between plant and moth is very high (Kawakita and Kato, 2006). However, dozens of endemic *Glochidion* species are known from the oceanic archipelagoes of Polynesia and Micronesia in the Pacific, some of which are >5000 km from the nearest continents. Co-dispersal is extremely unlikely because a *Glochidion* seed would require at least several years to grow into a mature plant, whereas an *Epicephala* larva would become an adult in a few weeks. However, despite this apparent barrier, *Epicephala* moths and *Glochidion* trees have independently colonized the remote Pacific and reassembled their interaction on each island where they are found today (Hembry *et al.*, 2012). Phylogenetic analysis indicates that not only have *Epicephala* been able to reassemble their interaction with *Glochidion* once, but that they have actually done so multiple times. There have been at least two independent colonizations, differing in age, of south-eastern Polynesian *Glochidion* by *Epicephala* (Hembry *et al.*, 2013a).

Ecological Biogeography of Interactions

Biologists have long remarked that just as different species and clades are found in different parts of the world, different interactions between these species and clades are also found in different parts of the world. However, quantifying these differences and searching for meaningful pattern in the biogeography of interactions has been more challenging.

Latitudinal Gradients in Interaction Types, Specialization, and Interaction Diversity

Since the beginnings of modern evolutionary ecology, it has been argued that major differences exist in patterns of interactions between the tropics and the temperate zones. The classic argument is that interactions in the tropics are more specialized than interactions in temperate or boreal zones (MacArthur, 1972; Janzen, 1973); a corollary is that biotic interactions are more important in the tropics than at higher latitudes (Dobzhansky, 1950; Fischer, 1960). Sometimes these hypotheses are thought to be linked with the well-demonstrated latitudinal diversity gradient, in which species richness increases with decreasing latitude. An ecosystem can pack in more species if each of them has a narrower ecological niche and interacts with a fewer number of other species. Consequently, understanding how biotic interactions and their specialization vary with geography is fundamental not only for understanding patterns of biodiversity and ecology, but to fundamental dimensions of biodiversity. Many estimates of the number of species depend heavily on assumptions about the degree of specialization of parasitic insects, mites, and nematodes and their animal or plant hosts (see Erwin, 1982 and Novotny *et al.*, 2002 for two famously contrasting views).

Objectively assessing patterns of specialization across geographic gradients is, however, hampered by the availability of comparable data from different regions of the world (Ollerton, 2012). As stated above, 'interactions' encompasses mutualism, antagonism, competition, and commensalism, and many of the best quantitative datasets are taxonomically and ecologically limited. Since the turn of the century, however, a number of groups of researchers have sought to empirically test the common assumption that tropical interactions are more specialized. These studies have primarily focused on terrestrial interactions between plants and their pollinators and seed dispersers, and plants and their insect herbivores. Some studies have found support for greater specialization in the tropics; these include plant–pollinator interactions generally (Olesen and Jordano, 2002; Trøjelsgaard and Olesen, 2013), hummingbird–plant interactions (Dalsgaard *et al.*, 2011), and host specificity in herbivorous Lepidoptera (moths and butterflies; Dyer *et al.*, 2007). In contrast, a recent large-scale review found evidence for the opposite pattern, that plant–pollinator and plant–seed disperser interactions are more specialized in the temperate zones than in the tropics (Schleuning *et al.*, 2012). Finally, other studies have suggested no important difference in specialization between the tropics and temperate zones for interactions such as plant–pollinator mutualisms (Ollerton and Cranmer, 2002), herbivorous insects and their host plants (Novotny *et al.*, 2006), and for niche breadth generally (Vázquez and Stevens, 2004). Biotic interactions appear to be stronger or more important on organisms' fitness in the tropics compared to in temperate zones (Schemske *et al.*, 2009), although this comparison is difficult to make quantitatively. Whether specialization is greater in the tropics than in temperate zones, and if so why, is likely to remain an area of active investigation.

Biogeography of Ecological Networks

One emerging area in understanding the ecological biogeography of interactions has been in examining geographic variation in ecological network structure. Ecological interactions within communities (such as food webs, plant–pollinator interactions, and seed–disperser interactions) can be envisioned as interaction networks, in which species that interact are connected via links. The specialization of species within these networks (above) is just one of many topological properties of these networks that may vary geographically. Like assessing geographic patterns of specialization in biotic interactions, this is a major area of recent growth and attention in interaction biogeography.

Cross-system comparisons have revealed that major, qualitative patterns in network structure often do not vary geographically among comparable interactions. For instance, plant–pollinator interactions are generally both 'nested' (consisting of a core of generalists and asymmetric specialists who interact with these generalists) and 'modular' (broken into weakly connected subnetworks, or modules), regardless of the region of the world in which they occur (Bascompte *et al.*, 2003; Olesen *et al.*, 2007). However, a number of studies have begun to assess differences in network structure across geographic regions. Network-level specialization (described above; Dalsgaard *et al.*, 2011; Schleuning *et al.*, 2012) is only one topological property that has been examined and it appears to vary geographically with latitude in pollination and seed-dispersal networks. Other studies on pollination networks have suggested that connectance is lower at high elevations, generalization increases at high latitudes and elevations, modularity increases toward the tropics, and specialization increases on oceanic islands (Olesen and Jordano, 2002; Trøjelsgaard and Olesen, 2013). In contrast, host–parasitoid networks do not show latitudinal patterns after sampling effects are controlled for (Morris *et al.*, 2014). Finally, geologic history – island age, which dictates the maximum possible age of the interaction network on that island – has effects on plant–pollinator network structure in the Canary Islands (Trøjelsgaard *et al.*, 2013; **Figure 1**). Some of these disparate findings, particularly within pollination networks, may be explained by differences in the metrics examined. Specialization can either take the form of asymmetric specialists which interact with generalists, or symmetric specialists which interact with other specialists (Guimarães *et al.*, 2006), and asymmetric specialization has been argued to be important in permitting the positive correlation between niche breadth and latitude (Vázquez and Stevens, 2004). To date, only a few studies have examined spatial variation in network structure, and this is likely to be a major area for future research in the biogeography of interactions.

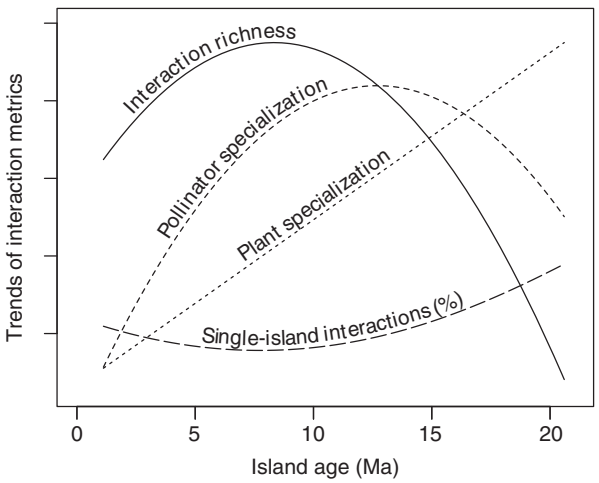

Figure 1 Summary of relationships found between structural properties of plant-pollinator networks (*y*-axis) and island age (*x*-axis), in the Canary Islands, reproduced from Trøjelsgaard, K., Báez, M., Espadaler, X., *et al.*, 2013. Island biogeography of mutualistic interaction networks. Journal of Biogeography 40, 2020–2031. All curves are normalized and *y*-axis values are omitted to show general trends. The different metrics (interaction richness, pollinator specialization, plant specialization, and proportion of interactions restricted to a single island) show differing trends with increasing island age.

Interactions on Islands

Biotic interactions on islands are typically thought to be more generalized than on continents (Carlquist, 1974; Kaiser-Bunbury *et al.*, 2010), although this interpretation has been rarely examined quantitatively and has been challenged by empirical analyses (Olesen and Jordano, 2002). Isolation of islands is thought to reduce successful colonization and establishment by new species in general and by specialists in particular, and the chance of extinction of any species once established on an island is also greater than in a comparable area on a continent (MacArthur and Wilson, 1967; Holt, 2010). These factors are thought to promote the prevalence of generalist species on islands, as well as the evolution of novel generalized interactions not seen on continents (lizards acting as pollinators and seed dispersers on islands being perhaps the most charismatic examples; Olesen and Valido, 2003). Specialized herbivorous insects certainly do exist on even very remote oceanic islands (either via colonization or *in situ* diversification; Hembry *et al.*, 2013b; Bennett and O'Grady, 2012); whether these assemblages are more or less specialized at the species level than on continents remain to be tested quantitatively. Interactions such as those hypothesized to have occurred between the endemic radiations of lobeliad plants and pollinating honeycreepers in Hawaii (Lammers and Freeman, 1986) suggest that given sufficient time and ecological opportunity, unique specialized mutualisms may be able to evolve even on remote oceanic islands.

Biogeography of Coevolution

Although often considered separately from the biogeography of interactions, much recent research in coevolution has focused on geographic variation in the evolution of interactions between different populations of two or more interacting species. Most investigators define coevolution as reciprocal natural selection between two or more species (Janzen, 1980; Thompson, 1994, 2005). The geographic mosaic theory of coevolution (GMTC; Thompson, 1994, 2005) postulates that coevolution between two species varies among their different pairs of populations. Some pairs of populations constitute coevolutionary 'hotspots,' where coevolution is actively occurring, whereas others constitute coevolutionary 'coldspots,' in which only one or neither species is evolving, and thus coevolution is not occurring. This spatial variation in coevolutionary outcomes arises as a result of abiotic selection gradients across the populations, differential gene flow between them, coevolution with different species (or different populations of the same species) among populations, or a combination of these processes.

Empirical tests of the GMTC have found that, as predicted by theory, interactions between two species often vary considerably in their traits and fitness outcomes for each species across their metapopulations. For example, the seeds of

lodgepole pines (*Pinus contorta*) in the Rocky Mountains of North America are fed upon by red squirrels (*Tamiasciurus hudsonicus*) but also by birds called red crossbills (*Loxia curvirostra* complex; Benkman, 1999; Benkman *et al.*, 2001; Edelaar and Benkman, 2006). In most parts of the Rockies, red squirrels are the most important predators of lodgepole seeds, so the cones evolve thick bases to avoid being easily gnawed off branches by squirrels. In isolated mountain ranges without squirrels (the South Hills and Albion Mountains in the US state of Idaho, and the Cypress Hills in the Canadian provinces of Alberta and Saskatchewan), crossbills are the most important predators of lodgepole seeds. Crossbills extract seeds from cones by prying apart scales with their bills while the cones are still attached to the trees. Instead of having thick bases, cones from these mountain ranges evolve thicker scales at the distal ends of their cones so that it is harder for crossbills to pry apart the scales. In these isolated mountain ranges, crossbills evolved deeper, shorter, and more curved bills which are more effective at extracting seeds from the cones available to them (Benkman *et al.*, 2001). Coevolution between crossbills and pines, in concert with both geographic isolation and the evolution of differences in their calls, has even driven these isolated crossbill populations to evolve into new species (Smith and Benkman, 2007). In this example, coevolution between pines and crossbills is actually promoting the speciation of crossbills in these outlying populations. Whether or not geographic isolation, in combination with coevolution, often promotes speciation is poorly understood and little studied, but may be an important future research area in the biogeography of interactions (Hembry *et al.*, 2014).

See also: Biogeography, Ecological Theories in. Biogeography, Evolutionary Theories in. Carbon Relations, the Role in Plant Diversification of. Coevolution, Introduction to. Commensalism, Amensalism, and Synnecrosis. Cospeciation. Dispersal Biogeography. Evo-Devo: Regulatory and Protein-Coding Evolution in Plant Diversification. Geographic Mosaic of Coevolution. Invasion Biogeography. Mutualism, the Evolutionary Ecology of. Predation and Parasitism. Vicariance Biogeography. Water Transport, the Role in Plant Diversification of

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Biogeography of Islands, Lakes, and Mountaintops; Evolutionary

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Overview

Studies of island biogeography are undergoing an invigorating transformation, from primarily ecological investigations founded in the equilibrium theory of MacArthur and Wilson (1967) toward a more general exploration of dynamic processes and patterns that span from generational to geological time (Whittaker *et al.*, 2008). By drawing on distributional, chronological, geological, and phylogenetic relationships among many kinds of data – for example, abundance, disparity, and diversity of alleles, species, and traits – this evolutionary biogeographical perspective (*sensu* Morrone, 2009, p. 1) aims to identify generalities in how ecological and evolutionary processes combine to produce current biogeographic patterns on diverse kinds of islands. While modified forms of the ecological equilibrium theory of island biogeography remain instrumental for investigating variations on extant patterns over short periods, the new general dynamic model links those ecological snapshots and thus animates the processes and patterns of change.

Theoretical Setting

The current theoretical transformation in island biogeography beckons integration of influential hypotheses that have grown up around islands. Relationships between species richness versus distance, distance-decay, and the classic species-area relationship (SAR; MacArthur and Wilson, 1967) – which embody processes such as migration, coupled with extirpation – may be united with evolutionary theories of bottlenecks, founder events, genetic drift, and founder-flush evolution that lead perhaps to peripatric speciation and punctuated equilibrium (Mayr, 1963; Gould and Eldredge, 1977). Evolutionary patterns emerging from ecological principles, such as the island rule, character displacement, adaptive radiation, reduced antipredator strategies, and decreased motility (e.g., Vermeij, 2004; Lomolino *et al.*, 2013) also should find their places within a general dynamic theory of island biogeography. In this article, we provide a brief overview of recent developments in ecological–evolutionary biogeographical research in diverse island situations.

Places and Players

Many kinds of places and organisms show patterns consistent with the aforementioned island theories: arthropods, lizards, and plants on oceanic islands, mammals on mountaintops, microbes in water holes, fishes and jellyfishes in lakes, and so on (**Figures 1** and **2**; Gillespie and Clague, 2009). This diversity emphasizes that island theory is not about a place alone, but rather the interactions between a place that has some level of isolation and a taxon (or many taxa) that shows the effects of that isolation. We explore these interactions using three of the most well-studied places that provide insular environments: islands, mountaintops, and lakes.

'True' Islands

Island theory has been inspired principally by studies of true islands, i.e., parcels of land completely surrounded by water. Their sometimes extreme isolation has led to exceptional biotas and earned them reputations as natural laboratories of evolution (Wagner and Funk, 1995; Losos and Ricklefs, 2009). The ecological–evolutionary dynamics of these, and other insular environments, are influenced by four key geographic characteristics: their mode of origin, isolation, size, and age (**Figure 3(e)**).

True islands are classified, coarsely, as continental or oceanic, based on their *geological origins*. Continental islands include fragments of continental crust or shelf that protrude above water; they may be connected to nearby mainland via land bridges during periods of lowered sea level. Oceanic islands instead have volcanic and/or tectonic origins and formed without connection to any mainland (Whittaker and Fernández-Palacios, 2007, p. 12). These differences influence the dominant processes structuring their biotas. Continental islands – fragments – begin with a substantial complement of mainland species, which after isolation are removed by extinction (or 'relaxation'; Simberloff, 1974), rapidly at first and at decreasing rates thereafter; the subset of species that persist may eventually become island endemics. In contrast, *de novo* oceanic islands begin devoid of life and must be colonized by species, added one-by-one into a newly formed landscape for evolution. The same place may be a different kind of island at different times; for example, a fragment may become a *de novo* island if stripped of its species (e.g., by submergence; Whittaker and Fernández-Palacios, 2007, p. 32). *De novo* islands, especially true oceanic islands, provide a theater for novel ecological and evolutionary diversification and some of nature's most striking adaptive radiations.

Once on an island, populations are subject to evolutionary dynamics that are determined in part by the *degree of isolation*. Islands that are close to the mainland – typically continental islands – will tend to be dominated by repeated colonization and extinction (MacArthur and Wilson, 1967) and influx of alleles from elsewhere. More isolated islands – usually oceanic islands – allow rare colonizers to escape the homogenizing influence of gene flow, thereby favoring the evolution of novel biotas (Losos and Ricklefs, 2009). There is, though, no single geographic distance that distinguishes islands with these

Figure 1 Some of the many species that inhabit islands. (a)–(c): Earthworm mice represent an adaptive radiation that is endemic to mountaintops in the Philippines. The radiation includes at least 50 species, at least 30 of which are restricted to Luzon Island. The species range from 15 to 220 g, including stout-bodied species that burrow through the soil to upright, gracile species that hop about on their hind legs along tiny trails. Up to seven species have been documented as occurring fully sympatrically on the 'sky islands' of northern Luzon. (a) = *Apomys musculus*; (b) = *Chrotomys whiteheadi*; (c) = *Rhynchomys soricoides*. (d)–(i): Representative Hawaiian *Tetragnatha* spiders showing three of four known ecomorphs of the spiny leg on older (Kauai, Oahu) and younger (Maui) islands; the taxa shown form different ecomorphs on the same island that are more closely related to each other than to the same ecomorph on different islands. (d) = *Tetragnatha kauaiensis*, Kauai; (e) = *Tetragnatha perreirai*, Oahu; (f) = *Tetragnatha pilosa*, Kauai; (g) = *Tetragnatha waikamoi*, Maui; (h) = *Tetragnatha kamakou*, Maui; (i) = *Tetragnatha quasimodo*, Maui. (j): Multiple subspecies of golden jellyfish, *Mastigias papua*, inhabit the coastal ocean and marine lakes in Palau. The ocean form (far left) is hypothesized to be similar to the ancestral medusae that colonized lakes after the Last Glacial Maximum and have since been evolving independently for between ~6000 and 15 000 years (second left to far right). (k)–(o): *Anolis* lizards have adaptively radiated on each of the four islands of the Greater Antilles: Cuba, Hispaniola, Puerto Rico, and Jamaica. Similar sets of microhabitat specialists with similar morphological adaptations (ecomorphs) have arisen on each island. All those shown evolved on Cuba: (k) = *Anolis alutaceus,* a grass-bush anole; (l) = *Anolis allisoni*, a trunk-crown anole; (m) = *Anolis mestrei*, a trunk-ground anole; (n) = *Anolis equestris*, a crown-giant anole; (o) = *Anolis angusticeps*, a twig anole.

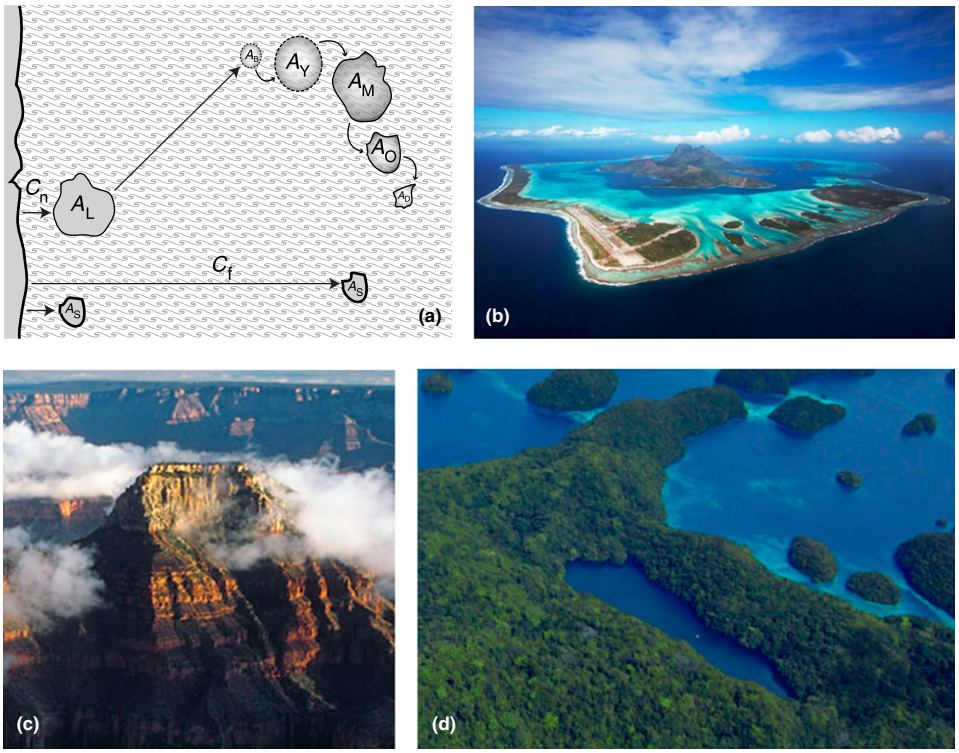

Figure 2 The geographic dimensions of islands, with some examples. (a) The basic geometric properties of islands: their colonization distance, $C_{(\text{near or far})}$, from the nearest mainland and their size which usually is measured as area, $A_{(\text{small or large})}$. These physical dimensions are considered to be causally related to biological characteristics of islands. For example, fewer species are able to travel – i.e., immigrate, I – to more distant islands; smaller area harbors fewer habitats and so fewer individuals, N, and fewer species, S; smaller populations in turn may elevate extinction rates, E; all rates may be species-specific. Intermediate islands may be stepping stones to more distant islands, and infrequently species endemic to islands may recolonize mainland areas. In the classical island biogeography theory of MacArthur and Wilson (1967), the basic physical dimensions are considered to be static and so the biological characteristics in equilibrium. However, the C and A of all islands change through time – for example, due to tectonic or volcanic activity, sea level change – with consequent impacts on I, N, S, and E. Thus, physical stasis and biological equilibrium may exist only as ecological 'snapshots' during a dynamic evolutionary history and future of islands, from birth $_B$, youth $_Y$, maturity $_M$, old-age $_O$, and toward death $_D$. (b) An oceanic island, Bora Bora; the land and reefs surrounding the extinct volcanic peak form gradually as the island subsides into the ocean. (c) A tabletop mountain, Arizona, also known as a mesa or tepui, which erodes through time. (d) A marine lake, 'Jellyfish Lake' in Palau, which formed, deepened, and increased in area after the Last Glacial Maximum as climate warmed, ice-caps melted, and sea level rose; since sea level stabilized ~6000 years before present, the lake has been getting shallower due to sedimentation. (b) http://www.louwphotography.com/South-Pacific/French-Polynesia/Bora-Bora/i-kPfDJT6/A, (c) http://blogs.mycentraljersey.com/arizonaexpedition/files/2013/07/Sky-Islands.jpg, (d) Christoph Gerigk.

effects. The greater a taxon's dispersal ability, the more geographically isolated an island must be to enter what MacArthur and Wilson (1967) called the "radiation zone", in which substantial biodiversity is generated within the island (or archipelago) from one or a few colonizing species (Gillespie and Baldwin, 2010).

The course of evolution in species following colonization of an insular environment is also influenced by *island size*. On small islands, species may undergo anagenetic speciation, evolving into a single new species and contributing to the exceptional endemism of the island biota without increasing species diversity on the island. On large islands, further within-island geographic or ecological separation of populations allows for cladogenesis, during which one species splits into two or more species. As with degrees of geographic isolation, the distinction between small versus large islands is relative, depending upon species' traits, particularly dispersal ability (Kisel and Barraclough, 2010). For example, the size threshold for cladogenesis is less than 100 km^2 for *Bulimulus* land snails on the Galápagos (Losos and Parent, 2010) but at least 3000 km^2 for Caribbean *Anolis* lizards (Losos and Schluter, 2000). Cladogenetic radiation can also occur at the scale of whole archipelagos, even if individual islands are below threshold sizes (Grant and Grant, 2010), because narrow straits separating islands can filter out potential migrants and thus resist gene flow. The ecological opportunities afforded by such islands can stimulate bursts of evolutionary radiation, which may decline through time as available opportunities are increasingly exploited (Mahler *et al.*, 2010).

These effects of origin, isolation, and size all take time to accrue; they may even change with time as the island itself changes (**Figure 3(e)**). Thus, the *age of an island* is the fourth major influence on ecological–evolutionary dynamics on islands. Incorporating island age into oceanic island biogeography is the major novel component in the new General Dynamic Model (GDM; Whittaker *et al.*, 2008). As the island

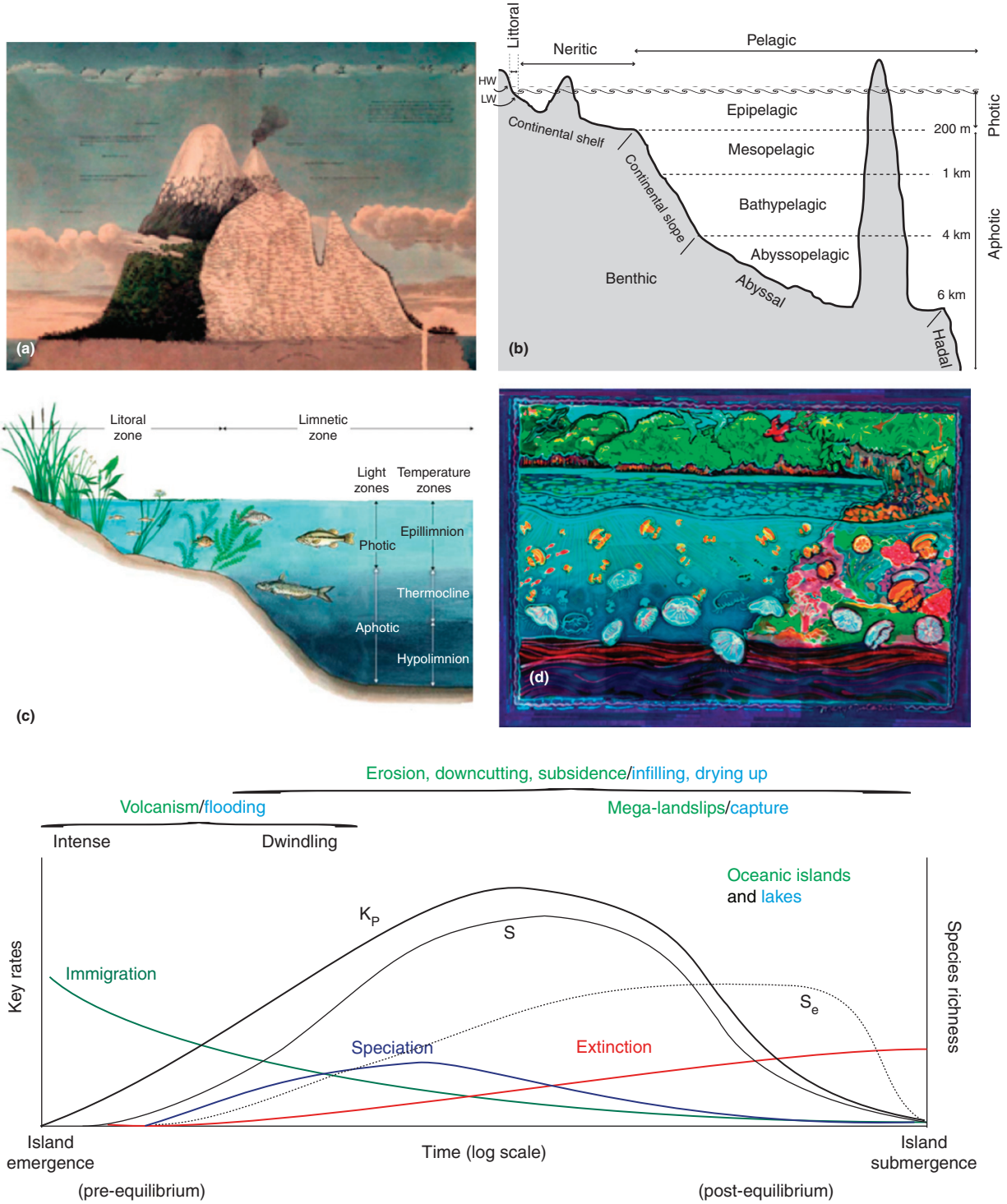

Figure 3 Environmental dimension of islands – horizontal and vertical zonation – and their interaction with time. (a) The classic description of altitudinal zonation of plants by Alexander von Humboldt. (b) Classification of islands by their positions in the sea, with continental or nearshore islands generally on a shallow shelf and distant oceanic islands rising from the ocean floor through multiple 'life zones' to the surface. HW, high water mark; LW, low water mark. (c) Freshwater lakes are similarly structured though typically on much smaller spatial scales, with nearshore littoral versus open-water limnetic regions, and depth zonation based on light, temperature, oxygen, and other factors that determine, and also maybe influenced by, the distributions of various organisms. (d) Marine lakes are structured in essentially the same manner as freshwater lakes, with horizontal and vertical zones being absent or present depending on the size and depth of the lake; the image shows rich eukaryotic life including jellyfishes in well-oxygenated illuminated surface-waters, above anoxic, cold, dark, chemically distinct bottom-waters inhabited by archaea. (e) Schematic of how the properties of islands may change through time according to the General Dynamic Model of oceanic islands; K_p – potential carrying capacity, S – species richness, S_e – endemic species richness (Whittaker *et al.*, 2008). (a) Chicago University Press. (c) http://imgarcade.com/1/lake-zones/. (d) Margo Vitarelli.

life span progresses and area, elevation, and topographic complexity increase, and environments change (e.g., Chadwick *et al.*, 1999), oceanic islands – specifically, those that form above geological 'hot spots' – shift from biotas composed primarily of immigrant species originating from older, nearby, islands, or from outside the archipelago, to those dominated by endemic species that diversified *in situ*. Islands that form along plate margins along subduction zones may persist much longer and grow to larger size, providing the opportunity for even greater speciation within the island or archipelago. The fates of islands and their biotas, though, may differ. Hot-spot islands will erode, subside, and their unique biotas disappear. Plate-margin islands more often will collide with, and become a permanent part of, the adjacent continent, which may reduce or provide new opportunities for the 'island' taxa.

The GDM provides a general framework linking the geological, geographical, ecological, and evolutionary dynamics of at least some 'life-stages' of many islands, especially oceanic islands. The geological time scale of the GDM, however, prohibits direct observation of evolutionary dynamics throughout a single island's life span. Moreover, generally poor fossil records on oceanic islands mean historical dynamics often cannot be documented retrospectively. Thus, most tests of the GDM depend on volcanic hot spot archipelagos – such as the Canaries, Galápagos, Lesser Antilles, and Hawaiian islands – that are composed of islands of widely varying age which can act as substitutes for different stages in the island life span. While some such studies find results consistent with predictions of the GDM, for example, that total and endemic species diversity should be a positive function of area and a hump-shaped function of island age, a number of exceptions suggest that not all archipelagoes follow the idealized GDM (**Figure 3 (e)**; Cameron *et al.*, 2013). Why islands, archipelagos, and lineages vary in their agreement with the GDM is a question ripe for both theoretical and empirical exploration. Fortunately, the occurrence of many archipelagos and islands around the world means that these natural laboratories often are replicated, providing opportunities to robustly test ecological–evolutionary hypotheses and to explore both the causes of shared patterns and also the reasons for unique outcomes.

Mountaintops

True islands have analogues in other terrestrial situations, including 'sky islands' and tepui. With increasing elevation, conditions tend to become cooler and wetter, often resulting in the presence of biological communities on mountaintops that are separated from each other by long distances and distinct communities living in warmer, drier conditions below.

That high elevation species often are composed of multiple separate populations isolated on various mountaintops was described first in Alexander von Humboldt's ground-breaking 1807 documentation of plant zonation on mountains in the Canary Islands and in the Ecuadorian Andes. That altitudinal 'life zones' or biomes exist sequentially up mountains was clarified by C. Hart Merriam in 1890, and the term 'sky island' was coined in the early 1950s and came into common usage by the 1990s (Warshall, 1995). Today, the term and concept of sky islands is applied broadly, to forested mountains in deserts, to the flat-topped steep-sided plateaus in northern South America called tepuis, to patches of cloud forest at the tops of the eastern arc mountains of Tanzania, and volcanic peaks in the Philippines; the term might also describe the tops of guyots and seamounts which are surrounded by deep ocean. As with true islands, the geological and biological history of each sky island greatly influences its species richness, level of endemism, and biological dynamics, and much variation in these and other factors is evident. However, because sky islands are connected by other terrestrial habitats through which at least some organisms can migrate, they have become known as 'habitat islands' rather than 'true islands' although these categories may have limited rhetorical value long term.

One of the first quantitative analyses of mountaintop biogeography employed extensive information from previous surveys of mammals on mountains in Utah and Nevada (Brown, 1971). As predicted for true islands, there was a strong correlation between mountaintop habitat island area and the number of species of small mammals that were montane specialists. However, there was no evidence for an effect of distance from a larger montane forested area – i.e., the degree of isolation from a montane analogue of 'mainland' was unimportant – indicating that modern mountaintop communities are fragments of formerly widespread communities that inhabited lower elevations in the western United States during the Last Glacial Maximum (LGM). That is, sky islands form the equivalent of continental shelf islands: in the absence of colonization, the biotas are gradually depleted by extinction, particularly on small sky islands where small populations are especially vulnerable. Moreover, sky islands may not be isolated in the same way as oceanic islands because surrounding lower elevations are inhabited by generalists such as bears and coyotes that often wander up to high elevations and may impact the dynamics of the small montane mammals and other organisms. The role of highly mobile organisms in modifying 'island' dynamics also has been emphasized for island-like marine habitats such as seamount-tops and reefs that are visited by itinerant sharks (Vermeij, 2004). It is now apparent that at least some colonization by some montane species probably has taken place since the LGM, offsetting relaxation in part. Conversely, studies of fossils show that the current absence of other species may be due to continued absence preceding the LGM rather than evidence of extinction since the LGM, as was initially assumed. While Brown's (1971) studies of sky islands of Utah and Nevada have become pivotal examples of nonequilibrial island dynamics and a model for investigations of other organisms on mountains in other parts of the world, subsequent research has shown that the development of current patterns was more complex than initially envisioned.

Nonequilibrial dynamics and long-term processes, especially evolution, as noted in the previous sections, are now some of the primary factors shaping new developments in island theory. Studies of mountaintops remain at the forefront of these developments, perhaps because they provide so many varied situations. For example, sky islands in North America provided a complex geographical and dynamic climatic landscape during the Pleistocene that may have promoted rapid speciation in *Melanoplus* crickets (Knowles, 2007). Tropical

mountain chains on continents and large islands provide situations in which speciation may contribute more to species richness than direct colonization, emphasizing that an evolutionary perspective on the dynamics of species richness is essential (Graham *et al.*, 2014). Furthermore, it becomes apparent that 'speciation' on islands can mean many things. For example, high elevation cloud forest on the Philippine island of Luzon supports at least 30 species of an endemic clade of 'earthworm mice' (which specialize in feeding on earthworms and other soft-bodied soil invertebrates) descended from an ancestral species that likely colonized lowland regions of the island about 8 million years ago. Speciation of the extant earthworm mice subsequently took one-to-several millions of years, and in some cases isolated mountaintops each host one of several closely related species indicating topographic isolation led to allopatric speciation, while in other cases a single sky island may host up to seven distantly related species in sympatry evincing multiple colonizations and character displacement in body size and feeding niche (Heaney *et al.*, 2016). These kinds of patterns have, in turn, led to recognition that the geological dynamics of mountain topography may change on timescales similar to those for the evolution of species. Thus, as in the GDM of oceanic island biogeography, the geological history of mountains is an essential component of conceptual models that attempt to describe long-term aspects of biodiversity on sky islands.

Lakes

Like islands and mountaintops, lakes come in a variety of sizes, shapes, types, and ages. Lakes are defined as depressions $\geq$2 ha filled with water – fresh, saline, or marine – that may persist for over 20 million years (Bengtsson and Herschy, 2012), although most are much shorter-lived. Lakes thus seem to offer similar potential for a range of ecological and evolutionary dynamics as evident on true islands and sky islands and represented in the GDM.

The majority of studies of lakes have been conducted in northern temperate freshwater systems which, like mountaintops, show mixed support for patterns predicted by equilibrium island theory. For example, meta-analyses indicate species richness increases with lake area (Arnott, 2009), probably as an effect of habitat heterogeneity, but a low effect size suggests that the lakes are not highly isolated. The slope of the SAR also is influenced by body size of the study taxa, which may be a proxy for dispersal ability, and thus the proximity of lakes influences patterns of connectivity and explains $\sim$20% of the variance in plankton community similarity. A similar percentage overlap in zooplankton community composition is explained by lake environment, presumably reflecting ecological filtering and natural selection (e.g., Arnott, 2009). Altogether, through time, the degree of isolation, the size of a lake, and the characteristics of the inhabitants each likely contribute to the formation – or lack thereof – of endemic populations, species, or species-flocks. Geologically young postglacial North American lakes may include only one or a couple of ecomorphs of sticklebacks (Schluter and McPhail, 1992), whereas ancient African Rift Lakes include many hundreds of species of cichlid fishes (e.g., Wagner *et al.*, 2014).

Some recent studies of tropical saline lakes have added a perspective from the underrepresented and by some metrics quite different marine realm (**Figure 1(d)**). Intriguingly, these marine lakes show many of the standard island patterns, but also share some of the increasingly common deviations from equilibrium theory. Modern marine lakes formed *de novo* after the LGM as rising sea levels forced water and propagules into low-lying inland valleys between $\sim$6000 and 15 000 years ago. Connections with the surrounding ocean 'mainland' still exist via underground tunnels and fissures, and the isolation of a lake likely is a function of interactions between the geographic and physical characteristics of a lake and the traits of potential colonists. Marine lakes show classical SAR and distance-richness curves in marine invertebrates, and the more isolated marine lakes harbor genetically isolated populations, subspecies, and species currently unknown elsewhere. However, phytoplankton have a flat or negative SAR, and population genetic and evolutionary analyses indicate nonequilibrial dynamics such as genetic drift during establishment and subsequent selection that generates novel morphologies and behaviors which change through time (Dawson and Hamner, 2005). Thus, populations in marine lakes show patterns reminiscent of common evolutionary trends in species inhabiting oceanic islands, including reduced swimming speed which mirrors reduced flight in birds and some insects, reduced size relative to ocean ancestors consistent with the 'island rule' (Lomolino *et al.*, 2013), and reduced coloration and vestigial morphology consistent with diminution of antipredatory devices associated with reduced predation in island settings (Vermeij, 2004). How such microevolution influences community processes through time and thus the form of the GDM currently is unclear.

Of special note, then, is that some lakes harbor rich sediment records that may allow direct tests of the GDM and evolutionary island theories. In lakes, dead or dormant organisms sink to the bottom where they may be preserved, and perhaps fossilized, in chronological sequence. By mining through these sediments, community composition, ancestral states, and anagenetic change can all be observed directly thus revealing actual evolutionary and biogeographic patterns through time, rather than relying on space-for-time substitutions using 'modern analogs.' For example, fossils in a Miocene lake reveal sequences of armor reduction in incumbent *Gasterosteus doryssus* sticklebacks, then invasion and replacement by a new highly-armored lineage which itself subsequently evolved reduced armor (Bell *et al.*, 2006; Hunt *et al.*, 2008). This paleontological succession parallels the biogeographic inference, from modern populations of *Gasterosteus aculeatus* (threespine stickleback), of multiple marine-to-freshwater colonization events and the *in situ* evolution of distinct ecomorphs including some with reduced armament (Schluter and McPhail, 1992; Schluter, 2000). Recent lake sediments also promise integration of ecological and evolutionary island biogeography through exploitation of seed banks and subfossils that permit 'resurrection' of putatively ancestral forms in modern experiments or paleogenomic analyses (Orsini *et al.*, 2013). Testing for repeated patterns in lake species, like repeated patterns in *Anolis* lizards and *Tetragnatha* spiders on true islands (Gillespie, 2004; Losos, 2009), will indicate the extent to which the ecological–evolutionary

characteristics of insular habitats are generalizable and therefore the possible explanatory power of the GDM in 'true' and other island situations.

Outlook

True islands, lakes, and mountaintops in some ways appear among the most different kinds of places. Yet they share key attributes such as clear bounds in space and through time, a degree of isolation, and oftentimes a simplified ecosystem; each may form *de novo* or by fragmentation (as may other kinds of islands). These 'natural experiments' will be invaluable to biogeography in the future, as in the past, because islands and island-like systems provide unparalleled opportunities for studying the many interacting mechanisms that influence ecological–evolutionary processes and determine general patterns of diversity. Islands and island-like systems also will be indispensible in providing numerous variations on those general themes that enable interactions to be deconstructed, reconfigured, and explored. In time, we suggest biogeography may distinguish places not in terms of whether they are true islands, mountaintops, or lakes, nor perhaps on the basis of their particular geographic location, nor the major branches of the tree of life to which they are home. Rather, biogeography will distinguish places to the extent they differ in a suite of ecological–evolutionary mechanisms (Figure 4) that shape the suite of organismal traits and ecosystem processes that are present at a particular place and time.

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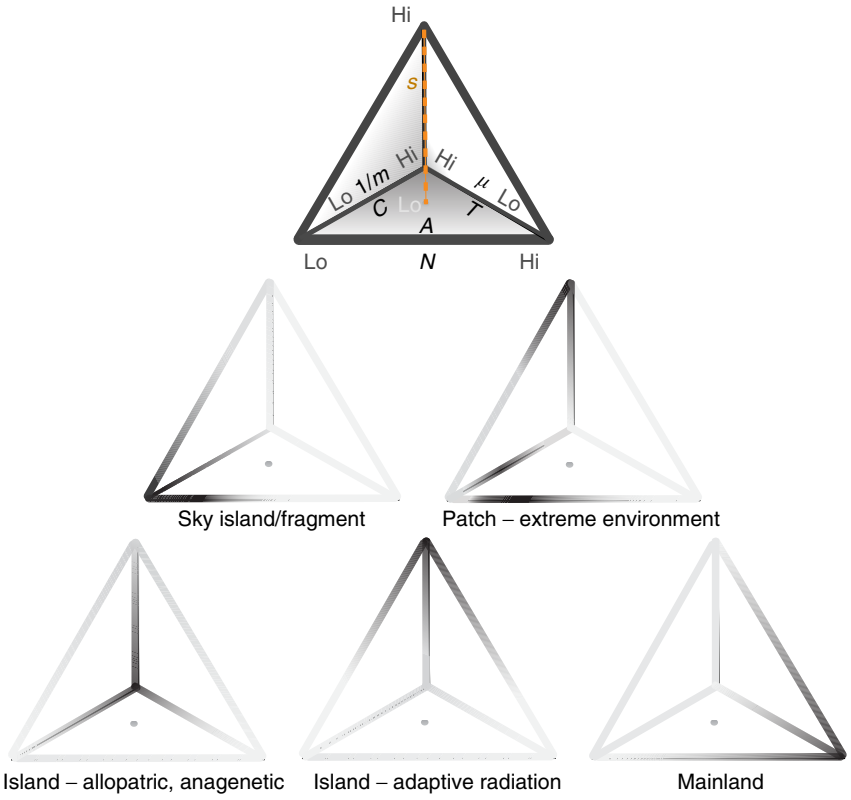

Figure 4 A conceptual diagram illustrating the biophysical nature of islands, island-like habitats, and mainland. 'Top'*:* A pyramidal volume represents interactions among the three key geographic characteristics of islands – colonization distance (*C*), area (*A*), and age (*T*, for time) – and the four principal mechanisms of evolution: migration (m), mutation (μ), genetic drift (a function of population size, *N*), and selection (s). The axes can be shaded to indicate the theoretical parameter spaces that describe various biogeographic situations. 'Middle row'*:* For example, patchy distributions may occur when habitats are recently isolated, as in the case of sky islands and continental islands, leading to small population size, ecological drift, and extirpations. Alternatively patches may result from extreme environments wherein strong natural selection counteracts modest levels of movement. In neither case has the habitat or population persisted sufficiently long to differentiate into an insular form. 'Bottom row'*:* Small islands that are remote, i.e., sufficiently distant to exceed the usual dispersal potential of a species, may harbor endemic species that evolve anagenetically in allopatry (or peripatry). Larger islands may foster adaptive radiations. The framework applies equally to true islands, mountaintops, and lakes, thus facilitating conceptual and quantitative comparisons among types of island. Note that an island may move through this parameter space with time, for example from a recently colonized island (left middle), through anagenetically evolving peripatric population (left bottom), to an adaptive radiation as the island size and habitat heterogeneity increases (center bottom).

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See also: Biogeography, Evolutionary Theories in. Biogeography, Patterns in

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Biogeography of Vertebrates

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Glossary

Adaptive radiation The evolution of ecological and phenotypic diversity within multiplying lineages, usually associated with the rapid production of high species diversity.

Ecological opportunity As organisms colonize new areas and encounter environments (islands, habitats) in which competitors or predators are absent, or in which new food resources can be exploited, novel adaptive processes can dive the evolution of new species as a response.

Gondwana The supercontinent, formerly composed of today's Africa, South America, India, Antarctica, Australia, Arabian peninsula, New Zealand, Madagascar, New Guinea, and other smaller landmasses.

Tetrapods The four-limbed vertebrates and descendants, including the living and extinct mammals, birds, amphibians, reptiles, and some extinct fishes.

Vicariance Passive divergence of evolutionary lineages, resulting from the formation of a barrier or geological processes; the splitting of formerly united species distributions via continental fragmentation and drift.

Biogeography is the study of the evolution of geographical distributions of organisms, a topic that has intrigued and bewildered theologians, philosophers, and biologists for thousands of years. We can trace the origins of biogeographical thought to early Greek philosophers like Plato (428–348 BC) and Aristotle (384–322 BC) and, eventually, to scholars like Linnaeus (1707–78) who first articulated theoretical concepts of the nature of species (*Essentialism* and *Typology*) and then logically asked why certain species occupied particular places. A century prior to the publication of Darwin's and Wallace's formulation of the idea of organismal evolution by natural selection (Darwin, 1859; Wallace, 1860), Georges-Louis Leclerc, Comte de Buffon (1707–88) clearly articulated the fundamental principles of natural selection but deliberately deemphasized their significance in light of societal and religious conventions of his day (sixteenth century Europe). Buffon observed distributions of extant organisms but also studied fossils, leading him to the inevitable conclusion that life changes with time, and varies geographically, often inhibited by physical barriers. Based on his observations, Buffon issued what has become known as Biogeography's First Principle (or 'Buffon's Law'), the idea that environmentally similar (but isolated) regions of Earth have distinct assemblages of animals. This simple observation, sometimes summarized as *The Uniqueness of Place* (Lomolino *et al.*, 2010), became so influential that, in large part, it spawned an Age of Exploration and more than a century of worldwide travel and discovery by several generations of European cartographers, mariners, botanists, and zoologists. Although the first generation of these explorers was primarily composed of botanists, or *Phytogeographers*, vertebrate zoologists soon joined their ranks and the field of *Zoogeography* (biogeography of animals) was born.

At the intersection of numerous physical sciences relating to both life and the geographical template on which it has evolved (Lomolino *et al.*, 2010), the field of biogeography addresses fundamental responses of life to the spatially variable biotic and abiotic environment. As the focus of many biogeographical hypotheses, physical barriers, environmental gradients, atmospheric and temperature extremes, seasonal, latitudinal and elevational variability, and interactions with other organisms are often key to understanding both the extent and limits of species distributions. On land, vertebrate animals respond in a wide variety of ways to environmental limiting factors, geographic barriers, and the presence of other species. The author's purpose here is to review a small subset of the many ways land vertebrates have responded – and continue to respond – to the geographic template. The author will discuss several bodies of work, which have influenced the development of the field in important ways. However, although biogeography might once have been viewed primarily as an historical science, it is now characterized by the melding of fundamental macroevolutionary and macroecological principles, their associated bodies of theory, schools of thought, and massive amounts of empirical data, now rigorously evaluated in an increasingly integrative, hypothesis-testing framework (Ladle *et al.*, 2015). Most of these biogeographical elements became so influential as a result of the careers, big ideas, and hard work of scores of individuals, working in disparate disciplines. Thus, students of biogeography necessarily must constructively gather information from a variety of sources to glimpse the 'big picture'; it is only relatively recently that textbooks with titles including the term '*Biogeography*' have been published (e.g., Lomolino *et al.*, 2010; this volume). To understand the discipline's foundational concepts, the reader is referred to these texts as a first reference, but then necessarily to the primary literature in geology, geography, earth history, phylogenetic systematics, ecology, and evolution.

The First Vertebrates

Vertebrates first appeared approximately 500 million years ago, during the Cambrian explosion. The first forms were fish-like jawless marine organisms with cartilaginous internal skeletons, and bony, plated, external armor; these were the Ostracaderms. True fish, with bony internal skeletons, and

eventually functional jaws, first evolved more than 450 million years ago in the Ordovician, becoming quite common in the Devonian. These early fish, the Gnathostomes, possessed moveable opposing jaws, teeth, paired appendages, and a modern vertebrate immune system. The development of an opposable and functional grasping jaw (derived from anterior gill support arches), and many additional associated feeding specializations, was a major advance that occurred in the Gnathostome fishes approximately 420 million years ago and allowed these organisms to exploit a broad array of feeding environments, and evolving into possibly as many as 65 000 to 75 000 species (Westneat *et al.*, 2005; Pough *et al.*, 2009), including the Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) which are further divided into the Sarcopterygii (lobe finned fish) and Actinopterygii (ray finned fish) of today. The lobe finned fishes include fossil and extant lungfish and coelacanths that we are familiar with today, but it is a pair of fossil taxa, *Eusthenopteron* and *Panderichthys*, that appears most closely related to the series' early tetrapods (e.g., *Tiktaalik*, *Acanthostega*, *Ichthyostega*, *Tulerpeton*; 390–360 mya) that made the evolutionary transition from an entirely aquatic lifestyle to an at least partially terrestrial, weight bearing, upright land vertebrate (Radinsky, 1987; Laurin, 2010).

Vertebrates invaded land at about 350 million years ago, giving rise to many forms we know from extant lineages and extinct fossils; possibly as many as 70 000 vertebrate species have been formally characterized (with tens of thousands more implied by trace fossils and other evidence). The next 10 million years was a period of major evolutionary radiations of amphibians, giving rise to two large groups, the large and robust labyrinthodonts and their slender, delicate relatives, the lepospondyls. Much of the initial Carboniferous and Permian fossil amphibian diversity went extinct at the end of the Permian (although a few lineages survived into the Triassic and one into the Jurassic), and a variety of hypotheses exist to explain how these dominant and diverse forms are related to the amphibians of today (the Lissamphibia: frogs, salamanders, and caecilians; Duellman and Trueb, 1994; Marjanović and Laurin, 2007; Sigurdsen and Green, 2011). Approximately 50 million years later, a lineage of labyrinthodonts evolved the amniotic egg and the ability to reproduce away from water. Together with the evolution of flexible scales composed of keratin, early reptiles were equipped to invade the interior of landmasses, arid environments (Laurin and Reisz, 1997).

Because of the timing of their initial diversification on land, terrestrial vertebrate diversification was initially impacted by the breakup of Pangaea and Gondwana (**Figure 1**), processes that resulted in isolation and unique early radiations of land vertebrates on individual landmasses. At 213 million years ago, the Triassic–Jurassic extinction event resulted in the loss of the labyrinthodont amphibians and most marine reptiles, leaving the majority of terrestrial vertebrate lineages limited to dinosaurs, who subsequently radiated evolutionarily into many of the vacant niches left empty following these extinction events.

As landmasses moved farther and farther apart, their vertebrate faunas became increasingly distinct taxonomically, and biogeographic patterns can be related to habitat types and paleoclimates to the extent that these can be reconstructed. The assumption that early vicariance, or cladogenetic divergence as a result of geological processes (splitting of formerly united species distributions via continental fragmentation and drift) primarily explained dinosaur distributions (Sampson *et al.*, 1998) was challenged by Sereno (1999), who suggested that local extinction and dispersal may have been major 'controls' of dinosaur land distribution, bringing into question the issue of whether a truly cosmopolitan dinosaur fauna ever existed. However, Upchurch *et al.* (2002) demonstrated that major vicariant divisions of dinosaur fauna were all a result of (1) the separation of Asia from Pangaea in the Early–Mid Jurassic; (2) the fragmentation of North America from Gondwana; (3) the separation of Australia (formerly eastern Gondwana) from Africa + South America (western Gondwana); and (4) the fragmentation of Africa from South America during the Early–Mid Cretaceous. Meanwhile, the signature of intercontinental dispersal and subsequent diversification becomes apparent in the fossil record and numerous examples of dinosaur dispersal have been documented (Rowe *et al.*, 2011; De Queiroz, 2014).

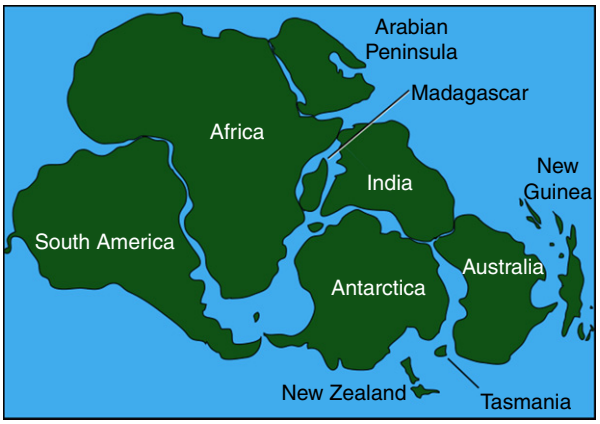

Figure 1 The breakup of the supercontinent Gondwana, 200–180 million years ago, with the outlines of today's major landmasses indicated. Adapted in part from Chatterjee, S., Goswami, A., Scotese, C.R., 2013. The longest voyage: Tectonic, magmatic, and paleoclimatic evolution of the Indian plate during its northward flight from Gondwana to Asia. Gondwana Research 23, 238–267.

Following the Cretaceous–Tertiary (K–T) asteroid impact at 66 mya, the loss of two-thirds of all Earth's diversity, and the extinction of all non-avian dinosaur diversity, a small group of terrestrial vertebrates (small mammals, birds, reptiles, amphibians) remained. In the absence of dinosaur and Mesozoic reptile dominance, birds and mammals soon flourished and occupied the majority of terrestrial niche space. The Paleogene (66–23 mya) marked a period characterized by the onset of many classic terrestrial mammal evolutionary radiations and biogeographic range expansions, presumably in response to the ecological opportunity presented by widespread extinctions of the dinosaurs. These include the sudden and prolific diversification of many clades of today: whales, bats, horses, and primates (Alroy, 1999). Extinctions were selective in some clades in particular areas. For example, marsupials largely disappeared from North America, and Laurasian

multituberculate mammals (a large group that had survived the previous 120 million years) went extinct, as did southern hemisphere Gondwanatherian mammals. Chiropterans, marsupials, and Cetartiodactyla (whales and even-toed ungulates), in contrast, may have diversified and expanded their global distributions in response to the K–T extinction (Bininda-Emonds *et al.*, 2007).

Plate Tectonics, Continental Drift, and the Paleotransport of Vertebrates

As discussed above, plate tectonics, rift formations, and the breakup of Pangaea, Laurasia, and Gondwana (**Figure 1**) had profound impacts on the distribution of a wide variety of early land vertebrate lineages. These tectonic events initially structured vertebrate assemblages, rendering land vertebrate species pools unique and identifiable on a continental scale. For example, as the southern continents successively fragmented, the taxonomically and biogeographically unique faunas became isolated and developed their own faunistic identities as a result of millions of years of isolation coupled with *in situ* evolution and faunal exchanges facilitated by plate collisions and landmass accretions. *Mesosaurus*, for example, was one of the first aquatic reptiles inferred to be a coastal habitat specialist, documented in fossil beds of southern Africa and eastern South America. Part of the celebrated '*Glossopteris*' floral/faunal assemblage, including *Lystrosaurus* (with an Indian, African, and Antarctica distribution) and *Cynognathus* (distributed in South America and Africa), the group has been cited as direct evidence in support of the hypothesis that the southern continents were once joined and that Gondwanan vicariance led to the primary patterns of geographical distribution in terrestrial vertebrates (Laurin, 2010).

Phylogenetic analyses of extant taxa have also been used to provide inference into the question of continental fragmentation and drift as a major initial causal factor for understanding land vertebrate distributions. The lungfishes (Lepidosireniformes) of South America, Africa, and Australia might appear to be an ideal group with a classic Gondwanan distribution; however, recent fossil evidence suggests that this group was formerly widely distributed, with a modern day distribution resulting from widespread extinction subsequent to the breakup of the supercontinents. Despite the many reinterpretations of previously assumed Gondwanan land vertebrate distributions, paleotransport of vertebrates on drifting continental fragments continues to be inferred in modern phylogeny-aided biogeographical inference and involving younger time scales (Bossuyt and Milinkovitch, 2001; Bossuyt *et al.*, 2006; Gamble *et al.*, 2008; Blackburn *et al.*, 2010; Siler *et al.*, 2012; Brown *et al.*, 2016). In a recent review and 'event-based' biogeographic analysis, Sanmartin and Ronquist (2004) demonstrated the surprising result that animals more closely followed order-of-Gondwanan landmass fragmentation predictions than did plants. Other single-taxon analyses have uncovered the apparent result of pure Gondwanan vicariance as a result of the tectonic fragmentation of former supercontinents. For example, Turner (2004) interpreted a phylogenetic analysis and documented distributions of crocodyliform taxa (notably, including both fossil and extant taxa) as evidence for pure Gondwana fragmentation affecting the primary diversification of a major class of vertebrates during the Mid to Late Cretaceous (see also Gamble *et al.*, 2008, for a similar example in geckos).

Vicariance and Dispersal: Theory versus Data

The influential biogeographer Léon Croizat is noted for his unwavering faith in the idea that most of the Earth's disjunct distributions could be explained by the breakup of former supercontinents, leading to isolation, divergence, and the formation of new lineages (vicariance). He famously summarized this with his dictum: "earth and life evolve together" (Croizat, 1962). Motivated and inspired by some of the Planet's most celebrated disjunct distributions, Croizat made major advances in Panbiogeography as he strove to explain shared patterns of endemism and provincialism. How could closely related taxa (species, genera, families) come to occupy separate and isolated geographical ranges on landmasses on either side of the world's major ocean basins? Operating in the absence of rigorous phylogenetic methods or today's sophisticated and time-calibrated estimates of evolutionary relationships, but with an appreciation of the emerging understanding of plate tectonics, Croizat was led to the conclusion that disjunct distributions were the direct evidence of vicariance. Focusing on patterns of endemism and disjunction shared by many unrelated organisms with a simple procedure he termed 'track analysis,' designed to summarize these instances of shared disjunctions, Croizat was convinced that virtually all groups of terrestrial plants and animals arrived at their current distribution by paleotransport on the fragments of former supercontinents. At the time, the standardization of a formal, repeatable method for analyzing distributions was novel and represented a substantial advance over the descriptive and taxon-by-taxon, idiosyncratic biogeographical studies that preceded. Croizat's work also heavily influenced the development of the school of Vicariance Biogeography (Nelson, 1973; Nelson and Platnick, 1981), a dominant perspective in the field for more than 30 years. According to this philosophical perspective, dispersal was virtually dismissed as exceedingly rare or improbable, barely considered except in passing, with the study of dispersal-related biology even ridiculed as being 'unscientific' and considered only by researchers who were prone to fantasy. In much the same way that we now look back at the period in the history of biogeography when workers inferred hypothetical landmasses (such as land bridges) on the basis of current day species distributions as overly simplistic and naively misdirected, an emerging mainstream of biogeographers similarly view Croizat's Panbiogeography and the Vicariance Biogeography school as an interesting but peculiar period in the history of the discipline. In retrospect, how could we possibly have dismissed the possibility (and ignored the evidence) that organisms do naturally disperse long distances, even across massive geographical barriers such as mountain ranges, deserts, and oceans (de Queiroz, 2005; Gillespie *et al.*, 2012)? As Lomolino *et al.* (2010) discuss, the period during which these ideas had mass appeal (1960s through 1980s) marks a time of great sociological and philosophical debate in systematics: a time

in which the impact of many novel contributions was somewhat diminished by strong, domineering personalities, who aggressively pursued winning in debate in order to promote their beliefs. Vertebrates, especially those embodying relative dispersal ability – amphibians, flightless birds, freshwater fish, etc., – figured heavily into this heated discussion.

Patterns: Endemism, Provincialism, Cosmopolitanism, and Disjunct Distributions

The search for explanations of biogeographic patterns – emergent properties of species distributions – inspired the first true biogeographers and contributed directly to the conception of evolution by natural selection (Darwin, 1859; Wallace, 1860, 1876, 1881). As the nineteenth century drew to a close and the newly acquired species distribution data from the age of exploration became available, vertebrate biologists noted a series of conspicuous shared patterns of species distributions. The 'Father of Biogeography,' A. R. Wallace, and noted vertebrate naturalist P. L. Sclater, are generally credited with having defined the major terrestrial biogeographical regions or 'realms' of the planet, among many other distribution patterns (Wallace, 1860, 1876; Sclater, 1858). Chief among these were the recognition of vertebrate endemism, biogeographical provincialism (or regionalism), and range disjunctions, all at varieties of scale.

The discovery and recognition of mammal, bird, amphibian, and reptile species with geographical distributions limited to a particular landmass like a small island (and not occurring anywhere else on Earth) is compelling evidence for the importance of historical events (such as colonization of a far away island by a vertebrate species) and the contribution of isolation to the processes of speciation. For example, the observation that the primate species the Red Slow Loris (*Loris tardigradus*) occurs only on Sri Lanka, that Melanesian Forest Frogs (genus *Cornufer*) are largely endemic and confined to islands of the Southwest Pacific, or that kangaroos and wallabies (marsupial mammals of the family Macropodidae) are limited primarily to the Australian continent, are all examples of the way in which varying taxonomic levels exhibit endemism at different scales. These examples contrast strongly with the nearly worldwide distribution of bats (order Chiroptera), a true case of cosmopolitanism in a terrestrial vertebrate species.

When many species or even an entire biota are influenced by such historical events, the resulting concordant patterns of endemism are often referred to as *biogeographical provincialism* or *regionalism*. Patterns of endemism are concentrated nonrandomly and usually are associated with particular areas. This co-occurrence of many endemic forms is sometimes also associated with particular biotic characteristics. Provincialism is a pattern that informs biogeographers about the significance of geographic barriers and their impact on numerous related and unrelated species. To return to the examples provided above, biogeographic provinces have been defined by isolation of biotas on islands (Bossuyt *et al.*, 2006). As a result of isolation, 12 named primate species or subspecies are endemic to the island of Sri Lanka, for example, and occur nowhere else on Earth (Nekaris and de Silva Wijeyeratne, 2009). Similarly, 95 + % of amphibians native to the Southwest Pacific's island archipelagos (the Solomon Islands, the Bismarcks, Admiralties, and Fiji) are endemic to those landmasses (AmphibiaWeb, 2015) and 90 + % of Australian mammals exhibit concordant distributions, primarily endemic to this landmass (Nowak, 1999).

Empirical Insights into the Biology of Long-Distance Vertebrate Dispersal

Well-known disjunct distributions in which vertebrate geographical ranges are discontinuous and usually divided by a substantial geographical barrier have inspired some of the most conceptually intriguing biogeographical studies and the discipline's most fervent debates. At the heart of these heated debates have been two basic alternate biogeographical hypotheses, namely the interpretation of biotic disjunctions as necessarily the result of ancient vicariance versus the possibility that these patterns lend support for the hypothesis of recent dispersal (e.g., Yoder and Nowak, 2006; Sanmartin and Ronquist, 2004). A third possibility in some systems, the interpretation of a formerly more widespread distribution, followed by extinction of populations or species in intermediate areas, has been invoked in terrestrial vertebrates exhibiting disjunct distributions on continents where climate change associated with glacial retreats caused shifts in major habitat types (e.g., mesic versus xeric forests; Nielson *et al.*, 2001).

The mechanisms of dispersal over marine barriers vary, and some authors have emphasized organismal or ecological traits that render certain vertebrates more prone to active or passive transport (Carlquist, 1965, 1966). For vertebrates capable of surviving exposure to salt water and sun, dispersal is achieved by simply floating and traveling passively with currents (Gerlach *et al.*, 2006). For land vertebrates of low relative dispersal ability and salt tolerance like nonvolant mammals, amphibians, or freshwater fish, the common assumption is the transport of animals on natural flotsam associated with strong storms. These include floating islands (large portions of river banks that have broken free, and floated out to sea), mats of vegetation, and/or log jams and tangled masses of tree trunks, which are often discharged from river mouths following flooding on land (King, 1962; Krause *et al.*, 1997). The occurrence of floating debris fields, coupled to the discharge of large amounts of freshwater, can create stratified freshwater 'lenses' (layers) of freshwater that form above denser salt water, and create temporary freshwater currents – all of which may favor the transport of salt-intolerance terrestrial vertebrates. Such mechanisms have been either directly observed (Censky *et al.*, 1998) or inferred by testing predictions with storm track data, localized flow patterns in the vicinity of river deltas, information from prevailing ocean currents, and phylogeographic data (Measey *et al.*, 2007; Bell *et al.*, 2015). The key to understanding the plausibility and importance of these supposedly rare or 'improbable' events is the simple fact that even seemingly unlikely phenomena may become easier for us to consider when their 'occasional' successful occurrence is multiplied by many millions of years (De Queiroz, 2014).

Several developments have contributed over the last three decades to the unraveling of the dominance of the Vicariance Biogeography paradigm; most of these directly involve

empirical studies of dispersal involving iconic land vertebrates (e.g., Townsend *et al.*, 2011). First, several of the most ardent promoters of this perspective have retired, moved on to other areas of research, or softened their perspectives. Additionally, the development of modern model-based molecular phylogenetic analysis has changed our understanding of the evolutionary relationships of most vertebrate clades, either radically by turning evolutionary trees on their head (e.g., squamate reptiles; Townsend *et al.*, 2004; Losos *et al.*, 2012), or by demonstrating widespread convergent evolution and repeated evolution of vertebrate body form (such as repeated evolution of elongate, limb-reduced/absent snake-like lizards), which misled previous morphological trait-based studies (Reeder *et al.*, 2015). Third, biologists have developed a better understanding of how vertebrates of seemingly low relative dispersal ability actually do achieve overseas dispersal (Carlquist, 1966; McDowall, 2002). Finally, as calibrated molecular divergence date estimation has become more reliable due to advances in methods and collection of large robust multilocus datasets, many vertebrate groups of apparent, ancient Gondwanan origin have simply turned out to be too young. In these instances where disjunct groups on either side of an ocean basin are many millions of years younger than the dated fragmentation of the landmasses in question, an explanation of vicariance becomes intractable.

One such clear case is illustrated by estimation of the split between Old World and New World monkeys. For a vicariance explanation to account for the distribution of these African and American taxa, the divergence between the clades would have to approximate the final separation of Africa and South America (~110 mya). One recent time-calibrated phylogenetic analysis dated their divergence at somewhere between 50 and 26 mya (Springer, 2012), meaning that the ancestors of New World monkeys (now about 55 species) crossed the Atlantic at about 40 mya, much too late to permit the former vicariance scenario.

Ratite birds (the Rheas and Tinamous of the Americas, the Ostriches of Africa, the Emus and Cassowarys of Australasia, and the Kiwis and extinct Moas of New Zealand) are a classic and often invoked case of diversification via vicariance (Cracraft, 1974). Their phylogenetic relationships (a monophyletic clade), the preponderance of flightlessness in most species, and their distributions on separate southern isolated Gondwanan landmasses, has been invoked as a classic case of diversification necessarily resulting from continental-scale vicariance (Craw *et al.*, 1999). Dispersal via powered flight in contrast was comfortably discounted due to the clade's tendency toward large bulky bodies and the inability of extant species to fly. At the center of these assumptions were the giant extinct Moas of New Zealand, which vicariance biogeographers confidently pointed out could not possibly have reached New Zealand by any means other than overland colonization when this island was still connected to Australia and Antarctica. However, a recent phylogenetic analysis using a massive genomic dataset recently has resulted in a major rearrangement of the group's family tree. Together with a analysis of ancestral evolutionary state reconstruction Phillips *et al.* (2010) estimated that flightlessness actually might not have been the ancestral condition in this group, leading to a revised interpretation of multiple recent losses of flight in individual lineages. This opens the door to the very real possibility that the ancestors of these iconic large-bodied flightless birds may have, in fact, dispersed to some of their current locations via powered flight over open oceans – after which they subsequently evolved large body sizes and lost the ability to fly. The case emphasizes the importance of evaluating alternate evolutionary hypotheses in conjunction with biogeographical studies. And, just because many ratite birds are large and flightless today does not necessarily mean that their ancestors were giants, incapable of flight. And once these seemingly 'self-evident' constraints are relaxed (i.e., giant flightless Moas 'obviously' could not have flown to New Zealand), biogeographers can consider the alternate explanations (in this case, dispersal) for ratite Gondwanan distributions previously assumed to have resulted from pure vicariance (Craw *et al.*, 1999; Phillips *et al.*, 2010; De Queiroz, 2014).

Another frequently invoked case of Gondwanan vicariance is the classic case of more than 1600 freshwater cichlid fishes inhabiting southern portions on a number of continents. Because of their distribution and an early branching pattern mirroring the order of fragmentation of Gondwanan landmasses, cichlid fish have been widely accepted as a group, whose distribution arose from Gondwanan vicariance, starting at 135 mya. Friedman *et al.* (2013) presented a combined but independent paleontological and molecular-clock estimate for the time of the clade's origin that soundly rejected the assumed Gondwanan vicariance scenario in cichlid fishes. In this case, both stratigraphic distribution of fossils and the age inferred from a robust time-calibrated analysis of DNA sequence divergences both agreed on a Paleocene (65–57 mya) origin of the group, suggesting a primary role for dispersal in generating the observed distribution of cichlid fish. Although the case of cichlid fish left some questions unanswered (the Friedman *et al.*, 2013, analysis did not include fossil taxa), ichthyologists generally agree that the Rainbowfishes, the Gobies, and the Killifishes are most likely all pure Gondwanan in origin.

Yoder and Nowak (2006) reviewed the entire terrestrial flora and fauna of Madagascar, a celebrated and spectacularly isolated landmass, formerly tucked into the heart of Gondwana (**Figure 1**), and often cited as a prime example of a biota derived from pure vicariance, preventing exchange with African fauna following the separation of the island from this continent 135–125 million years ago. Reviewing all available molecular divergence data the authors concluded that the vast majority of the fauna – including freshwater fish and amphibians with their presumed low relative dispersal abilities – was derived from descendants of more recent (Cenozoic) transoceanic dispersal events from Africa.

Many similar and well-studied vertebrate groups, once widely accepted as clear cases of Gondwanan or Pangaean vicariance by virtue of their modern distributions and presumed poor dispersal abilities, have likewise failed to stand the test of molecular phylogenetics and modern divergence date estimation (Rowe *et al.*, 2010; Townsend *et al.*, 2011; Friedman *et al.*, 2013). Summarizing many of these studies, De Queiroz (2014), with reference to Croizat's dictum, stated: "Earth and life evolve together—except when they don't."

With time and new advances, biogeographers have come to routinely embrace necessarily more sophisticated analytical approaches and much more pluralistic interpretations.

However, the influence of Panbiogeography and Vicariance Biogeography is still felt in curious ways. For example, only recently have probabilistic modeling methods for biogeographic inference been developed with explicit parameters that model long-distance dispersal to a novel area (such as a distant island, not previously or currently occupied by the clade of interest; Ree and Smith, 2008; Matzke, 2013a,b). The assumption is that this methodological shortcoming may be an historical and sociological artifact, dating back to a time when mention of the word 'dispersal' would earn a young biogeographer a swift and public rebuke from a siliverback member of the Vicariance Biogeography school.

Biogeographic Barriers, Interchanges, Gradients, and Filter Zones: Stepping Stones, Conduits, and Corridors

Biogeographic barriers, as discussed above, inspired the scholarship that resulted in the birth of the field and the first articulation of biogeography's first principles (Wallace, 1860). The inferred barriers themselves vary in assumed permeability, from the absolute apparent turnover characterized by Wallace's Line (Huxley, 1868) to the various biogeographic lines that divide the major realms of the planet (Wallace, 1860, 1876; Sclater, 1858). The universality of absolute biogeographic lines has been discussed at length (Mayr, 1944; Simpson, 1977) and is tempered with a wide variety of exceptions (Esselstyn *et al.*, 2010; Lohman *et al.*, 2011; Brown *et al.*, 2013).

The permeability of biogeographic barriers has been studied at length, mostly in the context as the concept was originally defined: impenetrable barriers across which land vertebrate dispersal is limited by marine channels (e.g., freshwater fish or amphibians, believed to be wholly incapable of dispersal over salt water). For example, Esselstyn *et al.* (2010) analyzed taxonomic and phylogenetic evidence for all terrestrial vertebrates with ranges abutting either side of the northern end of Wallace's Line (i.e., Huxley's Modification of Wallace's Line; **Figure 2**) and argued that the immediate area (Palawan Island, and smaller landmasses to the north and south of this elongate corridor) more accurately meets the definition of a biogeographic filter zone and not an absolute biogeographic line or barrier. Classic biogeographic filter or 'transition' zones have been defined by linear island chains such as the Lesser Sunda islands of eastern Indonesia, or the Alaskan Aleutian island chain, stretching between distinct biogeographic provinces (Kricher, 2011; Lomolino *et al.*, 2010). In such systems, as the number of faunal elements from one end of the chain declines successively with distance form the source, the influence and origin of inferred elements from the opposing region increase (**Figure 3**; Carlquist, 1965; Lomolino *et al.*, 2010). Filter zones, whether island chains or matrices of suitable habitat surrounded by inhospitable habitat (such as the 'Sky

Figure 2 The Australasian faunal interchange zone is home to the most celebrated biogeographic lines (funal turnover zones or biogeographic barriers) on the Planet. Wallace's Line, as originally conceived (Wallace, 1860) illustrates the faunal turnover that occurs at the eastern edge of the continental Asian landmass, Sunda Shelf (light blue shading, left). This feature corresponds to the land that was exposed during Pleistocene periods of lowered sea levels. This biogeographic boundary is now recognized as the area of transition between Asian fauna and that of Wallacea, the transitional faunal zone that separates Asian from Australian fauna. Lydekker's Line is the equivalent feature on the Australian Sahul Shelf (light blue shading, right), and Weber's Line represents the geographic point of equivalency or balance between Asian and Australian faunas. Adapted from Mayr, E., 1944. Wallace's Line in the light of recent zoogeographic studies. The Quarterly Review of Biology 19, 1–14; Simpson, G.G., 1977. Too many lines; the limits of the Oriental and Australian zoogeographic regions. Proceedings of the American Philosophical Society 121, 107–120.

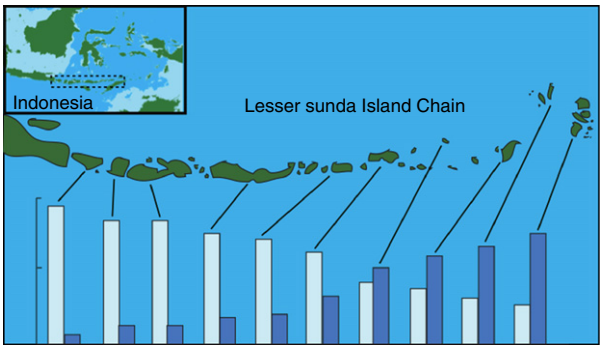

Figure 3 A terrestrial biogeographic filter zone is formed by areas of suitable habitat (such as dry land), separated or surrounded by an inhospitable matrix (sea water). The fauna of the Lesser Sunda islands of Indonesia (inset) illustrate the concept well: a chain of small landmasses separated by ocean channels, with the dispersal abilities of species demonstrated by their successful colonization of more and more distant islands. With dispersal from both ends of the chain of islands contributing to each island's fauna, the proportion of Asian species (light blue) nearly balances that of Australian species (dark blue) on midway through the chain. Adapted from Carlquist, S., 1965. Island Life. Garden City, NY: Natural History Press.

Figure 4 An example of an adaptive radiation in frogs from an island archipelago. Narrow-mouth frogs (genus *Kaloula*) have been characterized as an adaptive radiation, which began as their ancestors invaded an island archipelago (Blackburn *et al.*, 2013). From an inferred terrestrial mainland Asian habitat generalist, the Philippine *Kaloula* evolved into small, ephemeral pool breeding montane species ((a) *Kaloula walteri*), large-bodied open habitat burrowing species ((b) *Kaloula picta*), delicate scansorial shrub-breeding species ((c) *Kaloula conjuncta*), and tree-hole cavity dwelling taxa ((d) *Kaloula kalingensis*). Photographs by the author.

Islands' of the Madrean Archipelago; Manthey and Moyle, 2015) selectively illustrate the variation in relative dispersal abilities of the various taxonomic groups and the degree to which environmental filtering strongly structures community assembly along edges of biogeographic transition zones (Sommer *et al.*, 2014).

Other biogeographic island chains have been viewed as dispersal conduits or suitable habitat corridors, possibly allowing 'stepping stones' dispersal or 'island hopping' from one region to another (Carlquist, 1966). Dispersal conduits between continents and island archipelagos have been identified in empirical studies. For example, Diamond and Gilpin (1983) and Brown and Guttman (2002) (see also Brown and Siler, 2013) identified multiple chains of islands, which have facilitated faunal exchange of amphibians, reptiles, birds, and mammals between the mainland Southeast Asian continent and adjacent island archipelagos (see also Taylor, 1928; Inger, 1954). Comparative biogeographic analyses indicate that these series of terrestrial stepping stones have served as the primary source for mainland colonization of oceanic archipelagos (Brown and Siler, 2013; Brown *et al.*, 2013). The conservation significance of stepping stones and corridors of suitable habitat patches have been broadly discussed in insular terrestrial systems in terms of their importance for long-distance vertebrate dispersal – especially in light of species distributional shifts anticipated under climate warming scenarios as species track suitable habitats (Saura *et al.*, 2014).

Insular Extremes: Spectacular Vertebrates of Isolated Islands and Extraordinary Vertebrate Radiations in Island Archipelagos

Studies of vertebrate island biogeography have involved some of the planet's most interesting and unique forms of life. Three primary phenomena make this so. First, as previously discussed, the inferred origins of vertebrates on Earth's most isolated landmasses capture the imagination of evolutionary biologists and beg for an historical explanation that is inherently interesting – whether an ancient vicariance scenario or an inference of a spectacularly long-distance dispersal event.

Second, because of extreme isolation and the unique environments of islands, island vertebrates have evolved in many cases into bizarre forms, with phenotypic novelty accentuated and exaggerated by millions of years under unique selective regimes. Celebrated examples include island gigantism in tortoises, birds, and shrews, and at the other end of the spectrum, miniaturization in formerly large-bodied lineages like mammoths. Other examples of the 'Island Syndrome' involve inferences such as the secondary loss of flight in numerous lineages of birds after having arrived on islands, or other novel differences in life history, reproduction, demography, or behavior of island populations as compared to closely related species on the mainland (Adler and Levins, 1994).

Third, the uniquely replicated environments and at times unutilized resources (i.e., simplified food webs lacking top predators) of insular systems appear to have had an extraordinary impact on the process of diversification in vertebrates of island archipelagos. A disproportionate portion of the Earth's well-studied and truly spectacular adaptive radiations are found in islands or island-like archipelagos, and include highly celebrated clades, such as Darwin's finches in the Galapagos (Grant and Grant, 2008), the Hawaiian honeycreepers (Lerner *et al.*, 2011), New World direct-developing frogs (Heinicke *et al.*, 2008), the mantelid frogs of Madagascar (Vieites *et al.*, 2009), the great cichlid fish radiations of African rift valley lakes (Turner, 2007), lizards of the genus *Anolis* (Losos *et al.*, 1998; Mahler *et al.*, 2010), and the markedly diverse rodents,

narrow-mouth frogs, and forest frogs of the Philippines (Jansa *et al.*, 2006; Blackburn *et al.*, 2013; Brown *et al.*, 2015; **Figure 4**).

As such, it should be no surprise that islands not only spawn the big radiations, but they also inspire the big ideas. The geographic setting for much of our current speciation theory was the island archipelagos of the Pacific (Darwin, 1859; Wallace, 1860; Mayr, 1942), and some of evolution and ecology's most conceptually compelling, classic ideas were first conceived through the study of organisms on islands. These include topics such as equilibrium biogeography theory (Macarthur and Wilson, 1963), community assembly rules (Macarthur and Levins, 1967), adaptive radiation (Glor, 2010), ecological opportunity (Mahler *et al.*, 2010), evolution of supertramps (Toussaint *et al.*, 2013), taxon cycles (Wilson, 1961), and the Earth's great speciators (Diamond *et al.*, 1976; Moyle *et al.*, 2009). The importance of insular systems for development of conceptual models of processes of evolutionary biology cannot be over-emphasized (Vences *et al.*, 2009; R. Brown *et al.*, 2013; J. Brown *et al.*, 2014), and the study of land vertebrate biogeography historically has provided the inspiration behind the study of the Earth's most iconic island systems.

Future Prospects

The study of land vertebrate biogeography is at a crossroads, suggesting a synthetic paradigm shift has already begun with the availability of genomic data, the conceptual merging of traditions of historical versus ecological biogeography, and dampening of oscillations of a philosophical pendulum characterizing a history of debate between vicariance versus dispersalist perspectives. Although among the major branches of the Tree of Life the biodiversity of land vertebrates is comparatively well known, their distributions are less well documented, and an alarmingly large proportion of our understanding of mammal, bird, amphibian, and reptile distributional limits are approximations based on centuries-old, undocumented, or unverified observational data. This creates a challenge of great urgency for biogeographers charged with biotic inventories, surveys, and greatly needed follow-up surveys, and represents a major conservation concern in the face of species distributional shifts anticipated as a result of climate and other environmental change. Nevertheless, modern trends in the field of biogeography indicate that increasingly multidisciplinary approaches, massive integrative datasets, extensive collaboration, and increasingly global coordination and cooperation – where once competition prevailed – are becoming standard practice in the research programs of most vertebrate biogeographers. Because, relative to other groups, vertebrates are so well studied, with many aspects of their phenotype, genotype, and ecological characteristics understood by biologists, land vertebrates promise to serve as the model for biogeographers interested in studying integrated organismal biology through the application of multiple data streams.

See also: Land Animals, Origins of. Land Vertebrates, the Origin and Evolution of

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Biogeography, Patterns in

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This is a shared work with equal contribution by all authors.

Environmental Dynamics Affecting Evolutionary Processes and Biogeographic Patterns

Biogeographic patterns, as we observe them today are driven by current environmental settings interacting with influences from past abiotic environments as well as the characteristics and evolutionary history of current and ancient biotas (Ricklefs, 2004, 2006). The abiotic environment selects for certain biotic characteristics (traits), which enable species to utilize the prevailing environmental conditions in a certain habitat. The evolution of specific morphological, physiological, and behavioral traits enables species to occur even under environmental conditions, which are hostile for most other species (e.g., succulent or xeromorph leaf structures as an adaptation to perpetually dry conditions). Assuming that abiotic environmental conditions are the major shaping force of species' characteristics, species which co-occur in a certain habitat should evolve similar characteristics to cope with the prevalent environmental conditions (Osborn, 1902). In other words, environmental selection should enhance trait similarity among co-occurring species. Prominent examples for trait similarity are the independent evolution of giant rosette plants in tropical alpine environments, for example, on Hawaii, the Canary Islands, and in tropical alpine ecosystems of South America and Africa, or the vicariant evolution of succulence in Cactaceae in North and South America and Euphorbiaceae in Africa, Europe, and Asia. Indeed, biomes of the world can be characterized by species characteristics which evolved independently in different phylogenetic groups (Schroeder, 1998, e.g., giant rosette plants **Figure 1**), i.e., biome-specific traits such as xeromorphic leaves in Mediterranean ecosystems from the Chaparral in California, the Macchia in the Mediterranean basin, and the Fynbos in South Africa, are found in very different geographic regions of the world but under comparable abiotic environmental conditions (De Micco and Aronne, 2012). Thus, convergent traits selected for by similar

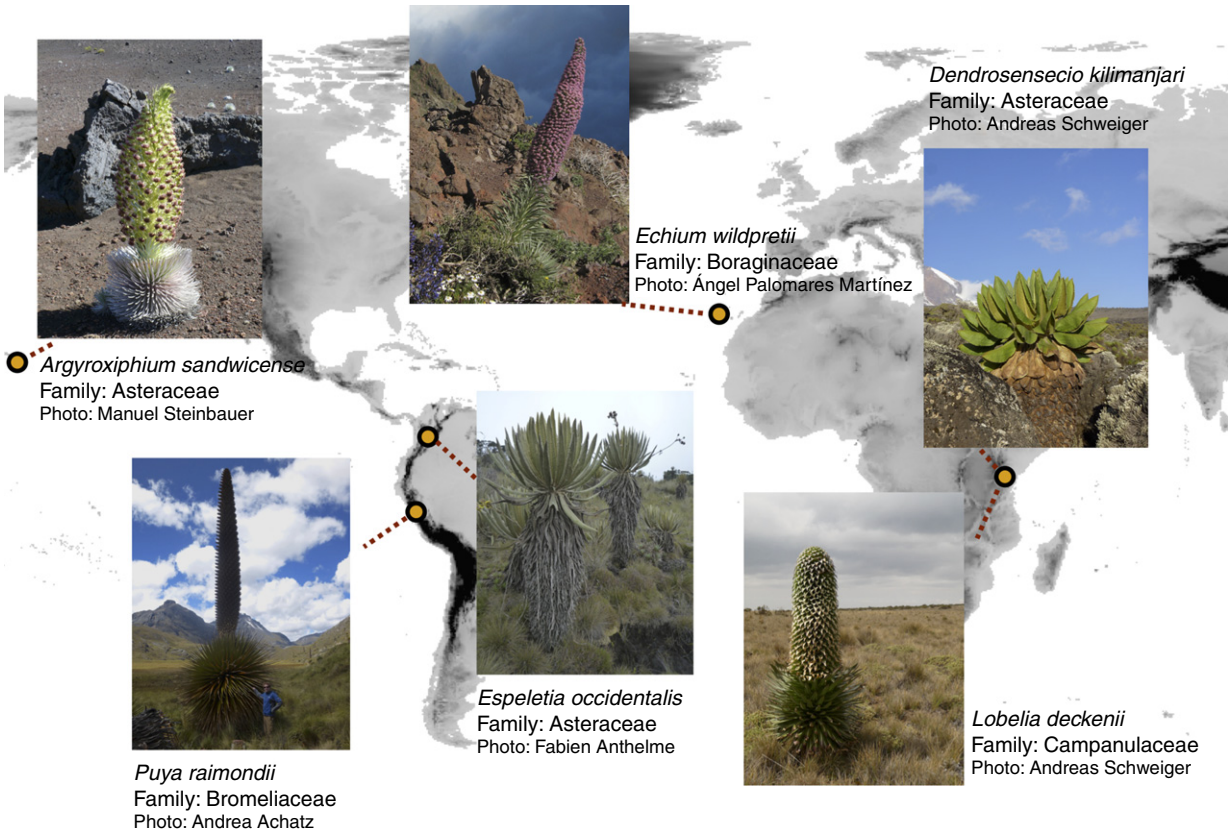

Figure 1 Giant rosette plants have independently evolved as adaptation to environmental conditions in (sub-)tropical high-elevation ecosystems.

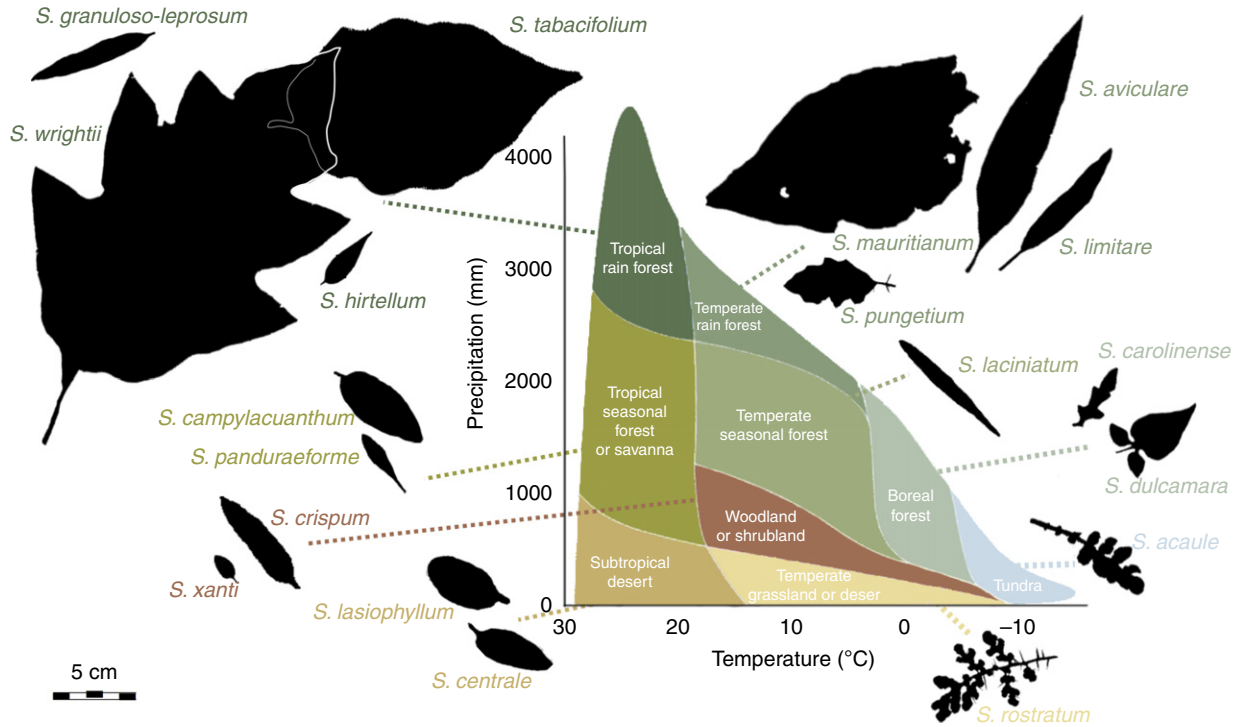

Figure 2 Leaf size and shape of closely related species (all genus *Solanum*) change as adaptations to environmental characteristics (illustrated here using the biome-classification proposed by Whittaker (1975)). Leaves were scanned from herbarium records.

environmental conditions are an impressive demonstration of the influence of environmental conditions on species characteristics. On the other hand, different environmental conditions in the different biomes of the world can lead to a high variety of morphological traits in closely related species (see Figure 2 for the genus *Solanum*, Solanaceae).

Although abiotic environmental conditions seem to shape large-scale patterns of species characteristics, interactions between species co-occurring in the same environment have to be assumed to be of similar importance for species trait evolution. In theory, two coexisting but very similar species inhabiting the same habitat should rapidly diverge or face extinction by competitive exclusion (Begon *et al.*, 2006). Thus, competition between similarly equipped species for the same resources has to be seen as an opposing force to environmental selection (MacArthur and Levins, 1967). Indeed, there are a number of examples of trait divergence in co-occurring species (e.g., *character displacement*, Brown and Wilson, 1956; Beans, 2014).

Character displacement driven by competition is, however, only one among many biotic mechanisms driven by the coevolution of species. Examples of species interactions affecting evolutionary processes include a variety of predator–prey or herbivore–plant interactions including the loss of herbivore defense mechanisms under the absence of herbivores on islands (Bowen and Van Vuren, 1997; Irl *et al.*, 2014). Species interactions and coevolution are suggested to be themselves strong drivers of evolutionary dynamics (*Red Queen* phenomenon, Van Valen, 1973).

Besides antagonistic interactions shaping species trait evolution, synergistic (mutualistic) interactions strongly affect the evolution of traits among coevolving species. These synergistic interactions among species were underestimated in the past but are nowadays believed to be a major shaping force of species trait evolution (Guimarães *et al.*, 2011). One of the most prominent examples of mutualistic interactions driving trait evolution of interacting species are the numerous very specific pollinator–plant interactions which are known for numerous orchid species (e.g., with orchid bees *Euglossini*, Roubik and Hansen, 2004). Coevolution driven by mutualistic interactions may results in visible spatial patterns of biotic organization like the *devil's gardens* in the Amazonian rainforest where ants living inside one specific tree (*Duroia hirsute*) kill all competitive vegetation in the surrounding, resulting in distinct clear cut patches in the otherwise dense tropical rainforests (Frederickson *et al.*, 2005).

In summary, environmental conditions in combination with synergistic and antagonistic biotic interactions shape evolutionary dynamics and biogeographic patterns. However, these evolutionary process and the resulting patterns are strongly influenced by the geographical context, which changes with time. Particularly plate tectonics as well as geomorphological events may alter geographic isolation and leaves its legacy in current distributions and characteristics of biota (Gillespie and Roderick, 2014). In addition, prevalent abiotic conditions of a certain environment are not temporally stable but vary strongly over time, especially on time scales that are relevant for evolutionary processes (Fernández-Palacios *et al.*, 2011). Thus, ecosystems have to be considered as complex adaptive systems, in which the current spatial arrangement of biotic elements (i.e., species) and their characteristics are the result of nonlinear system dynamics affected by

past environmental conditions and the ecological response to these conditions (Gell-Mann, 1994; Levin, 1998). Thus, causal drivers for current patterns of species distribution and trait characteristics may have time lags spanning from decades (Aggemyr and Cousins, 2012) to even millions of years (Kissling *et al.*, 2012).

Mechanisms, Structures and Scale Dependence of Biogeographic Patterns

Biogeographic patterns have fascinated scientists since the time of the early naturalists such as Alexander von Humboldt, but general interest of humans in these ecological patterns likely dates back several thousand years to the early human hunters and gatherers (Lomolino, 2001). Numerous ecological patterns were described since then at various spatial scales ranging from local patterns such as the above-mentioned devil's gardens to global patterns such as the latitudinal gradient of biodiversity. However, the description of biogeographic patterns is just the first step to understand the underlying, often invisible ecological processes driving the observable patterns. The understanding of processes causing biogeographic patterns is one of the most fundamental aims of ecologists and evolutionary biologists (Rosenzweig, 1995).

Processes driving ecosystem functioning which then result in observable biogeographic patterns strongly vary on temporal and spatial scale. It has long been suggested that procesess driving small-scale patterns act fast whereas processes driving large-scale patterns act over longer time periods (Brooks, 1988). Under this notion, biogeographic patterns emerging at continental scales can be adequately explained by long-term evolutionary processes such as speciation and extinction whereas patterns emerging at the community level can be best explained by short-term ecological processes or assembly rules acting at the population level (Cracraft, 1994). Biogeographic patterns and the underlying ecological processes have been studied for decades on distinct spatial and temporal scales without considering cross-scale interactions. However, ecological systems are complex with feedbacks between different hierarchical levels of organization and patterns emerging at the observed scale may be the result of mechanisms operating at different, unobserved scales (Levin, 1992; Gaston, 2000; see **Figure 3**). The importance of cross-scale interactions of ecological processes to understand biogeographic patterns across scales and, thus, whole ecosystem functioning are now increasingly recoginzed (c.f. Gunderson and Holling, 2002; Murphy *et al.*, 2007) but the study of cross-scale interactions is still underrepresented in ecological research.

Besides the overwhelming range of spatial and temporal scales on which ecological systems are observable, the scale of organisms themselves is impressive. Organismic body mass spans more orders of magnitude than the scale differences between the earth and the entire galaxy (from small microorganisms with 10^{-13} g to large plant species (10^{8} g), West and Brown, 2005). The scale of observation, thus, plays a crucial role when identfing biotic patterns and underlying explanations (Gaston, 2000). In addition, grain of observation and size of the study area may strongly influence observations (Rosenzweig, 1995; Steinbauer *et al.*, 2012). To sum it with Hutchinson's words, *"The concepts of scale and pattern are ineluctably intertwined"* (Levin, 1992).

Fundamental Evolutionary Patterns in Biogeography

Evolutionary processes drive fundamental biogeographic patterns on various organizational scales ranging from patterns in body sizes, for example, Bergmann's rule (i.e., an increase in body size with decreasing latitude), the distribution of life forms such as the latitude–elevation relationship of alpine treelines (Körner, 2012) or spatial patterns in species range sizes, for example, Rapoport's rule (i.e., increase of range size with decreasing latitude) and Stevens' rule (i.e., increase of elevational range with elevation, Stevens, 1992) to spatial

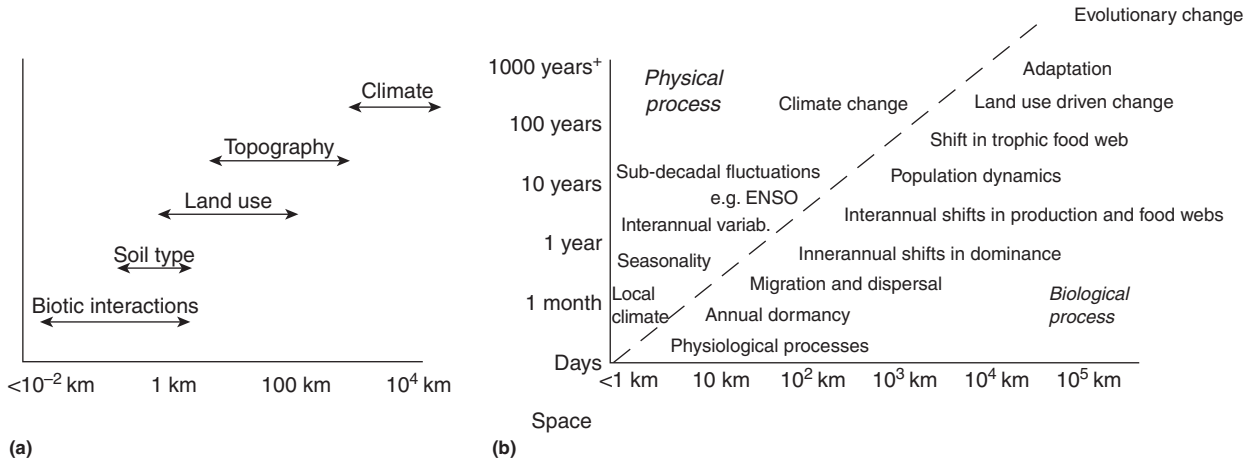

Figure 3 Schematic illustration suggested for processes and drivers operating at different spatial and temporal scales (a) adapted from Levin, D. A., 2003. Ecological speciation: Lessons from invasive species. Systematic Botany 28, 643–650 and (b) reproduced from Murphy, E.J., Watkins, J. L., Trathan, P.L., *et al.*, 2007. Spatial and temporal operation of the Scotia Sea ecosystem: A review of large-scale links in a krill centred food web. Philosophical Transactions of the Royal Society B: Biological Sciences 362, 113–148.

patterns in the structure of whole communities (i.e., species richness) such as the latitudinal diversity gradient (i.e., an increase of species richness toward the equator, Mittelbach *et al.*, 2007) and the elevational diversity gradient (i.e., an often complex pattern of species richness and elevation; Sanders and Rahbek, 2012). The following text and corresponding **Table 1** highlight some of the most prominent biogeographic patterns. None of the discussed causal mechanisms are uncontroversial. We classify the patterns according to their geographic origin into patterns based on latitude, elevation as well as island-specific phenomena as well as according to the affected biotic traits: species richness, morphology, and geography (including species dispersion and isolation).

Latitudinal Patterns

Latitude might be considered the single most prominent environmental gradient for species-related biogeographic patterns found on this planet. Indeed, a variety of theories exist that describe how species diversity, body size and body coloration, range size, or life forms vary with latitude. However, in many cases the mechanistic processes lying behind this latitudinal effect on the features of life are still unclear, leading to controversial and sometimes also contradicting explanations (Steinbauer, 2013). In the following we present some of the most noteworthy latitudinal patterns.

In our current world, most species are found in the tropics, while species richness decreases toward the poles. This phenomenon – already observed by Charles Darwin and Alfred Russel Wallace – has been termed the latitudinal diversity gradient (Mittelbach *et al.*, 2007) and is a general biogeographic pattern throughout a wide range of taxa (Hillebrand, 2004). Initially a high climatic stability in the tropics when compared to temperate regions has been suggested to enhance diversification (Wallace, 1878) either by higher specialization (Janzen, 1967) or coevolution (Schemske *et al.*, 2009) of tropical species, leading to the observable latitudinal gradient in species richness. However, empirical evidence of considerable climatic fluctuations in tropical regions challenges these hypotheses about the underlying environmental drivers of this biogeographic pattern (Mittelbach *et al.*, 2007). Not climate stability but rather climatic similarity to past environmental conditions has been proposed as an alternative explanation (Fine and Ree, 2006). The ancestors of current biota evolved under much warmer conditions than today. The adaptations to prehistoric warmer environments are suggested to be phylogenetically conservative (niche conservatism). As a consequence more species survived in the warm tropical climates whereas prehistoric climatic cooling in the higher latitudes lead to higher species extinctions and, thus, to lower species diversity (Wiens *et al.*, 2010). Another alternative explanation for this prominent biogeographic pattern is the increased availability of energy and thus, resources in the tropics compared to higher latitude which might enable the coexistence of more-individuals in tropical regions (Evans *et al.*, 2008), speed up evolutionary dynamics (Allen and Gillooly, 2006), or set a higher carrying capacity for species (Rabosky, 2014). Many of these explanations are not mutually exclusive, but the ongoing discussion shows the complexity when inferring processes responsible for observable patterns.

Morphological traits of species show distinctive changes along latitudinal gradients including species body size, size of species extremities, and species coloration. One of the most prominent, latitudinal patterns related to species morphology is an increase of body size with increasing latitude, named *Bergmann's rule* (Bergmann, 1847). In contrast to the latitudinal diversity gradient with many competing mechanistic explanations, this latitudinal pattern is related to species thermoregulation. The proposed reason is based on the simple biophysical assumption that larger animals have smaller surface-to-volume ratios than small ones and thus, require less energy for thermoregulation (McNab, 1971). This pattern is, however, only true for endotherms, for example, birds (Meiri and Dayan, 2003) and mammals (Ashton *et al.*, 2000), whereas ectotherms show unclear body size patterns, which either confirm or contradict this pattern or show no relation at all along latitudinal/elevational gradients (Shelomi, 2012).

The energy balance of ectotherms is strongly linked to body coloration (*thermal melanism hypothesis*, Clusella-Trullas *et al.*, 2007) which in turn is linked to body size (e.g., Schweiger and Beierkuhnlein, 2015 for Carabids). Thus, interactions of two thermoregulative, morphological traits varying with latitude, namely body size and coloration, may be one explanation for the inconclusive latitudinal patterns of body size in ectotherms. Thermoregulatory control mechanisms are also assumed to drive the increase of species extremities with decreasing latitude, which is observable, for example, for foxes (Genus *Vulpes*) or hares (Genus *Lepus*) and conceptualized in *Allen's rule*. Changes in body size, length of extremities, or coloration are not only observable along latitudinal but also elevational gradients, where thermoregulation is equally important.

The observed pattern that species' range size increases toward the poles has been termed *Rapoport's rule* (Stevens, 1989; Gaston *et al.*, 1998). There are indications that Rapoport's rule is restricted to the Northern Hemisphere (Rohde, 1996; Ruggiero and Werenkraut, 2007). However, the generality of this biogeographic pattern is highly controversial and still bears a lot of discussion.

Besides species latitudinal ranges, species elevational ranges have the tendency to change along latitude which is conceptualized in *Janzen's rule*. In 1967, Daniel Janzen hypothesized that elevational ranges of species (as a proxy of their thermal tolerance) increase from the tropics toward the poles (Janzen, 1967). He attributed this phenomenon to increasing seasonality along the latitudinal gradient. Because tropical species experience only little temperature variation throughout the year, it is advantageous for them to specialize their niche, i.e., develop small thermal amplitudes. In contrast, species at higher latitudes experiences a wide range of temperatures at any given location owing to the seasonal climate. For these species it is therefore necessary to develop a wide thermal niche in order to persist. As a consequence of higher species packing per unit elevation (or area), this seasonality-driven mechanism is used to explain hotspots of species richness in the tropics compared to higher latitudes. Support for this pattern has been found for ectotherms (Ghalambor *et al.*, 2006) and interestingly for vertebrates (McCain, 2009a), although recent findings suggest that seasonality dependence is altered by the species' life stage and physiology (Sheldon *et al.*, 2015).

Table 1 Selected spatial biogeographic patterns, important explanations, and controversies. References are not necessarily the authors first describing a pattern but often key reviews or summaries

		Name	*Pattern*	*Reference*	*Explanations*	*Discussions*
Latitude	Species richness	Latitudinal diversity gradient	Species richness decreases with latitude	Mittelbach *et al.* (2007), Fine (2015)	Climatic stability (Wallace, 1878) Diversity begets diversification (Van Valen, 1973; Gillooly *et al.*, 2004) Environmental fluctuations (Janzen, 1967) Niche conservatism (Wiens and Donoghue, 2004) More-individuals hypothesis (Wright, 1983; Evans *et al.*, 2008) Metabolic theory of ecology (Brown *et al.*, 2004) Species carrying capacity/energy (Rabosky, 2014)	Different nonexclusive explanations (Mittelbach *et al.*, 2007; Kraft *et al.*, 2011; Steinbauer, 2013; Harmon and Harrison, 2015)
	Species morphology	Bergmann's rule	Body size increases with latitude	McNab (1971)	Optimal surface-to-volumes ratio for thermoregulation (Bergmann, 1847) Dispersal capacity – energy relation (Blackburn and Hawkins, 2004)	Restricted to endotherms, ectotherms show unclear pattern (Mousseau, 1997)
		Allen's rule	Appendage increase with temperature	Allen (1877)	Thermoregulation	Many exceptions and little empirical support (Nudds and Oswald, 2007)
		Color related patterns	Yellow intensification of pollen with UV radiation Increasingly light-colored ectotherms with temperature	Stevenson (1985), Clusella-Trullas *et al.* (2007), Flenley (2011)	Yellow colors protect DNA from UV radiation induced mutations (Flenley, 2011) Thermoregulation in ectotherms (Clusella-Trullas *et al.*, 2007; Schweiger and Beierkuhnlein, 2015)	Colors often adapted to avoid visual detection or may serve other purposes
	Species geography	Rapoport's rule	Species' range size increases with latitude	Stevens (1989), Gaston *et al.* (1998)	Increase in niche width with latitude due to climatic fluctuations (Janzen, 1967; Stevens, 1989) More competition in species rich communities (Brown, 1995)	Only Pale- and Nearctic phenomenon (Rohde, 1996; Ruggiero and Werenkraut, 2007)
		Janzen's rule	Elevational range of species decreases toward the equator	Janzen (1967)	Increasing seasonality toward the poles results in larger thermal amplitude for a given high latitude species compared to tropics	Some ectotherm lineages show this pattern (Ghalambor *et al.*, 2006), unresolved for plants
		Alpine treeline	Subtropical double-maxima in upper limit of the arboreal growth form	Körner (2012)	Common isotherm seasonal mean temperature of 6 °C (Körner and Paulsen, 2004)	Islands show only a single maximum (Irl *et al.*, 2015)
Elevation	Species richness	Elevational richness gradient	Species richness peaks at mid elevations	Sanders and Rahbek (2012)	Mid domain effect/range overlap (Colwell *et al.*, 2004)	Lack of knowledge (McCain, 2009b), Likely no single

(*Continued*)

Table 1 Continued

		Name	*Pattern*	*Reference*	*Explanations*	*Discussions*
					Species-area relationship (Rosenzweig, 1995) Warm and wet climates (McCain, 2009b) Species interactions (Anthelme and Dangles, 2012)	mechanism (Pau *et al.*, 2012; Sanders and Rahbek, 2012)
	Species geography	Stevens' rule	Increase of species' ranges with elevation	Stevens (1992)	See Rapoport's rule	
		Mountain endemism	High percentages of endemic species on mountaintops	Steinbauer *et al.* (2012)	Isolation enhances speciation (Sky islands; Steinbauer *et al.*, 2013; Antonelli, 2015) Lower climate change velocity in mountains supports species survival (Sandel *et al.*, 2011)	Isolation not always increasing with elevation
Islands	Species morphology	Island rule (Island dwarfism/ gigantism)	Small animals colonizing islands tend to become larger, while large ones tend to become smaller	Lomolino *et al.* (2013), Carlquist (1967)	Absence of large predators on islands may support larger body sizes of smaller species (missing selection) Limited resource availability may select for small individuals	Clade-specific pattern, not general rule (Meiri *et al.*, 2008)
		Island woodiness	Herbaceous lineages evolve woody life forms on islands	Carlquist (1974), Lomolino *et al.* (2013)	Adaptation to low seasonality/frost (Carlquist, 1974)	Mechanism not really understood
	Species geography	Loss of dispersal ability after establishment on isolated islands	Species lose dispersal ability after establishing on islands	Darwin (1859), Carlquist (1967), Carlquist (1974)	High dispersal capacity enhances chance of being swept off the island (Carlquist, 1967; Gillespie *et al.*, 2012)	Lavergne *et al.* (2004), Vazacová and Münzbergová (2014)
		Shifts in plant reproduction	Species change plant reproduction after establishing on islands		Adaptation to low seasonality/frost (Carlquist, 1974) Adaptation to missing herbivores	
		Progression rule	Biota colonize younger islands after older islands	Funk and Wagner (1995), Roderick and Gillespie (1998)	Newly formed islands colonized from older existing ones (see e.g., Bess *et al.*, 2013) Priority effects promote early arriving species (Fukami, 2015)	Exceptions (Gohli *et al.*, 2015) and colonization history often more complex (Macías-Hernández *et al.*, 2013)
		Taxon cycle	Repeated range expansions and contractions of lineages in the order of 10^6 years, taxa initially colonize coastal habitats, but later adapt to highland conditions	Wilson (1959), Ricklefs and Bermingham (2002)	Coevolution with enemies of pests (Ricklefs and Bermingham, 2002)	Causal mechanisms under debate

Another, in the historical context of biogeography very prominent latitudinal pattern tackles the upper elevational range limit of tree species, which strongly decreases toward the poles. The *alpine treeline* is the fundamental limit of the arboreal growth form (arguably the most dominating life form in Earth's vegetation) and marks the transition from tree-dominated vegetation to treeless alpine scrub or grassland (Holtmeier, 2009; Körner, 2012). The alpine treeline demonstrates a nontypical latitudinal pattern, expressing its maximum elevation in the subtropics of the Northern (Miehe *et al.*, 2007) and Southern Hemisphere (Hoch and Körner, 2005) both around 4800–4900 m a.s.l. From the subtropical double-maxima, treeline elevation decreases toward the poles but also toward the equator (Hermes, 1955; Troll, 1961). In contrast, due to lower seasonality and cloud-induced lower solar radiation, treeline elevation on islands shows only a single maximum near the equator (Irl *et al.*, 2015). The treeline is formed by a wide range of different tree species (deciduous, coniferous, evergreen broadleaved) but a uniform thermal limit (seasonal mean temperature of approx. 6 °C, Körner and Paulsen, 2004) has been suggested for the treelines worldwide, which is independent of phylogenetic relationships, trait evolution, and biogeographic features and thus, might present a general explanation for this global, biogeographic pattern (Körner, 2012).

Elevational Patterns

In many respects elevation can be considered as the vertical representation of the (horizontal) latitudinal gradient (Lomolino, 2001). Thus, many concepts that apply to latitude have been transferred or adapted to elevational gradients. Elevational gradients are fundamental in shaping Earth's current species richness and have substantially contributed to the evolution of Earth's outstanding diversity of species and life forms. However, as is the case for latitude, elevation integrates a wide range of environmental features (e.g., temperature, precipitation, solar radiation, cloudiness, atmospheric pressure, etc.), making it quite difficult (and often impossible) to determine the actual underlying drivers and mechanisms.

Similar to the latitudinal patterns in species richness, the number of co-occurring species also shows distinct changes along elevational gradients. Species richness tends, in general, to show a mid-elevation maximum, even if a decline in species richness with elevation was assumed until the 1990s (McCain, 2009b; Karger *et al.*, 2011). In addition, the isolation of high elevation ecosystems is assumed to cause island-like situations leading to high ratios of unique endemic species ('islands in the sky,' Steinbauer *et al.*, 2012, 2013). Protection from high UV radiation may cause more yellow pollen at high elevations (Flenley, 2011) and more generally, any change in the environment along elevational gradients may result in characteristic trait compositions (like the common decrease in growth height in ecotypes of one species with elevation).

Species ranges sizes also change along elevational gradients – conceptualized in *Stevens' rule* (Stevens, 1992). Stevens' rule can be seen as an extension of Rapoport's rule to elevational gradients (i.e., a thermal gradient comparable to the latitudinal gradient). Stevens' rule has been documented for a wide range of taxa such as butterflies in the USA (Fleishman *et al.*, 1998) or orchids on the island of La Réunion (Jacquemyn *et al.*, 2007). Similar to Rapoport's rule, support for Stevens' rule is fragmental and the generality of this rule is debated.

Island-Specific Phenomena

Islands are considered nature's 'test tubes' (Losos and Ricklefs, 2009), acting as model systems for the development of a wide array of fundamental theories in ecology, biogeography and evolution (e.g., Darwin, 1859; Wallace, 1880; MacArthur and Wilson, 1967; Whittaker *et al.*, 2008). In addition, islands play an important role in the conservation of global biodiversity, not because of their exceptional species richness (Kreft *et al.*, 2008) but owing to their high degree of endemism (Kier *et al.*, 2009). Due to island-specific features such as small area, isolation and genetic impoverishment, islands are particularly vulnerable to land use change (Caujape-Castells *et al.*, 2010), invasive species (Kueffer *et al.*, 2010), and climate change (Harter *et al.*, 2015). For these reasons, the evolutionary patterns found on islands deserve a special focus, as we would like to demonstrate in the following.

Islands are ecosystems surrounded by a matrix inhospitable for terrestrial biota (Whittaker and Fernández-Palacios, 2007). Particularly studies on volcanic oceanic islands have stimulated the development of novel theories and concepts which have been successfully transferred to other systems such as inland lakes, caves, coral reefs, habitat fragments, or continental mountain systems. In the following, we would like to present and discuss four important features related to the geography and morphology of island taxa: island dwarfism and gigantism, island woodiness, dispersal capacity loss of island species, and the taxon cycle.

One of the most striking patterns related to the morphology of island animals is the island dwarfism and gigantism. The body size of island species (esp. animal species) typically becomes much larger or smaller than the body size of their closest relative on the continent, ultimately reaching an optimum size dependent on island-specific features (Lomolino, 1985; Lomolino *et al.*, 2012). This phenomenon has been observed in a wide range of different taxa (non-volant mammals, bats, passerine birds, snakes, and turtles, Lomolino, 1985). Both features are often referred to as the *island rule* (Van Valen, 1973; Lomolino, 1985). The island rule is particularly pronounced in paleo-endemic species, highlighting the evolutionary effect of time being isolated from potential predators and competitors (Lomolino *et al.*, 2013). However, recent studies indicate that the island rule is only clade-specific and not a general rule, if phylogenetic measures are applied (Meiri *et al.*, 2008).

Whereas animals are likely to get either smaller or larger on islands, plant species tend to get woody. The phenomenon is conceptualized under the term *island woodiness*. Shrubby or tree-like growth forms often characterize endemic plant species on oceanic islands, although they originate from herbaceous lineages (Carlquist, 1974). The phenomenon of island woodiness (or secondary woodiness) has been widely observed in many different genera throughout many oceanic island archipelagos, for example, the genera *Rumex* and *Sonchus* on the Canary Islands, *Echium* on the Canary Islands, Madeira, and

the Cape Verdes (Lens *et al.*, 2013), *Opuntia* and *Scalesia* on the Galapagos Islands (Hamann, 2001), the silversword alliance of Hawaii (Baldwin, 1997), or in the genus *Dendroseris* (or 'tree lettuces') of the Juan Fernández Islands (Moreira-Muñoz *et al.*, 2014). The common explanation for this ecological pattern is strongly related to the environmental conditions that characterize oceanic islands. Due to the low seasonality on oceanic islands adaptations to winter frost are not a competitive advantage. Thus, plants face an evolutionary advantage if they do not dieback or cease photosynthetic activity during parts of the year to avoid frost but rather produce biomass throughout the whole year. Thereby, woody plant tissue is advantageous because the initial high investment into the assimilation of carbon results in stable and durable structures with competitive advantages over herbaceous and especially annual species (Carlquist, 1974).

Besides the distinct effects of island features on plant and animal morphology, it also limits the dispersal ability of island endemic species. Long-distance dispersal often associated with nonstandard events (e.g., storms, oceanic rafts, etc.) is a prerequisite for biota to colonize oceanic islands in the first place (Heleno and Vargas, 2015). However, poor dispersal ability is a characteristic feature of endemic species on oceanic islands (Carlquist, 1974). Flightless birds, for example, the dodo of the Mascarenes (Livezey, 1993) and many species of the avifauna of New Zealand (Duncan and Blackburn, 2004), or poor dispersing plant species (Carlquist, 1967) are typical examples. Several explanations have been presented arguing why endemics are poor dispersers. On a spatially restricted island surrounded by an unfavorable environment the production of dispersal agents (diaspores) with high dispersal capacity would result in most passively dispersed diaspores landing in the ocean (Carlquist, 1967). Thus, the production of high dispersal diaspores is not a successful strategy for the proliferation of a species, resulting in disadvantages in terms of energy and competition. Further, long-term climatic stability on islands was suggested as one reason why good dispersal capacity is not a necessary trait for survival, when compared to more dynamic continental systems (Cody and Overton, 1996).

The *taxon cycle* (Wilson, 1959) is one of the key models implementing changes on evolutionary rates in an island biogeographical context. This conceptual idea initially used to explain patterns of diversity in ants on islands, suggests that the ant species successfully colonizing islands in Melanesia tend to be generalists adapted to marginal open habitats (Wilson, 1959). With time these generalist species tend to shift from marginal to forest habitats due to adaptation and specialization (Wilson, 1959). Extinction in the marginal habitats paves the way for a new wave of generalist colonizers (Pigot *et al.*, 2012). The cycle is suggested to reoccur about every 10^6 years (Ricklefs and Bermingham, 2002). Similar patterns have been observed for a wide range of taxa, for example, passerine birds (Jønsson *et al.*, 2014), the West Indian avifauna (Ricklefs and Cox, 1972) and Melanesian ants (Economo and Sarnat, 2012) but also North American highland fishes (Hoagstrom *et al.*, 2014) or freshwater decapods shrimps (Cook *et al.*, 2008) which strengthens the generality of this ecological process. Acknowledging differences in isolation of habitats within islands or continental landscapes in dependence of the environmental context (Steinbauer *et al.*, 2013), the taxon cycle seems to be not just an idiosyncrasy of oceanic islands but might be a general ecological process affecting biodiversity patterns on a global scale. New species successfully establishing in a landscape are likely preadapted to ecosystems also available in a larger context. With time those species may shift habitat (and specialization) utilizing the ecological opportunities provided by the ecosystems that are less inhabited because they are unique (or highly isolated) in a larger spatial context.

Conclusion

Biogeographic patterns, which can be observed in our current world, are the result of ecological (abiotic and biotic) processes interacting among various temporal and spatial scales. These cross-scale interactions of ecological processes have to be taken into account when studying biogeographic patterns and their underlying ecological driving forces. However, research on biogeographic patterns is up to now strongly restricted to certain temporal and spatial scales but hardly accounts for cross-scale interactions, which limits the full understanding of the relevant ecological processes leading to the expression of biogeographic patterns. Furthermore, the observation of biogeographic patterns itself is strongly scale dependent, which additionally blurs a general understanding of ecosystem functioning. Modern analytical methods in combination with the increasing availability and unification of observational data from various scales ranging from *in situ* field investigations up to earth observation via satellites bear the potential to overcome these scale-dependent problems and explicitly focus on cross-scale interactions among ecological processes and patterns. Another promising approach might be the investigation of scale-independent patterns (fractals) which can be frequently observed for geophysical structures (e.g., shorelines) but are understudied in ecology.

See also: Biogeography, History of. Biogeography of Islands, Lakes, and Mountaintops; Evolutionary. Phylogenetic Tree

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Bird Flight Origins

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Glossary

Airfoil A structure with curved surfaces designed to give the most favorable ratio of lift to drag in flight, used as the basic form of the wings, fins, and most aircraft.

Arboreal Pertaining to animals living primarily in trees, from the Latin, *arboreus*, of trees.

Archaeopteryx Also known as the urvogel, an extinct primitive bird of the Late Jurassic Period, having numerous bird-like characteristics including flight feathers, but with reptilian features such as teeth and a long bony tail.

Archosaur Any reptile of the subclass Archosauria, including the dinosaurs, pterosaurs, and crocodilians, characterized by an antorbital fenestra and two pairs of openings in the temporal region of the skull.

Avian Relating to birds, or things so related is called avian.

Cursorial Adapted for running, as the feet and skeleton of ostriches, horses, etc.

Dromaeosaur Any of a group of small- to medium-sized carnivorous maniraptoran dinosaurs that flourished during the Cretaceous Period (146 to 66 million years ago). Generally agile, lightly built, and fast-running.

Feather The diverse, flat epidermal appendages growing from a bird's skin and forming its plumage, typically consisting of a partly hollow horny shaft fringed with vanes of barbs that produce individual airfoils in flight feathers or remiges. Feathers are generally divided into categories of flight feathers and body contour feathers which produce laminar flow in flight and insulation.

Glider A volant animal with varied flight surfaces, including feathered wings, skin flaps, etc., capable of gliding from a higher to lower level by the action of gravity.

Jurassic The period of geologic time known as the second period of the Age of Reptiles or Mesozoic, spanning (200 to 145 million years ago) during which dinosaurs diversified and the earliest birds are known.

Maniraptora The group of bird-like theropods known as 'hand snatchers' were a clade of coelurosaurian dinosaurs including the dromaeosaurs birds.

Microraptor A genus of small, very bird-like, four-winged glider paravians (possibly dromaeosaurids) with avian pennaceous flight feathers. Numerous well-preserved fossil specimens have been recovered from the Early Cretaceous, Liaoning, China.

Pterosaur The group of flying reptiles of the Mesozoic Era, characterized by leathery, bat-like wings. Early groups had teeth and a long tail, but through the Cretaceous Period the teeth and long tail were lost.

Remiges (singular remex) Any of the large flight feathers of a bird's wing. Used also to describe hind-limb flight feathers of newly discovered Jurassic and Early Cretaceous gliders of China.

Scansoriopterygid Scansoriopterygidae is a family of very small four-winged glider archosaurs close to the ancestry of birds, known from well-preserved fossils unearthed in the Middle Jurassic Daohugou fossil beds of China. The skeleton is primitive and lacks any dinosaurian features.

Tetrapteryx A term introduced by naturalist William Beebe in 1915, describing his view of a hypothetical ancestral proavian, a small arboreal glider with four wings. His predication has recently been confirmed by discovery of four-winged microraptors and scansoriopterids and other fossils from the Early Cretaceous and mid-Jurassic of China.

Thecodont The varied groups of fossil quadrupedal or partially bipedal reptiles of the Triassic and persisting into the Jurassic Periods, having an antorbital fenestra and teeth set in sockets, and ancestral to crocodilians, pterosaurs and all groups of dinosaurs, as well as birds.

Theropod The group of 'lizard-hipped' or saurischian dinosaurs characterized by being obligately bipedal, with a large balancing tail and short arms with a hand reduced to three digits, I, II, III; theropod literally means 'beast-footed.'

Urvogel German term for the earliest known bird *Archaeopteryx* of the Late Jurassic Period, of the Solnhofen Limestone, Bavaria, approximately 150 million years old. It was in many features a fairly typical bird, but exhibited definitive reptilian characteristics such as conical teeth and a long bony tail.

Volant Relating to, or characterized by, ability to glide or fly.

Introduction

The origin of bird flight has intrigued people and engendered debate since the discovery of the then earliest bird, the Late Jurassic German urvogel *Archaeopteryx* in the early 1860s. This well-preserved fossil, sometimes called the Rosetta Stone of evolution, was discovered when the debate on Darwin's theory of evolution by natural selection was at a peak, only two years after the publication in 1859 of *The Origin of Species*, and an animal linking two major groups of vertebrates, reptiles and birds, was not likely to be quietly cataloged and slid into some dusty museum drawer. The first skeletal specimen, known as the *London specimen*, was purchased by the British Museum and described by Richard Owen in 1863. In 1867, the most famous *Berlin specimen*, preserved in spread-wing pose, was acquired by the Germany's Humboldt museum where it resides today (Figure 1). Until today, a total of 12 skeletal specimens (one unstudied) and a single feather have been recovered from the Upper Jurassic Limestone of Bavaria, along with a spectacular array of fossils that provide the most comprehensive window

available on Late Jurassic life (Wellnhofer, 2008). New discoveries of similar fossils coeval or of slightly older age from China have added immensely to our knowledge of flight origins (Feduccia, 2012).

Although debate on bird origins was intense, Thomas Huxley, Darwin's ally, sometimes called Darwin's Bulldog, saw the urvogel more as a link between reptiles and birds, and emphasized the small Solnhofen dinosaur *Compsognathus* (**Figure 2**) as a definitive link to birds, particularly based on the hind-limb skeleton, but he was equally enthusiastic about an avian ancestor among either Ornithischian (e.g., *Iguanodon*) or theropodan (e.g., *Megalosaurus*, *Compsognathus*) dinosaurs (Feduccia, 2012). Since for Huxley, birds were vaguely derived from 'dinosaurs' or perhaps their archosaur antecedents (thecodonts), the origin of flight must have been, albeit mysteriously, occurred from the ground up, and he viewed the living flightless ratites (ostrich, rhea, emu, etc.) as intermediates leading to modern birds. Today, however, we know that all flightless birds, regardless of ancestry, are derived from flying ancestors and still exhibit numerous flight adaptations (De Beer, 1956).

Figure 1 Complete fossil of *Archaeopteryx*, the Berlin specimen. Creative Commons. https://en.wikipedia.org/wiki/Archaeopteryx#/media/File:Archaeopteryx_lithographica_(Berlin_specimen).jpg.

Trees-Down versus Ground-Up Flight Genesis

In later editions of *Origin*, Darwin referred to *Archaeopteryx* as 'that strange secondary bird' in reference to its occurrence in the Jurassic Period, curiously de-emphasizing its importance in any dinosaurian nexus, and Huxley considered it an 'intercalary bird': "In certain particulars, the oldest known bird does exhibit a closer approximation to the reptilian structure than any modern bird…..The leg and foot, the pelvis, the shoulder-girdle, and the feathers…...are completely those of existing ordinary birds" (Huxley, 1868).

Archaeopteryx is a reasonably advanced avian, in profile and size an almost perfect superficial match for a modern magpie (*Pica pica*) or Australian pheasant cuckoo (*Centropus phasianus*) (Heinroth, 1923), so its relevance to bird flight can legitimately be questioned (**Figure 3**). *Archaeopteryx* falls in the middle of living birds statistically in important measures of wing function, as plotted in **Figure 4**, aspect ratio versus relative wing loading (Norberg, 1990), and in addition exhibits primary wing flight remiges with asymmetric vanes on the feathers, producing individual airfoils. Manfred Reichel's classic drawings of the urvogel in different life poses (Reichel, 1984) illustrate its possible diverse behaviors, terrestrial, trunk-climbing, and in full flight (**Figure 5**).

Huxley's 'dinosaurian' origin of birds model was initially accepted, but the publication of Heilmann's (1926) book

Figure 2 Skeleton of *Compsognathus*. Creative Commons. https://upload.wikimedia.org/wikipedia/commons/4/49/Compy.jpg.

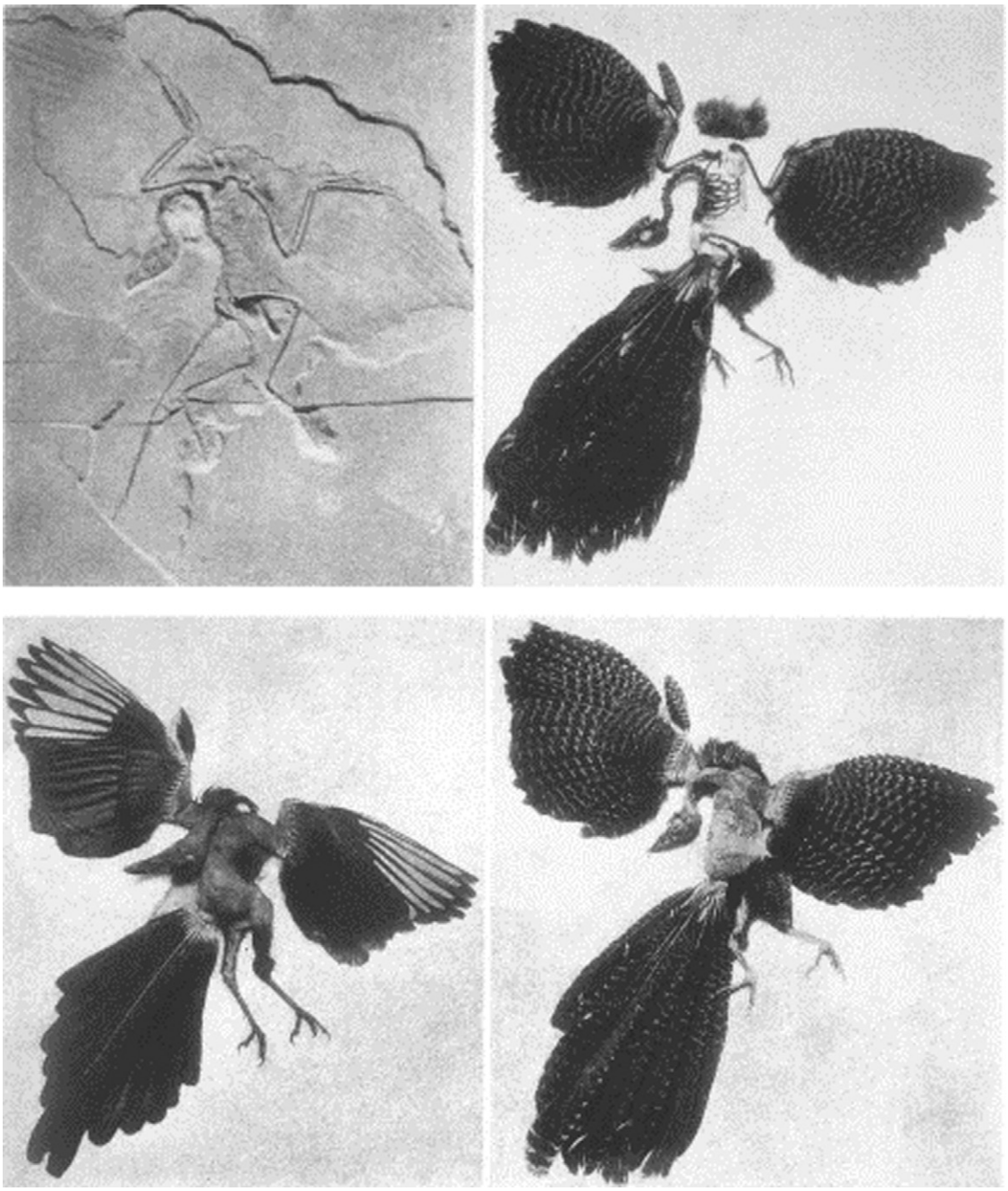


Figure 3 *Archaeopteryx* look-alikes. Upper left, the Berlin *Archaeopteryx*, compared with the Australian pheasant cuckoo (*Centropus phasianius*) (upper right), prepared brilliantly by Oskar Heinroth in 1923, in similar posture, showing the remarkable superficial similarity, including elliptical wings. However, the coucal is primarily terrestrial as indicated by its much flatter claws and distally frayed tail feathers and loosely constructed feathers of tail and wing. Below, similar comparisons of magpie (*Pica pica*) and the same coucal, showing similarity in profile. Magpies are equally at home on the ground or in trees. Reproduced from Heinroth, O., 1923. Die Flugel von Archaeopteryx. Journal für Ornithologie 71, 277–283; courtesy, Stefan Garthe for Journal für Ornithologie.

The Origin of Birds shifted focus to a group of primitive arboreal archosaurs then called 'thecodonts' (now more appropriately basal archosaurs), which are the more inclusive group of largely Triassic reptiles from which both groups of dinosaurs, pterosaurs, crocodilians, and birds are all ultimately derived. Heilmann thus favored a small tree-dwelling archosaur as the ancestor of birds, and thus favored a trees-down model for flight origin.

In the late 1960s, however, the debate was altered when Yale's John Ostrom discovered skeletons of a human-size Early Cretaceous dromaeosaurid theropod, *Deinonychus* (Figure 6), which exhibited innumerable bird skeletal features, including

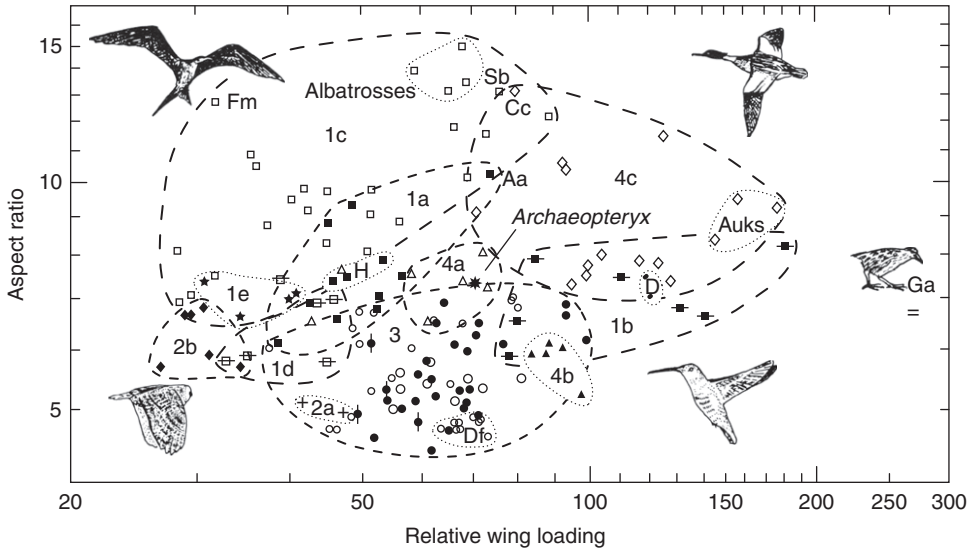


Figure 4 Aspect ratio vs. relative wing loading in various birds. The encircled foraging groups are defined solely by the similarity of flight and foraging modes, not by systematic affinity. Aa = *Apus apus* (swift); Cc = *Cygnus cygnus* (swan); D = diving petrels; Df = Darwin's finches; Fm = *Fregata maginificens* (frigatebird); Ga = *Gallirallus australis* (flightless New Zealand wood rail); H = swallows; Sb = S*ula basana* (bobby). Note particularly the position of the urvogel *Archaeopteryx.* Reproduced from Norberg, U.M.L., 2004. Bird flight. Proceedings of the 23rd International Ornithological Congress, pp. 921–935. Beijing: Current Zoology; courtesy, Ulla Lindhe Norberg.

a bird-like hand and wrist; the field would never be the same (Ostrom, 1969). Importantly, because this dromaeosaur and its kin like *Velociraptor* were thought to be entirely terrestrial (Chiappe, 1997), it rekindled the debate on flight origins, for Ostrom providing evidence that, somehow mysteriously, flight must have originated from the ground up (Ostrom, 1979). Most of these ideas date back to 1907 with an untenable running dinosaur model by Baron Franz Nopcsa (Nopcsa, 1907; **Figure 7(a)**). The problem, of course, was once aloft: where would the energy come from to keep the animal airborne? More recent models have used *Archaeopteryx* as a cursor, at early flight stages, being capable of takeoff from a running start, using already developed wings as a primary thrust generator (Burgers and Chiappe, 1999), but all ground-up models have been seriously challenged. Derek Yalden showed that *Archaeopteryx* and early birds are all arboreally adapted and have sharp claws highly recurved and adapted for trunk climbing, and match beautifully those of trunk-climbing birds and mammals (Yalden, 1985). All ground-up models have failed because of the architectural limitations of the pectoral apparatus of *Archaeopteryx* and putative avian ancestors, either theropod or archosaurian, which was incapable of a powerful, rapid upstroke of the wing. Another recent model by Dial (2003), called 'wing-assisted incline running' (or WAIR), uses the chukar partridge as a model. By this scenario, partridges (and presumably ancestral birds or their antecedents) vigorously beat their wings to run up steep or vertical substrates, even trees, and thus such birds could get off the ground. Yet, it is unclear what relevance this model might have to flight origins, and as noted above, early birds or any of their putative ancestors lacked the necessary pectoral anatomy for a strong, vigorous recovery stroke of the wing. Thus all ground-up models fail to explain flight origins in reasonable biomechanical terms, are 'gravity-resisted,' and have generally been discarded.

As with all these scenarios, Ostrom's main obstacle was how a bird could evolve elongate modern flight feathers and an avian wing if it were, as he saw it, first an earth-bound theropod dinosaur. He proposed a scenario whereby the flight feathers of protobirds elongated as insect traps and continued elongation until full wings and flight were achieved (**Figure 7(b)**). The other ground-up models noted above, rely on ancestors with a strong recovery stroke of the wing, absent in all putative avian ancestors, either dinosaurian or archosaurian (Feduccia, 2012). The Ostrom model has been highly criticized, no longer considered feasible; and along with more recent models now resides in the waste bin of theories that are biologically and biophysically unsound.

Mesozoic Experiments in Flight

During the Triassic and the preceding Permian, numerous, often bizarre, arboreal vertebrates experimented with the new aerial ecological zone for the first time. Notable among these Triassic archosaurs were *Longisquama* and *Sharovipterix* (**Figure 8**). *Longisquma* had elongate feather-like appendages (parafeathers) projecting from the upper mid-line of the back; it was probably a parachuter in butterfly profile, using its integumental appendages to break the fall. *Sharovipteryx* was a small gliding reptile, and has been reconstructed with various conformations of a leathery patagial gliding delta-wing, another early evolutionary experiment in the conquest of air. Among the menagerie of volant forms were numerous and anatomically varied gliding Triassic lizards including *Mecistotrachelos* from the late Triassic of Virginia, which like modern southeast Asian lizards of the genus *Draco*, glided on membranous wings supported by elongated ribs that could spread laterally to provide a gliding surface; and there are gliding

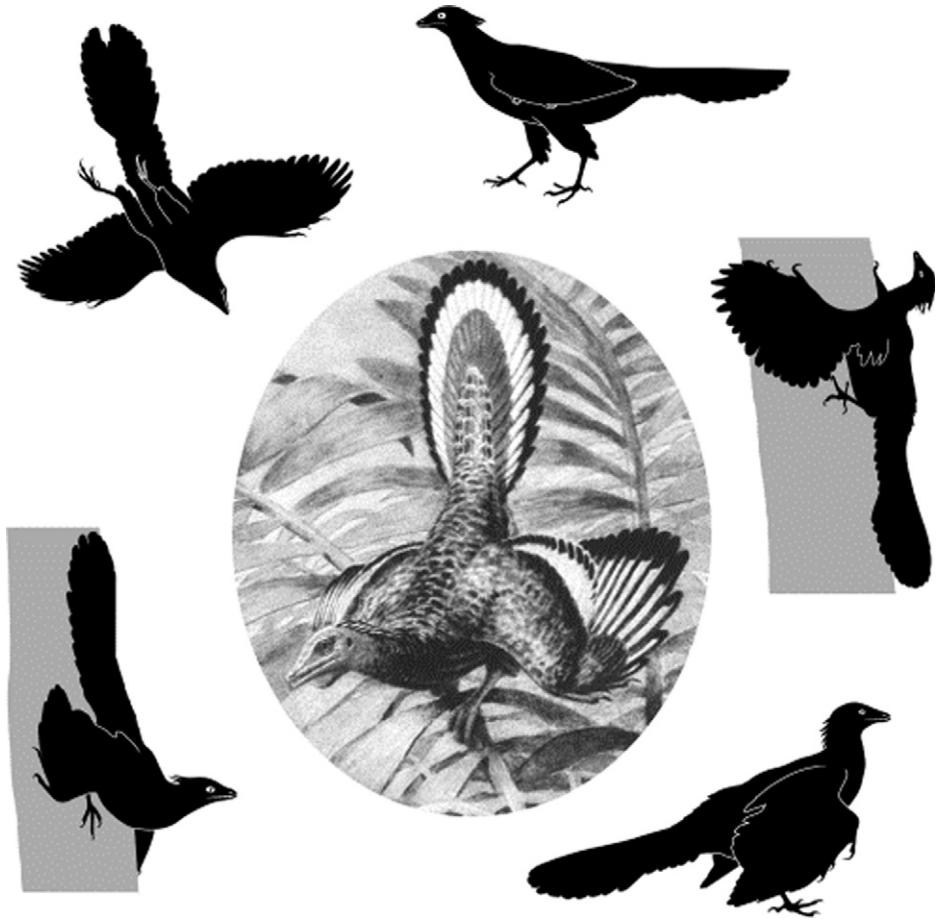
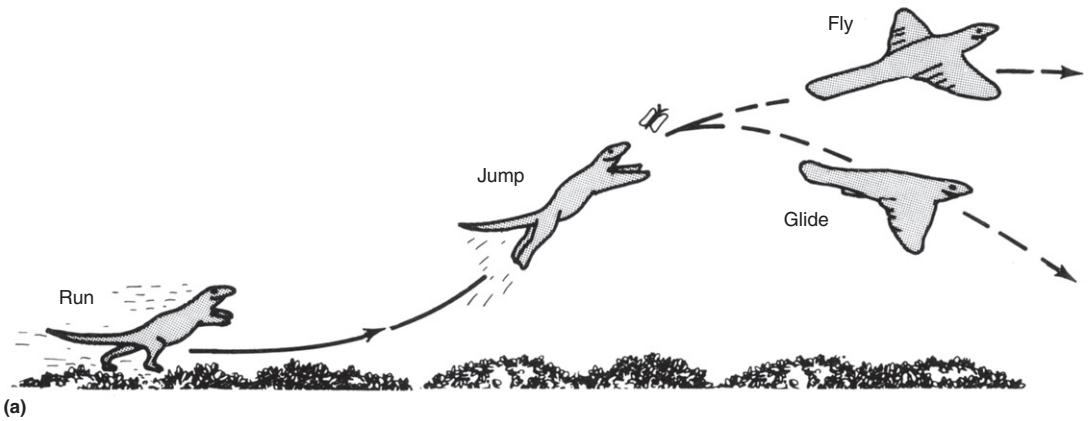


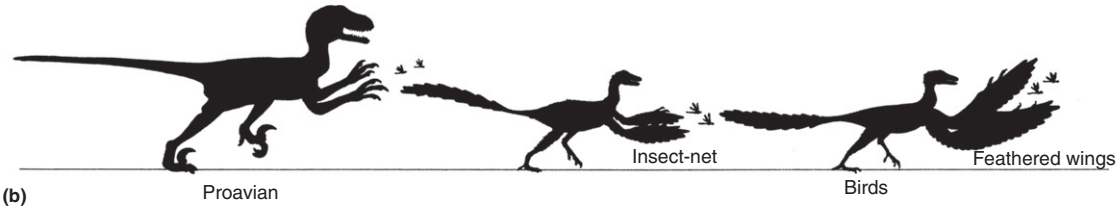
Figure 5 Adaptation of Gerard Heilmann's reconstruction of *Archaeopteryx*, surrounded by silhouettes from Manfred Reichel's classic drawings of the urvogel in different life poses, illustrating its possible diverse behaviors, terrestrial, trunk-climbing, and in full flight. Adapted from Heilmann, G. 1926. The Origin of Birds. London: Witherby; silhouettes modified from Reichel, M., 1984. Dessins (1896–1984). Basel: Geological Institute of Basel University.

Figure 6 Skeleton of *Deinonychus*, the specimen at the Field Museum, Chicago. Creative Commons. https://en.wikipedia.org/wiki/Deinonychus#/media/File:FMNH_Deinonychus.JPG.

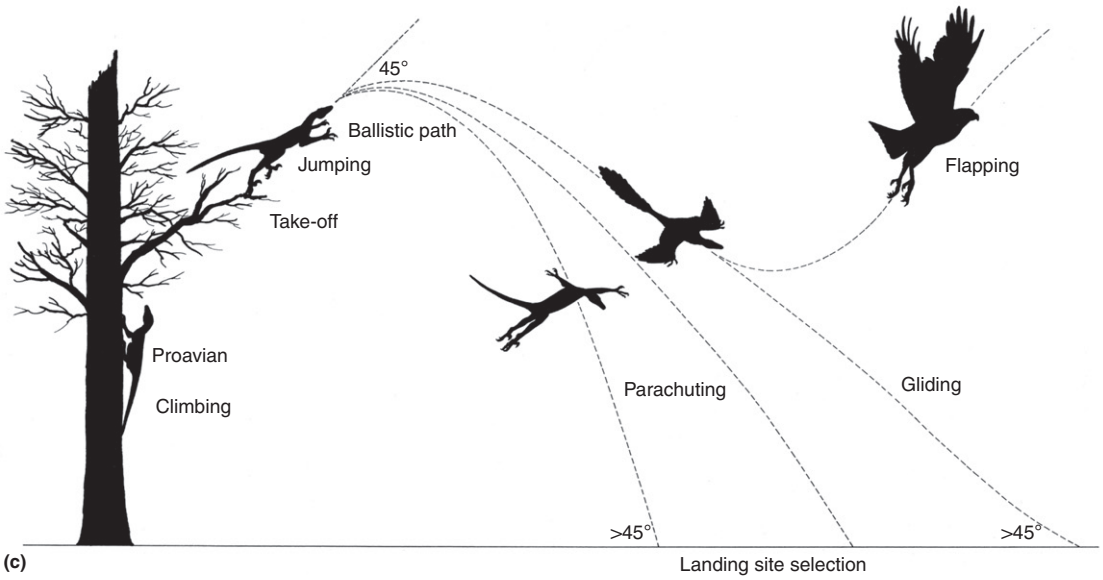
geckos and even parachuting tree frogs (*Rhacophorus*) and a gliding snake (*Chrysopelea*) of Southeast Asia. The first active fliers were the leathery winged pterosaurs of the Triassic, which evolved in great variety through the Cretaceous losing their long tails and teeth, and debate on the genesis of their flight closely paralleled the debate on bird flight, but now is known


Fly
Jump
Glide
Run

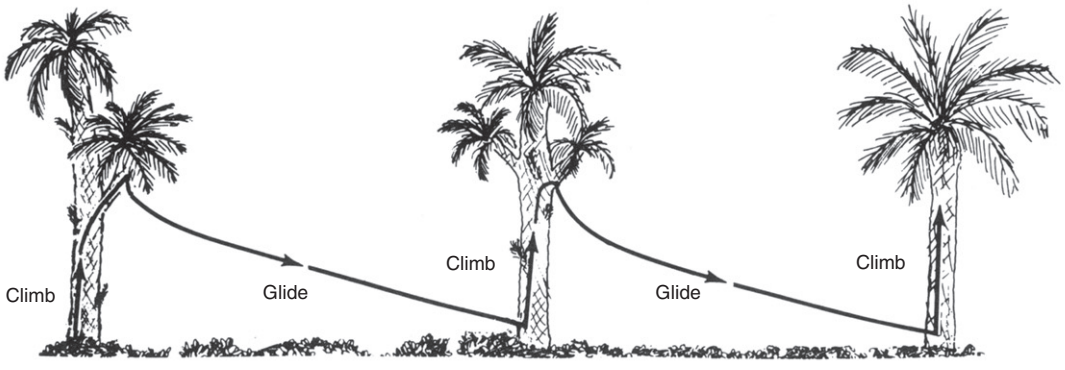
(a)


Insect-net
Feathered wings
Proavian
Birds

(b)


45°
Ballistic path
Jumping
Take-off
Flapping
Proavian
Climbing
Parachuting
Gliding
>45°
>45°
Landing site selection

(c)


Climb
Glide
Climb
Glide
Climb

(d)

Figure 8 Fossil (left) and restoration (right) of *Sharovipteryx*. Creative Commons. https://en.wikipedia.org/wiki/Sharovipteryx#/media/File:Sharovipteryx_mirabilis_fossil.JPG (left) https://en.wikipedia.org/wiki/Sharovipteryx#/media/File:Sharovipteryx.jpg (right).

to have originated, like that of birds, from the trees-down, via the arboreal model (**Figure 9**).

Patterns of botanical evolution during the Late Permian and the Triassic are relevant to the evolution of widespread experimentation with arboreality and with forms of flight among reptiles. Tall conifer forests emerged during this geological time span, and because angiosperms had yet to evolve, there were no vines or other similar plants. A somewhat similar but analogous situation exists today in the rainforests in Borneo where myriad gliding creatures have evolved. There are 30 or so gliding species in the rainforests of Borneo; these include four species of flying frogs, that use loose flaps of skin on their limbs as well as long web fingers to parachute. There are flying geckos that use webbed toes and flanges of skin on the legs and tail for gliding, and lizards that use their expanded rib cage for gliding; and even a ribbon-flat, paradise tree snake that flatten the body to propel for a distance of up to 30 meters (98 ft). These creatures are more aerodynamically agile than appreciated; the harlequin tree frog, for example, capable of 180-degree mid-air turns, and *Draco* lizards can make hundred-foot glides between trees, landing upright on tree trunks. Gliding mammals include flying lemurs (*Cynocephalus*) and 14 species of flying squirrels ranging in size from the 15 cm (6 in) pygmies to the red giant flying squirrel that can exceed one meter (3 ft) in length including the tail. In Amazonian rainforests, the only gliders are small squirrel monkeys that parachute from tree to tree while assuming the same flattened, spread eagle posture characteristic of all gliders; there are also Central American parachuting frogs. However, the diversity of Bornean forests is not there. One explanation is that the rainforests of Borneo, unlike those of the Amazon basin, Africa, or other similar regions, are dominated by giant dipterocarp trees, which fruit infrequently and unpredictably; this forces animals to expand the territory to be canvassed for food. Gliding offers an energetically cheap and efficient method of canopy travel for a variety of animals, relieving them of the long trip down to the ground and back up again. Also, the dipterocarp forests of Borneo are taller, have fewer lianas, and a more discontinuous canopy than Amazonian rainforests. In dense Amazonian rainforest canopies, gliding is more difficult and an organism can simply climb from tree to tree (Laman, 2000; Feduccia, 2012).

The Bornean rainforest suggests a possible general explanation for evolutionary experimentation in the aerial ecological zone during the Triassic, an evolutionary trend that produced not only the volant pterosaurs, but flying lizards, and strange experiments in flight such as the delta-winged *Sharovipteryx* and the butterfly-parachuter *Longisquama*.

Chinese Discoveries Refocus Debate

The urvogel *Archaeopteryx* was until recently the only Jurassic bird, and was the focus of almost all debate on the subject until the past few decades when the Lower Cretaceous Chinese Jehol Biota and equally well-preserved Jurassic deposits produced some equally significant fossils relevant to bird and flight origins (Zhou *et al.*, 2003). Among the most significant discoveries were fossils with both fore- and hind-limb wings (Zheng *et al.*, 2013), confirming the prediction by early naturalist William Beebe that modern avian flight was preceded by a four-winged stage which he called the *tetrapteryx* (**Figure 10**; Beebe, 1915).

Perhaps the most significant discovery was the very bird-like Early Cretaceous microraptors (*Microraptor* and allies, silhouette, **Figure 11**), four-winged gliders considered variously basal dromaeosaurs or early offshoots of the avian radiation. There are also Jurassic fossils variously considered troodontid

Figure 7 Illustrations showing the historically contrasting views on the origin of avian flight. (a) The ground-up or cursorial model. In this highly improbable model the early feathers would produce drag, which would have the undesired effect of slowing down the animal; then, once the animal was aloft, where would the energy come from to maintain flight? (b) An equally improbable scenario, John Ostrom's 'insect net model' which attempted to explain elongation of feathers in a non-flight context. (c) The trees-down or arboreal model illustrated by a trunk-climbing proavian (either a basal archosaur or a theropod) that began to jump from limb to limb, then parachute from trees, eventually beginning to glide as the angle of descent decreased from 45°. Gliding increased maneuverability and slowed landing. Following gliding, flapping would begin to prolong powered flight. The arboreal theory is intuitively facile, using high places or gravity as a source of power, converting potential energy into kinetic energy. (d) Illustration by Ulla Norberg illustrates the energetically least expensive manner to traverse a forest, by climbing and gliding. Panels (a) and (d): Adapted from Norberg, U.M., 1990. Vertebrate Flight. Berlin: Springer, courtesy Ulla Lindhe (Norberg)); (b) and (c): Reproduced from Chatterjee, S., 2015. The Rise of Birds. second ed. Baltimore, MD: Johns Hopkins Press; courtesy Sankar Chatterjee.

Figure 9 Bird and pterosaur flight origin went through a similar tortuous debate, but now the arboreal theory dominates paleobiological and ornithological thought. Here terrestrial locomotion in pterosaurs is shown above in Cretaceous *Pteranodon* (left) and *Pterodactylus* (right), and below, Jurassic *Rhamphorhynchus*, featuring teeth and long tail, later lost. In the center is H.G. Seeley's 1901 view of the basal, bipedal *Dimorphodon*, now disproved by finds of a uropatagium (skin stretching between hind limbs), quadrupedal tackways, and a specimen showing it was flat-footed. Kevin Padian in the 1980s erroneously revived Seeley's model with his view that pterosaurs were closely allied with theropods, which were obligately bipedal and therefore concluded that pterosaur flight originated from the ground up, now totally disproved. Silhouettes modified from Reichel, M., 1984. Dessins (1896–1984). Basel: Geological Institute of Basel University. Reproduced from Feduccia, A., 2012. Riddle of the Feathered Dragons. New Haven, CT: Yale University Press.

theropods or early birds, some, like the mid-Jurassic *Anchiornis*, with fully developed avian wings and a tetrapteryx flight bauplan. Forms such as the Jurassic four-winged glider scansoriopterids (Czerkas and Feduccia, 2014), originally described as coelurosaurs, stand at the base of the avian tree, and show very little in the way of theropod affinity. The recent discovery of a partially preserved fossil scansoriopterid named *Yi qi*, with feathers and a bat-like patagial flight membrane was an early evolutionary flight experiment, and adds additional mystery to the entire problem (Xu *et al.*, 2015). Many of these early Chinese fossils are often reconstructed erroneously as cursorial dinosaurs, but such a model is not tenable because the elongate hind-limb wing feathers would have been a hindrance in running and would have created detrimental drag for an animal preparing to take flight. Instead, all these animals were trunk-climbers, may have been parachuters or gliders at varied stages of development, and beautifully illustrate how the origin of flight may have occurred via the biophysically facile, 'gravity-assisted' arboreal model with wing-assisted climbing or WAC (Chatterjee and Templin, 2012; Chatterjee, 2015), as opposed to the improbable, 'gravity-resisted' cursorial model.

Conclusions

Ground-up or cursorial (from Latin *cursus*, running) theories for flight origin are historically based on the belief that birds are derived from earth-bound theropods, incapable of tree climbing (Chiappe, 1997). The varied versions of this model have all failed because if running or jumping were involved, any elongation of feathers would produce drag and therefore

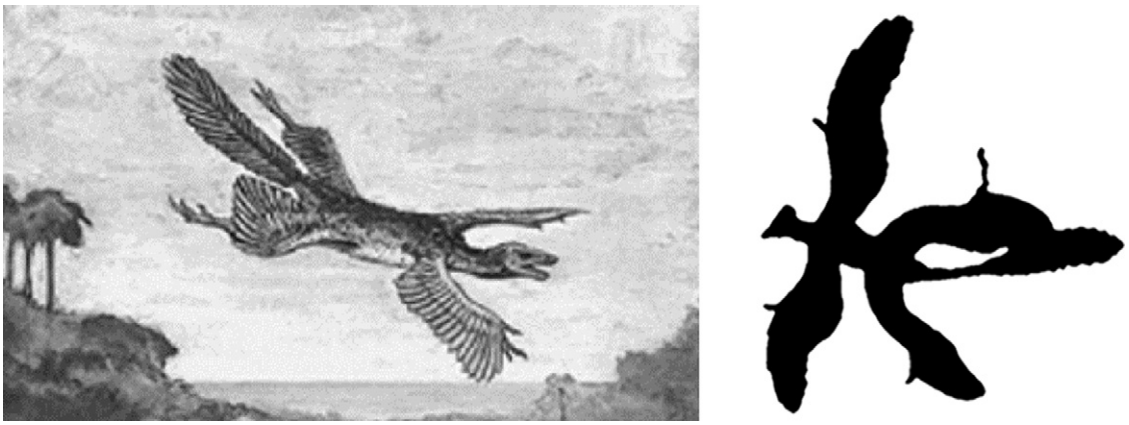


Figure 10 Beebe's (1915) hypothetical *tetrapteryx* stage in avian flight, remarkably confirmed in recent years by the discovery of microraptors, four-winged avian-like, basal dromaeosaurid gliders from the Jurassic and Lower Cretaceous of China. Silhouette of the Early Cretaceous four-wing glider *Microraptor* shown on the right in silhouette. Left: reproduced from Beebe, W., 1915. A tetrapteryx stage in the evolution of birds. Zoologica 2, 39–52.

Figure 11 The holotype of *Microraptor gui*. Creative Commons. https://en.wikipedia.org/wiki/Microraptor#/media/File:Microraptor_gui_holotype.png.

slow down the organism while running, and hence the term 'gravity-resisted'. While paleontologists have in recent decades embraced the view that birds evolved from terrestrial maniraptoran dinosaurs typified by the dromaeosaur *Velociraptor*, and as noted some still hold the view that flight evolved from the biophysically improbable ground-up model, there is no reason that many of these maniraptorans could not have climbed trees (Zhou, 2004). Most early workers, however, favored evidence for a small, arboreal basal archosaur as the logical ancestor, with flight having evolved via the biomechanically facile trees-down model from small arboreal archosaurs (Norberg and Norberg, 1989), or even arboreal maniraptoran theropods, which may prove to be early avians (James and Pourtless, 2009). The simple trees-down 'gravity-assisted' model has been used without exception by myriad land vertebrates that have developed flight.

O. Able in 1911 suggested an extension of the arboreal hypothesis, that both dinosaurs and birds descended from tree-climbing archosaurs (Able, 1911), and a version of this idea has been carried into modern times by Zhonghe Zhou who has termed the new version the dinosaurian trees-down model for avian flight (Zhou, 2004). However, there is still dispute as to whether the Chinese Jurassic and Early Cretaceous four-winged gliders are true theropods or early avians (Feduccia, 2012), and the recently discovered scansoriopterids from the Middle Jurassic of China are close to suitable tree-dwelling, archosaurian ancestors for birds (Czerkas and Feduccia, 2014), but are devoid of any theropod skeletal characters.

Whatever the true ancestor is, the most reasonable explanation for the origin of flight, not only in birds, but in all other vertebrates, is the arboreal or trees-down model (Chatterjee and Templin, 2012; Chatterjee, 2015). The requisites for flight origin are small size, because without that restriction, no integumentary structures (such as feathers) will have any effect on breaking the fall; and high places, primarily trees. High places allow such animals to take advantage of the cheap energy provided by gravity (**Figure 4**). Not only the climbing adaptations of early birds but their possession of a hind toe (hallux) for perching indicate that

they were largely arboreal, and their possession of brain enlargement, particularly a highly developed cerebellum, acute visual sense, and neurosensory development for hearing and equilibrium (inner ear), all point to an arboreal setting. The arboreal theory for flight origins, unlike the cursorial theory, has the advantage of beginning to explain how all the microstages in flight origins were individually selected for, including the progression of arboreal jumping or leaping, parachuting, gliding, leading to maneuvering powered, flapping flight.

See also: Amniotes, Diversification of. Birds, Diversification of

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Birds, Diversification of

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Glossary

Aves The class of vertebrate animals comprising all living species of birds.
Cretaceous The geological period lasting from 145 to 65 million years ago.
Divergence time The age (often denoted in million years ago) of time of evolutionary separation between two taxonomic units.
Eocene The geological epoch lasting from 56 to 34 million years ago.
Eocene–Oligocene extinction The geological transition encompassing the end of the Eocene and the beginning of the Oligocene period (34 million years ago) marked by large-scale faunal and floral extinction (that included most stem members of modern bird families).
Paleocene The geological epoch lasting from 65 to 56 million years ago.
Paleogene The geological period lasting from 65 to 23 million years ago, encompassing the Paleocene, Eocene, and Oligocene epochs.
Paraves The evolutionary lineage that contains all living and extinct birds and those maniraptoran dinosaurs that are immediate ancestors of birds. This lineage itself is comprised of two groups, one (Avialae) containing fossil and modern birds, and one (Deinonychosauria) containing dromaeosaur (including microraptors), and troodontid dinosaurs.
Phylogeny The reconstruction of the evolutionary history (relationships) of a group of species.
Radiation The increase in taxonomic biodiversity over a short period of time, involving rapid morphological evolution and new ecological exploitation.
Taxonomy The biological classification of a group of organisms/species.

General Introduction

Though birds are not better adapted to their environment than other animals, to human eyes they appear almost enviously so. Birds have perfected the art of flying and singing in more colorful ways than any other organism (Figure 1). Humans continue to be fascinated by the lush variety in bird behaviors, food capture, and their ability to thrive in almost any environment. Throughout human history, birds have played many important symbolic roles and served as inspiration for art. However, in today's time, birds are perhaps best suited as symbols of conservation and extinction. This article serves to introduce the reader to the general aspects of bird appearance (what anatomical features make a bird a bird), origins (what is the ancestor to birds?), phylogeny and timeline (when did the various types of living birds separate and diversify?), and the importance of birds to the welfare of humans. In order to provide a scientific framework to introduce these issues and better recognize current threats of extinction, these topics will be discussed through an evolutionary lens. To best appreciate the variation among the roughly 9500 living species of birds (Aves), it is useful to have an understanding of the humble origins among dinosaurs, followed by subsequent radiations into now extinct toothed birds, and later on, beaked birds. This journey addresses the questions of where and when birds evolved from reptilian ancestors, and how (the mechanisms through which) this was accomplished through evolutionary time. Once these questions are answered, the biodiversity of living birds is better understood as an immeasurable set of exemplary cases of specialization and ecological niche filling in evolution. With this vision, the importance of preserving birds for the future of humanity, existing knowledge gaps, and opportunities for research are highlighted briefly.

Figure 1 A male blackbird (*Turdus merula*) singing. Creative Commons. https://en.wikipedia.org/wiki/Bird_vocalization#/media/File:Solsort.jpg.

The Origin of Birds

Birds are commonly defined by a combination of powered flight ability and the possession of feathers and beak. Indeed, the combination of these three characteristics in the animal kingdom is found only in birds. To uncover evolutionary history through time, one must also consider the fossil record, i.e., evidence of once extinct organisms ancestral to living species. In this context, it can be seen that not all extinct

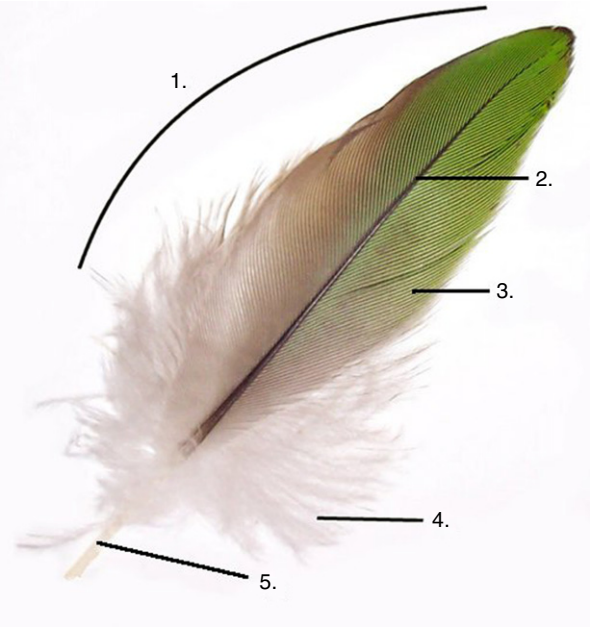

Figure 2 Parts of a feather. (a) vane, (b) rachis, (c) barb, (d) afterfeather, and (e) hollow shaft, calamus. https://en.wikipedia.org/wiki/Feather#/media/File:Parts_of_feather_modified.jpg.

Figure 3 Reconstructed skeleton of *Troodon* sp., a bird-like troodontid. Creative Commons. https://en.wikipedia.org/wiki/Troodontidae#/media/File:Troodon_Perot_Museum.jpg.

birds displayed the three 'bird' traits and that other extinct organisms, particularly dinosaurs, were the first to evolve feathers and additional anatomical traits once considered unique to birds, for example, the semilunate carpal wristbone and the wishbone-shaped furcula. The presence of lightweight feathers is so intricately tied to today's fluffy birds that it is hard to imagine that some of the more robust dinosaurs were also covered with feathers (**Figure 2**). The insight of some dinosaurs being feathered is relatively new, linked to many discoveries of exceptionally preserved fossils from China starting in the late 1990s and early twentieth century (Norell and Xu, 2005). Through these discoveries, research has concluded that feathers evolved originally for purposes other than flight (Prum and Brush, 2002), via a filamentous-pinnate-branching feather order (Clarke, 2013). Despite this ancient origin, modern flight feather anatomy remains a relatively recent phenomenon unique to birds (Feo *et al.*, 2015). Current analyses of comparative anatomy that have included the known varieties of feathered and non-feathered dinosaurs, modern and ancient birds, and other ancient reptile lineages, consistently show that birds are descendants of a particular lineage of dinosaurs, the maniraptorans (Smith *et al.*, 2015). This group of dinosaurs is one of several theropod (meat-eating) dinosaur lineages that also includes *Deinonychus* and *Velociraptor* (Dromaeosauridae) and the bird-like Troodontidae (**Figure 3**), and as a whole (a group termed Paraves) is a close cousin of *Tyrannosaurus rex*. Thus, based on current scientific consensus, birds are (living descendants of) dinosaurs.

A second consensus is appearing from the most recent comparisons of earliest bird fossils and dinosaurs: that of a muddled line between what is called a bird (avian dinosaur) and what is called a non-avian dinosaur. While this zone of evolutionary innovation is blurry and taxonomically confusing, it points out several interesting evolutionary phenomena that put the origin of birds into a sound framework: (1) birds never show close evolutionary relationships to primitive non-dinosaurian reptiles (Xu *et al.*, 2014; Smith *et al.*, 2015); (2) birds are an evolutionary outcome of continued miniaturization (decrease in body size) in a particular lineage of dinosaurs, the theropods, that already showed a tendency toward miniaturization (Turner *et al.*, 2007; Benson *et al.*, 2014; Lee *et al.*, 2014); (3) Paravian dinosaurs did not show size reduction in forelimb proportions, thus giving the first birds relatively long forelimbs (Puttick *et al.*, 2014), which promoted the evolution of flight; (4) rapid anatomical innovation occurred during the evolutionary period immediately following the origin of birds (Brusatte *et al.*, 2014); (5) many 'bird' traits are first observed in theropod dinosaurs (Makovicky and Zanno, 2011; Xu *et al.*, 2014); (6) the evolution of birds from dinosaurs is an evolutionary successful outcome from a time where several dinosaurs experimented with flight (Han *et al.*, 2014; Xu *et al.*, 2015). For example, microraptoran dinosaurs (**Figure 4**) close to the origin of birds had four instead of two wings (Xu *et al.*, 2003), using their long leg feathers as added flight devices. Gaps in our knowledge remain on the relative relationships of troodontids and microraptoran dinosaurs to birds (Xu *et al.*, 2014). Where they part of birds or closely related to them? Resolution of these issues is important because it answers what the first bird looked and behaved like (Godefroit *et al.*, 2013). Did birds evolve flight from gliding down trees like bats and other animals did, or by taking off from the ground? The answer is yet unclear.

Figure 4 The holotype of *Microraptor gui*. The arrows indicate the presence of feathers in this specimen. Creative Commons. https://en.wikipedia.org/wiki/Microraptor#/media/File:Microraptor_gui_holotype.png.

Figure 5 The restoration of the extinct penguin lineage, *Waimanu manneringi*. Creative Commons. https://en.wikipedia.org/wiki/Waimanu#/media/File:Waimanu_BW.jpg.

Diversity of Extinct (Non-Modern) Birds

Immediately preceding the boom of Chinese feathered dinosaur finds, a similar renaissance took place in our understanding of the varieties of now extinct bird lineages that ultimately gave rise to modern birds (Chiappe and Dyke, 2002). These birds are generally distinguished from modern birds by having teeth, thus lacking a true beak. Several fossil lineages are known and intricately studied, and these include primitive weak-flying birds with long tailbones such as *Archaeopteryx* (currently 11 specimens known; Foth *et al.*, 2014) and *Jeholornis* (Zhou and Zhang, 2003). More advanced toothed birds include the short-tailed exquisite flying birds (Chiappe, 2007) and are represented by the well-known and abundantly found Confusiusornithids (Chiappe *et al.*, 2008), Enantiornithids, Ichthyornithids, and Hesperornithids, and numerous unique fossil genera (e.g., *Gansus*, *Apsaravis*, *Ambiortus*, *Patagopteryx*). While many of these distinct fossil bird groups have been known since the mid-1990s (Chiappe, 1995), the evolutionary tree of toothed birds is becoming better understood with each new fossil find. This conclusion applies particularly to the taxonomy of Enantiornithine birds, the ecological diversity of toothed birds not directly ancestral to modern birds, and the anatomical traits of those extinct toothed birds (Ornithurae) that ultimately gave rise to modern (living) birds.

Timing the Evolutionary History of Modern Birds

With so much detail laid out in the fossil record about the origin and early ecological diversification of toothed birds, it is surprising to see little to no direct evidence for the events leading up to the wholesale extinction of all toothed birds at the end of the Cretaceous period (Longrich *et al.*, 2011;), and in particular for the (reason for) survival of modern beaked birds Robertson *et al.*, 2004). The fossil record here is scant (e.g., Fountaine *et al.*, 2005; Brocklehurst *et al.*, 2012), and for this reason, genetics-based studies have provided particular insight where fossils alone have been less able to do so. One must keep in mind that molecular insights provide indirect evidence of (or reconstruct rather than show directly) evolutionary history in contrast to the fossil record. Thus, to reconstruct phylogenetic relationships and divergence times of the major groups of modern birds, collaboration among paleontologists and molecular biologists has been especially sought (e.g., Dyke and van Tuinen, 2004; van Tuinen *et al.*, 2006; Ericson *et al.*, 2006; Slack *et al.*, 2006; Jarvis *et al.*, 2014).

Few fossils point to the presence of modern birds in the Cretaceous. Although contentious, several fossil representatives of the waterfowl (duck) lineage Anseriformes are known from the latest Cretaceous (Clarke *et al.*, 2005; Kurochkin *et al.*, 2002) of South America and Asia. The next earliest fossil finds are from the earliest Paleocene, following the end-Cretaceous extinction event in New Zealand, and belong to the penguin lineage *Waimanu* (**Figure 5**, Sphenisciformes; Slack *et al.*, 2006). Combined with a phylogenetic framework for living birds, the molecular clock can be used to constrain the age of Anseriformes and Sphenisciformes according to their fossil record and infer ages of other phylogenetic divergences. Using this approach, many analyses have been performed, which yield a mid-Cretaceous initial divergence among modern birds (reviewed in van Tuinen, 2009) followed by either Cretaceous diversification of many distinct bird orders (e.g., Cooper and Penny, 1997; Paton *et al.*, 2002; Brown *et al.*, 2008; Brown and van Tuinen, 2011; Pacheco *et al.*, 2011), Cretaceous and Paleogene diversification depending on order (van Tuinen *et al.*, 2006; Pacheco *et al.*, 2011), or entirely Paleogene diversification of orders (Ericson *et al.*, 2006; Jarvis *et al.*, 2014). The disparity among these findings is likely a result of varying use of fossil priors and perhaps also saturation in molecular data (van Tuinen and Torres, 2015). More analyses testing these parameters will be forthcoming in the near future. Until then, it is plausible to assume a mid-late Cretaceous divergence among the primitive modern lineages Paleognathae and Neognathae (despite absence of Cretaceous fossil evidence for Paleognathae) that is inferred from fossil evidence of the age of the Ornithurine outgroup, of the age of Neognath birds, and the consistent observation of a distinct branch length (indicating strong phylogenetic support from unique molecular differences) at the base of Neognath and Neoavian birds. Furthermore, diversification of the majority of distinct birds orders is likely post-Cretaceous (uncertain for Anseriformes, Galliformes) and

Figure 6 The eagle is a Neognath and the moas are Paleognaths. Creative Commons. https://en.wikipedia.org/wiki/Evolution_of_birds#/media/File:Giant_Haasts_eagle_attacking_New_Zealand_moa.jpg. https://en.wikipedia.org/wiki/Evolution_of_birds#/media/File:Giant_Haasts_eagle_attacking_New_Zealand_moa.jpg.

for some may be an ecological response to Eocene–Oligocene extinction (van Tuinen *et al.*, 2006). These scenarios are consistent with the overall fossil record of modern birds and with major branch length patterns seen in various molecular datasets.

Phylogeny of Modern Birds

The timing of the divergences between the major lineages of modern birds is dependent on knowledge of the phylogenetic relationships among these lineages. Fortunately, much progress in phylogenetic reconstruction of the modern bird tree has recently been made through analysis of taxonomically large and character-rich datasets (reviews in Torres and van Tuinen, 2013; Jarvis *et al.*, 2014; Mayr, 2014). At its base, the group containing all modern birds consists of two distinct lineages, the Paleognathae (tinamous and ratites) and the Neognathae (all other birds) (**Figure 6**). Neognathae itself consists of two distinct lineages, the Galloanserae (waterfowl and gamefowl, **Figure 7**) and all remaining birds (20+ orders). This latter grouping contains the Neoavian birds, which appear to have rapidly diversified into all known ecological types observed today. The latest evidence from the fossil record and genetics concurs that the diversification of an ancestral Neoavian bird into today's songbirds, parrots, aquatic birds, raptors, shorebirds, and other distinct lineages happened in a matter of only a couple million years (Ericson *et al.*, 2006; Jarvis *et al.*, 2014; Mayr, 2014). Exciting recent genetic consensus is building on a separate origin of, on the one hand, a group containing pigeons and relatives united with flamingos and grebes and on the other hand a group containing all other neoavian birds. The separation of these two groupings of ecologically disparate birds implies massive ecological and anatomical convergence in the aquatic realm (Fain and Houde, 2004). Strong genetic evidence further exists for the independent acquisition of raptorial lifestyles (Ericson

Figure 7 A male specimen of Ceylon junglefowl (*Gallus lafayetti*), a representative of Galloanserae. Creative Commons. https://en.wikipedia.org/wiki/Galliformes#/media/File:Flickr_-_Rainbirder_-_Ceylon_Junglefowl_(Gallus_lafayetii)_Male.jpg.

et al., 2006; Jarvis *et al.*, 2014). Morphological analyses support some of these findings but complete agreement is still lacking. Also, phylogenetic results from multi-locus genetic studies are often sensitive to several parameters and a complete phylogenetic tree at the ordinal level is not yet statistically secure enough. More importantly, family-level and species-level genetic and morphological studies are still anticipated for many bird lineages, and thus a full modern bird tree of life is still several years away. Despite the lack of a fully resolved phylogeny for birds, the major outer branches have now been established. This framework will prove essential in the assessment of the evolutionary origins and ecological, behavioral, and geographical breadth seen among birds, but also in promoting the conservation of birds currently under threat.

Conclusion

With today's scientific knowledge about the evolution of birds, it is easy to get excited about them as lone survivors of the dinosaurs, to observe and study them as a large spectrum of variations around a theme involving flight, high metabolic rate, and numerous specializations in food capture. As visible and audible gems, birds too are ever-present facets of daily human life, providing humans easy access to food, inspiration, and admiration. For all these reasons, it is increasingly important that we recognize the current danger of losing many wonderfully unique birds to extinction. Some bird families show particular prevalence to being under threat yet funds for putting existing conservation plans in action are frequently lacking. Without immediate action, many threatened birds may soon be lost forever. Given projected future climate scenarios for the next 50 years, it is not unlikely that a considerable portion of bird diversity that originated through a rapid evolutionary radiation over a span of a couple million years, may go extinct at a much higher, indeed alarming, rate due to human-caused habitat loss and environmental change (e.g., Pimm *et al.*, 2014). For the sake of building upon an already sound scientific knowledge base and acknowledging the importance of birds to many aspects of humanity, new students of ornithology are fortunate to build a

career during a time where data gathering is needed more than ever, whether this involves studying live birds in the field or captivity, fossil birds in the rocks, or genetic aspects of birds and their parasites in the lab.

See also: Amniotes, Diversification of. Bird Flight Origins

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C

Cambrian Explosion: A Molecular Paleobiological Overview

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Glossary

Bilateria Bilateria is the lineage of all animals except the sponges, jellyfishes, corals, comb jellies, and placozoans (the plated animal). Bilateria share a common ancestor (i.e., it is a clade) and it is characterized by a primitive bilaterally symmetrical body plan. That is, the bilaterian last common ancestor was bilaterally symmetrical.

BST Burgess Shale Type deposits. These are fossil deposits with exceptional preservation. Fossils are preserved in BST as carbonaceous compressions with or without mineralization of tissues such as guts and muscles.

Cambrian The first period of the Phanerozoic eon is called cambrian. The Cambrian spans the interval between 541 and 485 Mya. It is characterized by abundant fossil animals that can be unambiguously assigned to extant (living – not extinct) animal phyla.

Clade A group of species sharing a common ancestor is called clade.

Crown group Given any living lineage (say the placental mammals), the crown group includes all living members of that group, their last common ancestor, and all fossil members of the same group that descended from the same common ancestor.

Ediacaran This is the last period during the Neoproterozoic. The Ediacaran spans the interval between ~630 and 541 Mya. It is characterized by the emergence of macroscopic organisms in the fossil record, including animals.

Fossil biomarkers Chemical fossils extracted from sediments that may be specific to particular lineages of life and can constrain their origin in deep time in the absence of a fossil record.

Ga Billions of years ago. This acronym is used when defining a single point in time (e.g., 1.5 Ga).

Gya Billions of years ago. This acronym is used when defining a time interval (e.g., 1–1.5 Gya).

Ma Millions of years ago. This acronym is used when defining a single point in time (e.g., 541 Ma).

Metazoa A synonym for animals is called metazoa (sponges included).

Molecular clock An abstraction of the general observation that the number of differences in the genomes of two related species is a function to the time since they shared a common ancestor is called molecular clock.

Mya Millions of years ago. This acronym is used when defining a time interval (e.g., 541–520 Mya).

Relaxed Molecular Clock Methods These methods represent improvements over the Strict Clock Method (see below) and assume that the rate at which changes accumulate in the genomes of different species is lineage dependent. That is, each branch in a phylogenetic tree is assigned its own rate of change.

Stem group This group includes all taxa more closely related to an extant group than to any other extant groups, but that diverged from the extant group before the last common ancestor of the extant taxa was alive.

Strict Molecular Clock Method This was the first methodological implementation of the molecular clock. It assumed that the rate at which mutations accumulated in genomes was the same across all the species in the considered phylogeny.

Taphonomy The study of what happens between an organism death and its discovery as a fossil is called taphonomy. It includes the effects of decay and diagenesis.

Total group The Crown plus Stem group is called total group.

Trace fossils Behavioral fossils, such as burrows and borings, and feeding marks.

Introduction

Historically, the fossil record has been the principal source of information to understand animal evolution. Based on this information the Cambrian period (541–485 Mya), the first period in the Phanerozoic eon, has long been considered the cradle of animal life. More recently, the genomic record has also been used to study animal evolution and our current understanding of the origin and early evolution of animals is derived from a combined interpretation of the fossil and the genomic record of life (see Erwin *et al.*, 2011).

Herein a 'Molecular Paleobiological' overview of the Cambrian explosion and the origin of animals is provided that integrates molecular and fossil evidence to derive a

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picture based on a critical interpretation of both sources of information.

The Cambrian Fossil Record

The base of the Cambrian (541 Ma) is defined by the synchronous first appearance of *Treptichnus pedum*, a trace fossil with probing vertical and horizontal movement representing the first evidence of extensive vertical movements within a substrate. Only Bilateria have the complex muscle systems and the turgid body necessary to vertically borrow within sediments in such a fashion, and *Treptichnus* is widely accepted to represent the first unambiguous evidence for the widespread existence of Bilateria in the fossil record. Recent experiments using extant animals showed that *Treptichnus* traces were most likely produced by a priapulid-like worm (Vannier *et al.*, 2010). Priapulida are a small extant animal phylum including only eight genera with an extensive Cambrian fossil record (Smith *et al.*, 2015). They have a distinctive body plan and together with the Arthropoda (e.g., insects, spiders, crustaceans, millipedes, and centipedes), the Nematoida (round and Gordian worms), the Loricifera, the Kinorhyncha (mud dragons), the Tardigrada (water bears), and the Onychophora (velvet worms) are members of one of the three major bilaterian clades: the Ecdysozoa. Accordingly, *Treptichnus* indicates that the first two major splits in the bilaterian tree of life (separating Deuterostomia, Lophotrochozoa, and Ecdysozoa – i.e., the three main bilaterian animal lineages) happened before the Cambrian. Consequently, also divergences between non-bilaterian animal lineages (sponges, the elusive placozoans (the plated animal), jellyfishes, corals, and comb jellies), that predated the separation between the Bilateria and their sister lineage (jellyfishes, corals, and most likely also comb jellies), must also have happened in the Precambrian (**Figure 1**). The conclusion is that while the

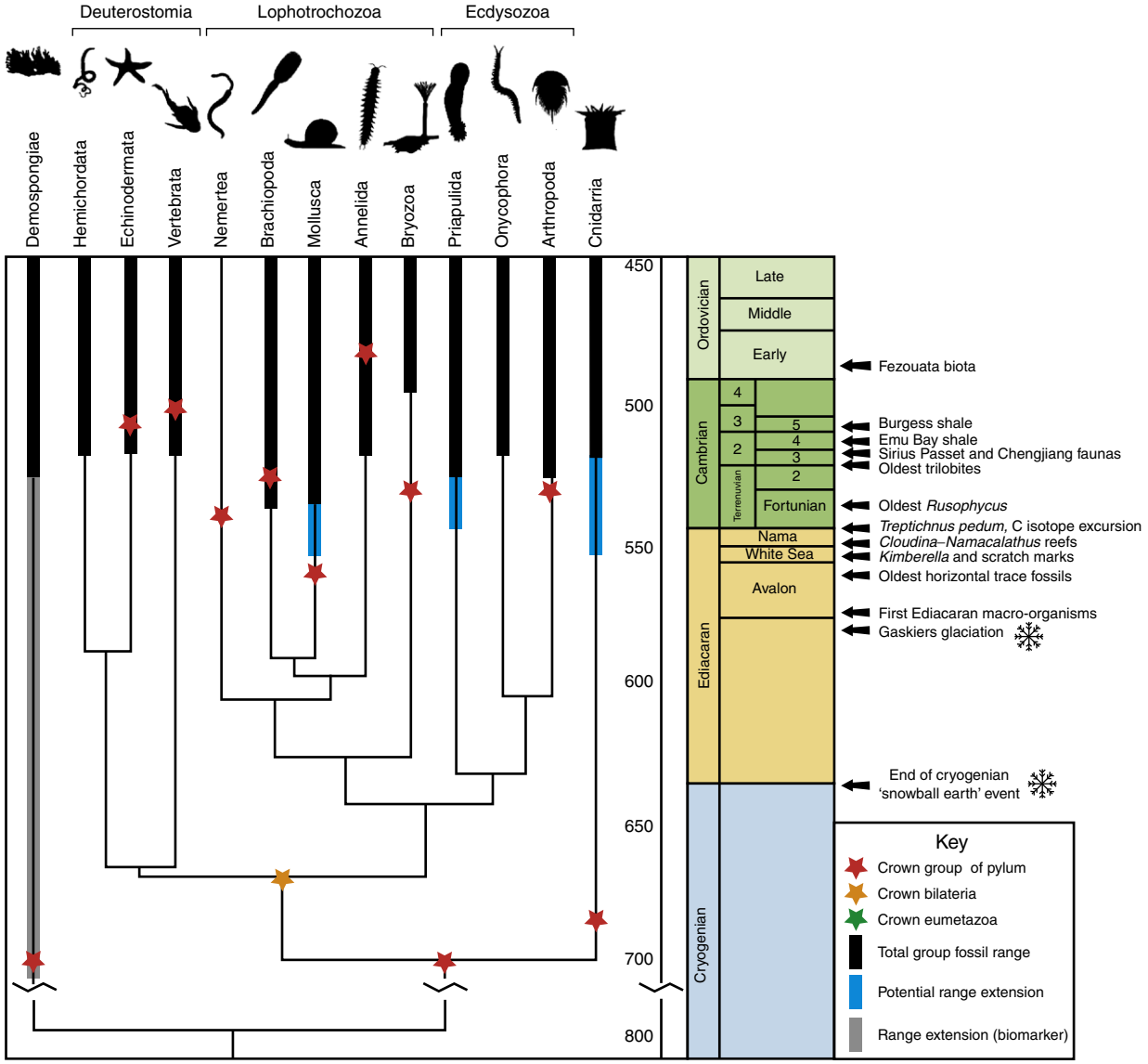

Figure 1 Molecular time tree of animal evolution after Erwin *et al.* (2011). Positions of key events on timescale to the right are approximate.

Cambrian has long been considered the cradle of animal life, fossil evidence now indicates the animal originated in the Precambrian. What is unclear is whether they originated deep in the Neoproterozoic (in the Cryogenian), or much more recently, after the last of the snowball earth event, in the Ediacaran. While animals origins might be as deep as 750 Ma (Erwin *et al.*, 2011; dos Reis *et al.*, 2015), the Cambrian still represents a crucial period in the history of animals, with the Cambrian explosion now being interpreted as the time when crown group animal lineages underwent their diversification (Erwin *et al.*, 2011). That is, the Cambrian is the time when lineages generally recognized as classes and in some cases phyla in the Linnaean hierarchy emerged.

The Cambrian fossil record is characterized by its famous 'weird wonders.' These are exceptionally well preserved fossils, from Burgess Shale type (BST) deposits that occur from Cambrian Stage 2 onwards, and include iconic species like *Anomalocaris*, *Halkieria*, and *Hallucigenia* (**Figure 3**). BST deposits also provide evidence of soft-bodied animal groups otherwise poorly known or unknown from the fossil record and place a minimum age on their origin. These include sipunculans (Huang *et al.*, 2004), tunicates (Chen *et al.*, 2003), hemichordate worms (Caron *et al.*, 2013), and chaetognaths (Vannier *et al.*, 2007). BST biotas are now known to be geographically and temporally widespread (**Figure 1**) and new localities are still being discovered (Caron *et al.*, 2014). Cambrian fossils from BST deposits, as well as the older 'small shelly fossils' (**Figure 3(f)**), and the more recently discovered small carbonaceous fossils (Butterfield and Harvey, 2012), represent exceptional windows into the early evolution of the extant animal phyla. Many Cambrian organisms belong to the stem groups of the extant phyla (for explanation, see **Figure 2** and Glossary), and consequently their morphology provides key information regarding the common ancestors of extant animal phyla, and the sequence of character transformations that led to the origin of their morphologies.

BST fossils are among the best-preserved invertebrates fossil known, and their importance for understanding animal evolution is undoubted. However, their evolutionary interpretation is not without controversies. For example, Conway Morris and Peel (1995) presented a revolutionary interpretation of *Halkieria* and used it to link the stem lineages of molluscs, annelids, and brachiopods. They proposed that *Halkieria evangelista* (**Figure 3**) was a stem-group brachiopod, advocating homology between the anterior and posterior shell and the shells of brachiopods. In this scheme annelids also evolved from a *Halkieria*-like animal, with the chaetae (bristles) of stem annelids (**Figure 3**) suggested as derived from the mineralized sclerites of halkieriids. However, the body plan and sclerite morphology of *Halkieria* has many similarities with aculiferan molluscs – chitons and their relatives (Vinther and Nielsen, 2005), and *Halkieria* is perhaps better explained as a stem aculiferan, phylogenetically predating the evolution of intermediate shell plates as seen in extant chitons (Vinther, 2015). Furthermore, the most primitive stem-group annelids have simple chaetae (Parry *et al.*, 2015), that are very different from the sclerites of halkieriids.

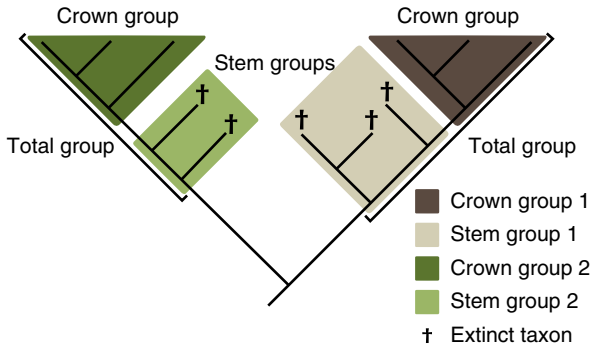

Figure 2 Graphical explanation of the stem-group, crown group, and total-group phylogenetic concepts.

Cambrian ecdysozoans have been extensively studied and include several unique extinct morphotypes, like the iconic Anomalocaridids (**Figure 3**), Cambrian apex predators with grasping frontal appendages and acute vision (Paterson *et al.*, 2011). Anomalocaridids are stem arthropods and provide evidence for the complexity of early animal ecosystems. *Tamisiocaris*, an anomalocaridid from Sirius Passet, has an appendage morphology indicative of filter feeding, but is well nested within this predatory lineage (Vinther *et al.*, 2014). The acquisition of a filter feeding ecology from a large-bodied predatory ancestor is known in modern animals (e.g., the whales) and *Tamisiocaris* thus indicates that familiar evolutionary trends and ecological phenomena known from well-understood animal lineages had been established as early as Cambrian Stage 2.

Investigations of the Cambrian ecdysozoan fossil record have uncovered candidate stem and crown group members of many of its constituent phyla. Such taxa include character combinations that can be useful to infer the morphology of the last common ecdysozoan ancestor. The long problematic *Hallucigenia* (**Figure 3**) has now been identified to belong in the onycophoran stem lineage. *Hallucigenia* has been shown to possess onycophoran like claws, but it possesses a foregut armature lined with a circlet of elements, pharyngeal teeth, and mouthparts similar to those of taxa like the priapulids (Smith and Caron, 2015). This would suggest that the last common ancestor of Ecdysozoa most likely had priapulid-like mouthparts.

Trilobites (**Figure 3**) are important Paleozoic euarthropods and first appear in Cambrian Series 2 (**Figure 1**; Liñán *et al.*, 2015). Despite their robust, mineralized skeletons, there is a lag between the origin of trilobites and their first appearance as fossils, mirroring general trends in the fossil record of animals. When trilobites first appear, they are already separated into distinct geographic faunas, suggesting a significant hidden evolutionary history (Lieberman, 2002).

It is now known that by the end of the Cambrian, all of the phyla with a mineralized skeleton must have existed. Subsequently, the Great Ordovician Biodiversification Event recorded a diversification of animals at taxonomic ranks below phylum level and also the increase of ecological phenomena like bioerosion (Servais *et al.*, 2010). The causes of the Cambrian explosion have been the subject of intense debate and range from biotic factors such as an intense predator–prey arms race to abiotic factors including the end of snowball Earth glaciations (Marshall, 2006). The role of oxygen has been particularly prominent in this discussion, and an increase in oxygen concentrations is often invoked as a driver for the

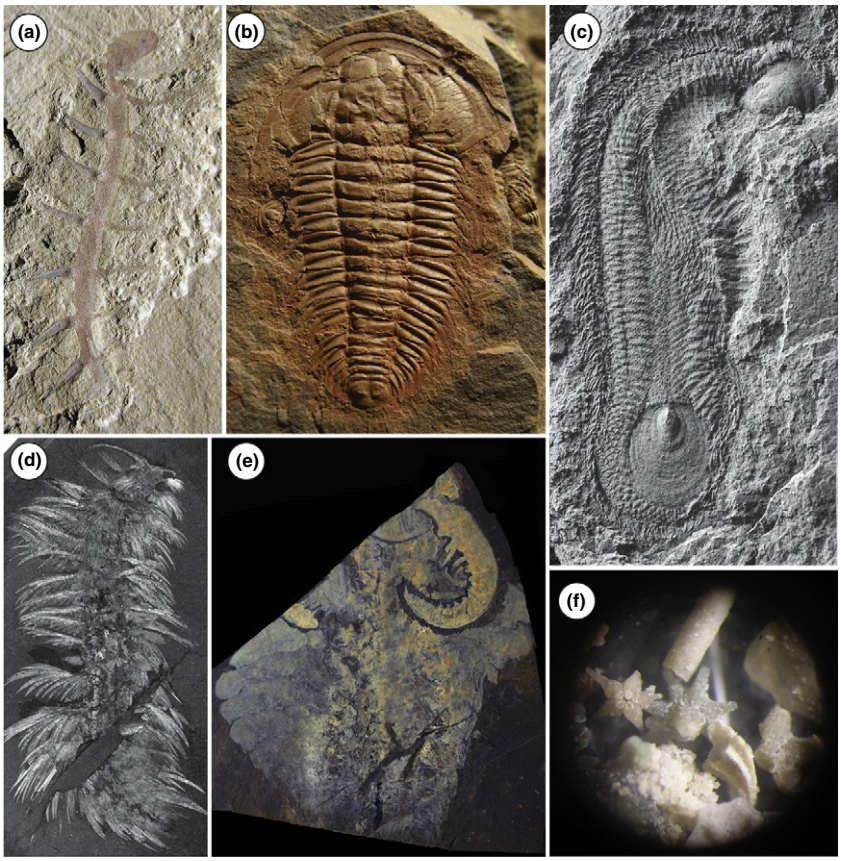

Figure 3 Cambrian fossils. (a) *Hallucigenia fortis*, a stem Onycophoran from the early Cambrian Maotianshan Shales; (b) *Redlichia takooensis* an early Cambrian trilobite from South Australia; (c) *Halkieria evangelista* an early Cambrian mollusc; (d) *Canadia spinosa*, from the middle Cambrian Burgess Shale; (e) *Anomalocaris canadens* from the middle Cambrian Burgess Shale; (f) Examples of small shelly fossils (SSFs).

origin of animals in the Neoproterozoic, for a review see Sperling *et al.* (2015).

While BSTs, small carbonaceous fossils, and the small shelly fossils offer important insights into the morphology of the earliest members of the major bilaterian lineages, Cambrian fossils are mute on the origins of animals and on the origin of the Bilateria, as these groups had a deeper (Precambrian) history. To attempt understanding the origin of animals we must thus look deeper to the Ediacaran fossil record and beyond.

The Ediacaran Record and the Origin of Animals

The Ediacaran (631–541 Mya) is the period preceding the Cambrian. Paleontological and genomic evidence suggests that minimally this is the period during which the deepest divergences within Bilateria and perhaps Metazoa took place (see above, Erwin *et al.*, 2011; dos Reis *et al.*, 2015).

When Darwin penned the origin of species, he lamented the absence of body fossils in strata older than the Cambrian (then part of the Silurian period) and consequently the lack of direct fossil evidence for the origin of animals. Darwin's disdain for the fossil record led him to assert that 'no organism wholly soft can be preserved.' Fortunately, this prediction proved to be false, and there is a wealth of soft-bodied fossils from Ediacaran rocks that document animal life before the Cambrian. When such sites were discovered, the belief that Precambrian rocks were barren led to the conclusion that the rocks, and consequently the fossils they preserve, could not possibly be from this time. This changed in 1958 with the discovery of *Charnia masoni* (Ford, 1958) in Charnwood forest in Leicestershire from unequivocally Precambrian sediments. Subsequently similar fossils, such as those from the Ediacara Hills in Australia, were later recognized to be Precambrian in age.

The oldest of the classic 'Ediacara biota' type macrofossils are known from the Avalon assemblage of Newfoundland, which appear a few million years after the Gaskiers glaciation (582–580 Mya). This assemblage contains many taxa of the rangeomorphs, a group to which *Charnia* belongs. The Avalon assemblage is then followed by the White Sea (~560–550 Mya) and Nama (~550–541 Mya) assemblages (Waggoner, 2003) that are characterized by distinct biotas with some taxonomic overlap. The White Sea assemblage is the most diverse while the Nama is significantly more taxonomically depauperate (Darroch *et al.*, 2015). The Ediacaran macrofossils were initially interpreted as relatives of extant taxa such as jellyfish and sea pens (Glaessner, 1984). Many of these interpretations were then overturned as they were based only on superficial similarity, see Antcliffe and Brasier (2007) for an

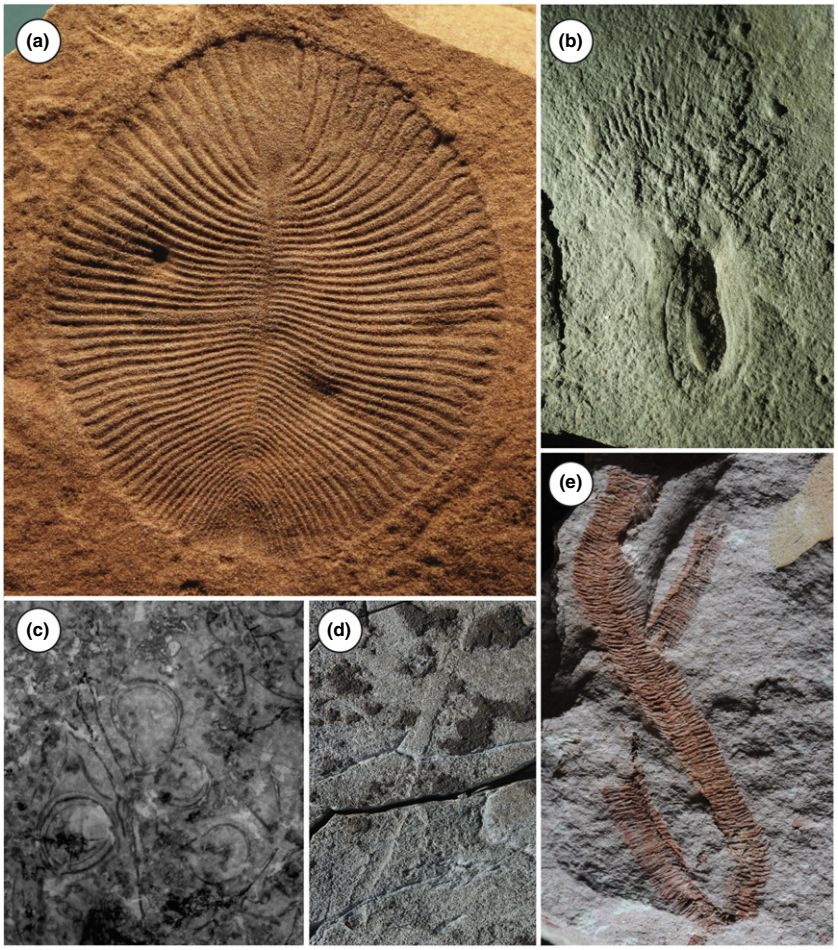

Figure 4 Ediacaran fossils. (a) *Dickinsonia costata*, Flinders Ranges, South Australia; (b) *Kimberella quadrata* from the Erginskaya formation; (c) *Cloudina sp.* from the late Ediacaran of Brazil; (d) Surface trail from the Mistaken Point Formation, Newfoundland; (e) *Corumbella werneri* a tubular cnidarian like body fossil from the late Ediacaran of Brazil.

example, leading to further reinterpretations of these fossils. Alternative interpretations have viewed these organisms as potentially belonging to every multicellular eukaryotic lineage, including a proposed independent and wholly extinct experiment in multicellularity known as the Vendobionta (Seilacher, 1992). While at least some ediacarans are considered metazoans, such as *Kimberella* and *Corumbella* (see below), the affinities of many Ediacaran organisms, like the rangeomorphs, are still unclear.

There is perhaps no Ediacaran fossil more iconic, or with a taxonomic history more tumultuous, than *Dickinsonia* (**Figure 4**), which has been considered a polychaete worm (Wade, 1972), a placozoan like animal (Sperling and Vinther, 2010), a lichen (Retallack, 1994), a cnidarian (Valentine, 1992), a protist (Seilacher *et al.*, 2003), and total group (see Glossary and **Figure 2**) Bilateria (Gold *et al.*, 2015). Due to the simple construction of these fossils, functional morphology and development may be helpful tools in determining their affinities. For example, if passive mechanisms for the formation of *Dickinsonia* feeding traces can be ruled out (Mcllroy *et al.*, 2009), *Dickinsonia* can be assumed to have been motile and muscular and consequently would have to be of a grade of organization higher than the sponges. Sperling and Vinther (2010) presented a more precise hypothesis for the affinities of *Dickinsonia* based on its mode of feeding. *Dickinsonia* fossils suggest that it was feeding with its ventral surface. This is because trace fossils with opposite relief to *Dickinsonia* fossils are generally associated with these fossils. This suggests that the whole ventral surface was digesting and absorbing organisms from the microbial mat over which *Dickinsonia* was crawling (Sperling and Vinther, 2010). Among extant taxa, feeding with the ventral surface is a phenomenon restricted to placozoans, hence the suggestion of a possible close affinity between these taxa. It is plausible that other enigmatic Ediacaran organisms fed through their external body surface as well, given that organisms like the rangeomorphs lack evidence for a mouth or gut and have a high surface area to volume ratio (Laflamme *et al.*, 2009).

From an evolutionary perspective, perhaps the most important among the Ediacaran fossils are *Kimberella* (Fedonkin and Waggoner, 1997) and annulated tubular fossils like *Corumbella werneri* (Pacheco *et al.*, 2015). *Kimberella* is generally interpreted as a total group mollusc, also because some *Kimberella* fossils have been found in association with scratching traces on microbial mats compatible with a radula-like feeding apparatus. *Corumbella werneri* shares ultrastructural similarities with both extant coronate scyphozoans (the

'crown' jellyfish – phylum Cnidaria) and the extinct conulariids (Pacheco *et al.*, 2015), potentially bridging the morphological gap between these putatively closely related groups (Van Iten *et al.*, 2006). Fossils like *Corumbella* may be the oldest animal body fossils, and if it that was the case they would extend the evidence for the existence of Cnidarians (*Corumbella*) and perhaps Bilateria (*Kimberella*) to ~550 Ma and ~558 Ma, respectively.

The ecology of the Ediacaran was different in several crucial respects to that of the Phanerozoic. Substrates were dominated by microbial matgrounds which give way to Phanerozoic style mixgrounds across the Ediacaran–Cambrian transition (Buatois *et al.*, 2014). Trace fossil diversity is limited to horizontal grazing trails and burrows (Mángano and Buatois, 2014) as well as horizontal (**Figure 4**) and vertical structures attributed to cnidarian grade animals (Liu *et al.*, 2010; Menon *et al.*, 2013). The marked difference between Ediacaran and Cambrian trace fossil assemblages highlights the fact that although motile animals were certainly present in the Ediacaran, the diversification of bilaterian clades close to the Precambrian–Cambrian boundary changed the taxonomic make up of marine communities, the geochemical environment of the substrate (with the onset of bioturbation), and the ecological interactions between organisms. While the matground environments typical of the Ediacaran do persist into the early Cambrian, the Ediacaran macroorganisms are conspicuously absent (Buatois *et al.*, 2014). This suggests that their disappearance is not the closure of a taphonomic window due to the demise of matgrounds, but instead represents a faunal turnover or mass extinction which is also supported by studies of late Ediacaran diversity and geochemistry (Darroch *et al.*, 2015).

Biomarker Evidence for a Pre-Ediacaran Origin of Animals

While there are no animal-like body and trace fossils older than ~600 Ma, fossil biomarker extends the possible existence of sponges deeper into the Cryogenian but not earlier than the late Tonian, maximally to 713 Ma (Love *et al.*, 2009). 24-isopropylcholestane has been suggested as a biomarker for Demospongiae, which is the largest of the four lineages of extant sponges (Love *et al.*, 2009), see **Figure 1**. The veracity of sponge biomarkers has been recently challenged (Antcliffe, 2013) and successfully defended (Love and Summons, 2015). The biomarker record demands a substantial gap in the sponge body fossil record, of approximately 240 Ma (Sperling *et al.*, 2010). However, early sponges might have been no more complex than simple colonies of unicellular eukaryotes that might have been difficult to fossilize as multicellular aggregates. Interestingly, the biomarker records allow for a substantial reconciliation of the molecular divergence times of the evolution of animals, and the fossil record.

Evidence from the Genomic Record

Our understanding of animal evolution has been revolutionized by the increasing application of molecular sequence data to unraveling animal phylogeny. Old concepts like Articulata (a clade of arthropods and annelids) have found little support from molecular systematics and have been replaced with Lophotrochozoa (a clade of animals with a trochophore larva or a lophophore), and Ecdysozoa (the clade of the animals that molt their cuticle). The fossil record lends support to these concepts, with fossils known that share characters with both Onycophorans, Arthropods, and Priapulida within Ecdysozoa (e.g., *Hallucigenia* – see above), and fossils that highlight the similarity between molluscs, brachiopods, and annelids (Butterfield, 1990; Conway Morris and Peel, 1995).

The relationship between molecular divergence time estimates and the Cambrian explosion have a checkered history, with early studies recovering divergence dates for both the origin of animals and of the major split within Metazoa in the Mesoproterozoic (~1.5–1 Gya – e.g., Wray *et al.*, 1996). However, such studies used simplistic assumption about the molecular evolutionary process, and practices that had been strongly stigmatized in subsequent papers. Notable among these was Graur and Martin (2004): 'Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision,' that single handedly put to rest 20 years of dubious molecular clock studies. Recent studies using recently developed (relaxed) molecular clock methods and multiple calibrations have recovered dates more congruent with the fossil record (e.g., dos Reis *et al.*, 2015; Erwin *et al.*, 2011; Parfrey *et al.*, 2011). Erwin *et al.* (2011) found that deep divergences in the animal tree of life occurred in the Precambrian, but less than 800 Ma – i.e., animals are not older than the Cryogenian or the late Tonian, while the diversification of the crown groups of the animal phyla occurred within or just before the Cambrian (**Figure 1**). This confirms the Cambrian explosion was a real evolutionary event; even if it did not correspond with the origin of animals. There are caveats to interpreting such studies, however, as uncertainties in the internal phylogeny of clades render it uncertain whether or not the crown node has been properly bracketed, such as in annelids (Parry *et al.*, 2014). Further on a more recent study by dos Reis *et al.* (2015) have exposed the sensitivity of such analyses to calibration and have questioned the precision that is achievable in molecular clock analyses deep in the animal tree of life (dos Reis *et al.*, 2015). While studies such as (dos Reis *et al.*, 2015) are welcome and represent important sanity checks, it is interesting to note that they confirmed a possible maximal age for the origin of animals not deeper than the Cryogenian, and diversification of the animal phyla close to the Cambrian or within the Cambrian. That is, the study of dos Reis *et al.* (2015) confirmed a Neoproterozoic (<800 Ma), rather than Mesoproterozoic (~1.5 Ga) origin of animals and the Cambrian radiation of the crown members of the animal phyla.

Conclusions

Both the fossil record and molecular divergence times support a significant diversification of animals close to the Precambrian–Cambrian boundary. From this perspective the Cambrian explosion has to be considered a real evolutionary event, rather then a mere increase in fossil abundances. The Cambrian fossil record documents the early history of numerous bilaterian phyla

but provides no information on the origin of the groups deeper in the animal tree. To gain information about the origin of lineages that diverged closer to the root of the animal tree we have to look at the Precambrian record and at the genomic record. Sponges are the earliest diverging animals (Pisani *et al.*, 2015) and are known from Precambrian (Cryogenian) fossil biomarkers and cnidarians are known from the Ediacaran, broadly in line with the predictions of molecular divergence times. Overall, we can conclude that there is a reasonable agreement between molecules and the fossil record, and that what we could call a 'Molecular Paleobiological Approach' is allowing a congruent picture for the origin and early evolution of animals to emerge, even if convergence of independent lines of evidence is not quite there yet. We are however hopeful that further paleontological explorations and improvement in molecular clock methods will in the next decades lead to a complete understanding of the origin and early evolution of animals.

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See also: Animal: What Is an Animal?. Developmental Paleontology and Paleo-Evo-Devo. Metazoans, Origins of. Molecular Evolution, Models of

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Glossary

Calvin–Benson Cycle Series of chemical reactions that constitute the light-independent phase of photosynthesis; occur in the chloroplasts; and use CO_2, H_2O, and ATP/NADH to produce glucose.

Enabler Trait of an organism which facilitates the evolution of another trait.

Epiphytism Plant life strategy involving growing on the surface of another plant.

Molecular dating Technique used to date biological events based on the number of changes accumulated in DNA molecules, calibrated with absolute time data from the fossil record.

Monophyletic A group of organisms consisting of all the descendants of a single ancestral species.

Photorespiratory cycle Series of energetically costly reactions which recycle the three carbon product that results from the oxygenation reaction of RuBisCO.

Photosystem Protein complexes which facilitate the conversion of light energy into chemical reducing power during photosynthesis.

Phylogeny The study of the patterns of branching of populations of organisms into subpopulations or new species.

Stomata Pores found in the epidermis of plants through which exchanges with the atmosphere occur.

It is remarkable that almost all assimilation of inorganic carbon into food chains around the world is performed by a single enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Raven, 2013). This enzyme is used by all photosynthetic organisms for the fixation of atmospheric CO_2 in the Calvin–Benson cycle, which constitutes the light-independent phase of photosynthesis. Despite this quasi-universality, the enzyme seems rather poorly suited for the current conditions of Earth (Tcherkez *et al.*, 2006). Rubisco is estimated to have evolved more than 2.7 billion years ago (Nisbet *et al.*, 2007), on a version of planet Earth that was very different. The atmosphere of this time was extremely rich in CO_2 and almost devoid of O_2 (Kasting, 1993). Rubisco happened to evolve with a propensity to confuse the O_2 and CO_2 substrates, two featureless molecules (Tcherkez *et al.*, 2006). This was not problematic in the O_2-free environment of Rubisco early history and was consequently not counter-selected. However, O_2 became very abundant in the atmosphere following the expansion of photosynthetic organisms some 2.4 billion years and CO_2 levels continuously decreased during Earth's history (Bekker *et al.*, 2004; Kaufman and Xiao, 2003). The emergence of an atmosphere where O_2 is more abundant than CO_2 revealed the flaws of Rubisco to natural selection (Sage, 1999; Christin and Osborne, 2013). When O_2 is abundant, it will be incorporated in a significant proportion of Rubisco reactions. The products of O_2 fixation by Rubisco are toxic and need to be recycled by the photorespiratory cycle (Ogren, 1984). This cycle consumes energy to release CO_2, and can therefore be considered a wasteful process. In total, up to 29% of light energy is dedicated to photorespiration in the current atmosphere (Skillman, 2008), which strongly decreases plant productivity in conditions where relative CO_2 concentration is low (Zelitch, 1973).

Despite its flaws, Rubisco was never replaced by a better CO_2-fixing enzyme, even though some exist in other pathways (reviewed in Rothschild, 2008). This is probably because it was too integrated in the photosynthetic metabolism, which happens to be the most successful autotrophic process. Rubisco enzymes with higher specificity were however gradually selected, which came at the expense of catalytic efficiency (Tcherkez *et al.*, 2006; Young *et al.*, 2012). In conditions where CO_2 depletion is strongest, this evolutionary fix reached its limits and plants had to find additional tricks to prosper. One of these is represented by the carbon-concentrating mechanisms (CCMs), which solve Rubisco's deficiencies by concentrating CO_2 around the enzyme, reducing the relative concentration of oxygen and therefore the amount of photorespiration (Sage, 1999; Christin and Osborne, 2013). In land plants, the most frequent CCMs are C_4 and crassulacean acid metabolism (CAM) photosynthesis, two adaptive novelties that represent exceptional evolutionary and ecological success stories. In this article, we will review the history of C_4 and CAM plants, from their evolutionary origins to their recent diversification across the globe.

C_4 and CAM Photosynthesis, Two Adaptations that Reduce Photorespiration

C_4 and CAM operate using the same biochemical mechanism, but diverge in their spatiotemporal organization. They use an enzyme other than Rubisco to fix atmospheric CO_2 into organic compounds, namely phosphoenolpyruvate carboxylase (PEPC). This enzyme has no affinity for O_2, but its product cannot be directly integrated into the Calvin–Benson cycle. Instead, the resultant four-carbon product is transformed and transported via different carbon shuttles until CO_2 is finally released by one of three possible decarboxylating enzymes to feed Rubisco and the Calvin–Benson cycle (Osmond, 1978; Hatch, 1987). Other enzymes are then involved to regenerate the intermediate compounds of the cycles (**Figure 1**). These additional enzymatic reactions increase the energetic cost of carbon fixation (Kanai and Edwards, 1999).

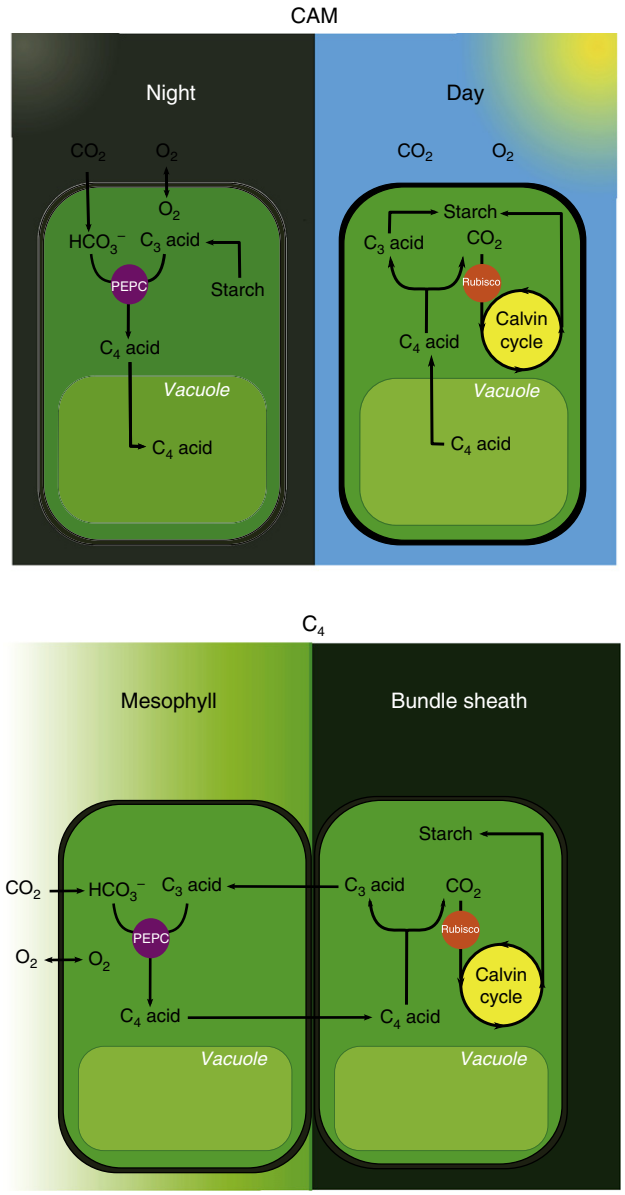

Figure 1 Schematic of C_4 and CAM cycles. In classical CAM, gas exchanges with the atmosphere take place at night. At this time, PEPC is expressed and catalyzes the fixation of atmospheric CO_2 (in the form of HCO_3^-) to a C_3 acid to create a C_4 acid, which is stored in the vacuole (usually in the form of malate). In the day, PEPC activity is downregulated and Rubisco activity is upregulated. The C_4 acid leaves the vacuole and is decarboxylated, releasing CO_2 which can be fixed by Rubisco and used to synthesize starch via the Calvin cycle. In C_4, gas exchanges with the atmosphere take place primarily in the mesophyll cells. There, PEPC is expressed and catalyzes the fixation of atmospheric CO_2 (in the form of HCO_3^-) to a C_3 acid to create a C_4 acid, which is actively transported into the bundle sheath cells. Here it is decarboxylated, releasing CO_2 which can be fixed by Rubisco and used to synthesize starch by the Calvin cycle. Note in both cases the separation of Rubisco activity and atmospheric oxygen.

In both CCMs, PEPC acts as an additional filter on the atmospheric gases that can reach Rubisco. The main consequence of this filter is that mostly CO_2 is available for Rubisco, and photorespiration is strongly decreased (Skillman, 2008). This effect requires a segregation of PEPC and Rubisco activities, and an isolation of Rubisco from atmospheric gases. This is achieved spatially in C_4 plants and temporally in CAM plants (Figure 1).

In C_4 plants, PEPC and Rubisco activities are synchronized and happen during the light period, when the photosystem is active and provides ATPs (Hatch and Osmond, 1976). PEPC activity is localized in compartments within the leaf that are in direct contact with atmospheric gases, which reach them via diffusion through the stomata (Lundgren *et al.*, 2014). Rubisco activity is segregated in compartments that are nested deeper within the leaf, where contact with the atmosphere is limited. In most C_4 plants, PEPC is localized in mesophyll cells while Rubisco is segregated in bundle sheath cells, which surround the veins and are encircled by mesophyll cells (Figure 1; Lundgren *et al.*, 2014). This segregation can however be

achieved within a single cell, in which case PEPC and Rubisco are segregated in different areas generated through a reorganization of subcellular components (Edwards *et al.*, 2004).

The main effect of the C_4 trait is to decrease photorespiration, but this benefit is partially offset by the extra energetic cost of the C_4 reactions, so that it is advantageous only in conditions where photorespiration rates are high (Ehleringer and Björkman, 1977). While low atmospheric CO_2 concentration is a necessary precondition for photorespiration, temperature plays an important role too. The solubility of CO_2 decreases faster with temperature than that of O_2 and the specificity of Rubisco decreases with temperature, so that relative O_2 concentration increases with leaf temperature (Ku and Edwards, 1978; Jordan and Ogren, 1981). C_4 is consequently mainly advantageous in warm climates, where most C_4 plants are distributed (Ehleringer *et al.*, 1997). Aquatic plants can similarly gain an advantage from the C_4 trait in high-light, high-temperature environments with limited dissolved CO_2 (Keeley and Rundel, 2003). Because C_4 plants can photosynthesize at low CO_2 concentrations, they can also maintain carbon assimilation despite limited exchange with the atmosphere. This allows for a closure of stomata, which limits water loss and provides an advantage in arid and saline conditions (Osborne and Sack, 2012). Finally, the C_4 trait increases the number of CO_2 molecules fixed per Rubisco protein, which improves nitrogen-use efficiency and can confer an advantage in nutrient-poor environments (Brown, 1978). In summary, the C_4 CCM is advantageous in all conditions where the benefit of reducing photorespiration offsets the additional energetic requirements (Sage *et al.*, 2012; Christin and Osborne, 2014).

In CAM plants, PEPC and Rubisco activities are not synchronized. Instead, the fixation of atmospheric CO_2 happens during the night, and the produced C_4 acids are stocked as malate in the vacuole. CAM is often associated with succulence, where large vacuoles allow the storage of high concentrations of malate (Nelson and Sage, 2008). The CO_2 is extracted from malate during the light period to feed Rubisco and the Calvin–Benson cycle, whose activity is synchronized with the photosystem (Osmond, 1978). The main consequence of CAM is to allow closure of stomata during the day, as atmospheric gases are sequestrated in the plant during the night (Osmond, 1978). Stomatal opening leads to water losses through transpiration, which is exacerbated during the warmth peaks of the light period. With water-use efficiencies up to sixfold higher than non-CAM plants (Borland *et al.*, 2009), CAM plants are consequently highly adapted to arid climates and saline environments, and several plants develop a CAM cycle only during periods of drought or high salt (Winter and Holtum, 2014). Some CAM plants can, in periods of extreme drought, keep their stomata closed throughout the diurnal cycle and recycle respiratory CO_2, which does not sustain growth but allows the plant to maintain functionality until more water is available (Lüttge, 2004). CAM species are also present in dry microenvironments in otherwise well-watered climates – in tropical forests, epiphytism is a life strategy that is linked to limited water availability and there are consequently many CAM epiphytic orchids, bromeliads, and ferns (Griffiths, 1989). CAM can even be advantageous when plants are fully immersed in water, which restricts CO_2 uptake during the day due to the low diffusivity of CO_2 in water (Keeley, 1998; Pedersen *et al.*, 2011). When CO_2 fixation continues during the day, the night fixation of CO_2 by PEPC extends the period of CO_2 uptake, potentially up to 24 h. This process has also been shown to enhance carbon uptake in bromeliads in cloud forests where dew can inhibit gas exchange (Pierce *et al.*, 2002). As in C_4, the additional reactions of CAM have an energetic cost and CAM plants are generally associated with low growth rates (Lüttge, 2004). Closing stomata comes at a cost, as it prevents oxygen produced during photosynthesis to efflux from leaves, potentially increasing oxygenic stress toward the end of the day (Lüttge, 2002). In summary, CAM is associated with a variety of lifestyles, and similar to C_4, can be associated to a number of ecological factors that all result in different ways from low CO_2 availability (Edwards and Ogburn, 2012).

Evolutionary Origins of C_4 and CAM Photosynthesis

The C_4 and CAM CCMs are traits of impressive complexity, which result from the coordinated action of multiple anatomical and biochemical components. Despite this complexity, each of them evolved multiple times independently. Over the last 15 years, phylogenetic efforts have elucidated the relationships between C_4 plants and those lacking this trait (e.g., Giussani *et al.*, 2001; Kadereit *et al.*, 2003, 2012; Grass Phylogeny Working Group II, 2012). These efforts have identified numerous monophyletic C_4 groups separated by other photosynthetic types in the phylogenetic trees. While some phylogenetic patterns might be interpreted in some cases as either multiple C_4 origins or fewer origins followed by losses of the C_4 trait (Duvall *et al.*, 2003; Ibrahim *et al.*, 2009), anatomical and biochemical differences among monophyletic C_4 groups as well as differences in the identity of genes co-opted to evolve the C_4 trait clearly point to a predominance of C_4 origins over losses (Christin *et al.*, 2010), and it is estimated that C_4 originated more than 62 times independently in angiosperms (Sage *et al.*, 2011). The number of CAM origins is not known with confidence, mainly because establishing whether specific plants are able to perform CAM can be challenging. For instance, some plants can switch to CAM depending on the environmental conditions or have a CAM cycle contributing to only part of their carbon assimilation (Winter and Holtum, 2014; Winter *et al.*, 2015). In such cases, determining the photosynthetic type requires detailed physiological analyses. Despite this uncertainty, CAM is present in distant phylogenetic groups, including lycophytes, gymnosperms, monocots, and eudicots, and multiple origins are established in some groups (e.g., Crayn *et al.*, 2004; Bone *et al.*, 2015). Overall, it is estimated that the tally of CAM origins might even exceed the number of C_4 origins (Edwards and Ogburn, 2012).

The apparent paradox between the complexity of CCMs and their recurrent origins is likely explained by the presence of CCM-like components in ancestors lacking these physiological adaptations. First, enzymes of the C_4 and CAM pathways exist in all plants, although they are ancestrally responsible for other functions (Aubry *et al.*, 2011). They can in some cases be already abundant in photosynthetic organs,

and it has been reported that C_4-like cellular localization of some enzymes existed before C_4 photosynthesis (Hibberd and Quick, 2002; Brown *et al.*, 2010). The C_4-specific expression of some enzymes, moreover, co-opted pre-existing regulatory mechanisms (Brown *et al.*, 2011). Low levels of CO_2 fixation in the dark, a possible precursor to CAM, have similarly been detected in some plants (Ikeda and Yamada, 1981; Winter and Holtum, 2015), and the similarity of the reactions controlling stomata opening to CAM has been suggested as a possible source of genetic material for CAM evolution (Cockburn, 1981). C_4-like anatomical features also existed before the C_4 physiology (Muhaidat *et al.*, 2011), and it has been shown that C_4 emerged from groups of plants that possessed C_4-like bundle sheaths (Christin *et al.*, 2011b; 2013). Succulence, which is associated with enhanced water storage as well as providing a large vacuole for malate accumulation, has been suggested as an enabler of CAM evolution (Sage, 2002) and indeed osmotically active malate accumulation in the vacuole can potentially facilitate water uptake (Lüttge, 2004).

The evolution of CCMs consisted of the co-option of all the required anatomical and biochemical components. It is established that this happened in a stepwise manner for C_4, with the existence of evolutionary stable intermediates (Sage, 2004; Christin *et al.*, 2011b; Sage *et al.*, 2012). These intermediates include plants with different degree of C_4 physiology, such as several yellowtops (*Flaveria*), some heliotropes (*Heliotropium*), and the perennial wall-rocket (*Diplotaxis tenuifolia*; Sage *et al.*, 2011). They are characterized by a weak CCM that relies on the segregation of photorespiratory reactions between the mesophyll and bundle sheath cells (Sage *et al.*, 2012). This trait, referred to as the C_2 pathway, uses anatomical features that are close to the C_4 requirements (Sage *et al.*, 2014) and might be advantageous in warm and dry conditions (Vogan and Sage, 2011). It therefore bridges the gap between the ancestral condition and C_4 plants, and models have suggested that the C_4 trait can be assembled through successively advantageous mutations from a C_2 ancestor (Heckmann *et al.*, 2013; Mallman *et al.*, 2014). Low levels of night-time CO_2 fixation, including recycling of respired CO_2, coupled with mostly Rubisco-based daytime photosynthesis, allows to reduce stomatal conductance during the day and thus improve water-use efficiency. This physiological strategy can lead to the evolution of better integrated CAM systems, which further limit water losses, therefore potentially acting as evolutionary intermediates (e.g., Sage, 2002; Edwards and Ogburn, 2012). The concentration of CCM-like components in some lineages of plants eases the transition toward C_4 or CAM photosynthesis, and likely explains both the repeated origins of CCMs and their clustering in some parts of the phylogenetic tree (Sage, 2001; Sage *et al.*, 2011; Edwards and Ogburn, 2012; Christin and Osborne, 2013).

Selective Pressures and Species Diversification

While many environmental factors can be linked to the selective advantages of CCMs, changes in atmospheric gas concentrations are thought to be a necessary precondition for C_4 and CAM photosynthesis. Indeed, C_4 is predicted to gain an advantage at high temperatures only in extremely low CO_2 concentrations (Ehleringer and Björkman, 1977; Ehleringer *et al.*, 1997) and the advantages of CAM in arid conditions are similarly tightly linked to CO_2 levels (Edwards and Ogburn, 2012). Molecular dating confirmed that all C_4 plants evolved in the low-CO_2 atmosphere that persisted for the last 30 million years (Christin *et al.*, 2008; Christin *et al.*, 2011a), a time that might also have seen the emergence of some CAM groups, although earlier origins are possible (Edwards and Ogburn, 2012, Keeley *et al.*, 2012). While low CO_2 seems a necessary precondition to CCM evolution, it is not sufficient, and other factors that exacerbate photorespiration probably promoted the evolution of C_4 or CAM in the different lineages. In grasses, C_4 photosynthesis evolved in open habitats of the warm regions (Osborne and Freckleton, 2009; Edwards and Smith, 2010), while C_4 origins in Caryophyllales happened in dry and saline environments (Kadereit *et al.*, 2012). In Bromeliaceae, CAM origins were more frequent in epiphytic taxa (Givnish *et al.*, 2014), and aridity is generally seen as the main driver of CAM evolution (Keeley *et al.*, 2012).

The evolutionary origins of CCMs are not linked in time to their ecological dominance. While C_4 origins are spread during the last 30 million years, the rise of C_4-dominated ecosystems is apparent in the fossil record in the last ten million years, and was driven mainly by C_4 grasses, which replaced either forested or open biomes depending on the geographical location (Edwards *et al.*, 2010). This ecological dominance is also linked to increased numbers of species. The C_4 trait has indeed be shown to increase diversification rates, but again, this occurred long after the initial emergence of C_4, suggesting that C_4 diversification was influenced by other phenotypic traits and ecological changes in addition to the photosynthetic type (Bouchenak-Khelladi *et al.*, 2014; Spriggs *et al.*, 2014). The Miocene, which saw the rise to dominance and increased diversification of C_4 grasses, also witnessed the convergent radiation of the major CAM lineages, including cacti and other groups typical of arid climates (Arakaki *et al.*, 2011; Horn *et al.*, 2014). The selective pressures for the origins of the C_4 and CAM CCMs are therefore likely decoupled from those that increased their ecological success. While Oligocene CO_2 decline and local ecological conditions promoted the origins of CCMs, the aridification and expansion of open habitats during the Miocene likely triggered the expansion and species diversification of both CCMs.

Effect of CCMs on the Ecological Niche

Both CCMs confer a number of physiological characteristics that are potentially influenced by other attributes of the plant and can confer advantages in various conditions depending on how the new photosynthetic types are integrated within the organism. It is therefore not surprising that the ecology of plants with CCMs reflects that of the ancestors lacking such CCMs (Edwards and Ogburn, 2012; Christin and Osborne, 2014). The ecology of plants with CCMs is however also influenced by the changes that happened after the origin of the CCM. In grasses, inferences of the ancestral ecological niches concluded that C_4 lineages shifted to slightly more arid conditions compared to their ancestors (Edwards and Smith, 2010), and C_4 has been shown to statistically increase the rate of transition to both arid and saline

habitats (Osborne and Freckleton, 2009; Bromham and Bennett, 2014). C_4 can therefore be considered as a niche opener. A recent study of C_4 ecological effects within a single species complex showed that C_4 initially broadened the niche without shifting it (Lundgren *et al.*, 2015). This would allow young C_4 groups to explore new areas of the ecological space, with a possible subsequent specialization to more extreme conditions (Christin and Osborne, 2014). The evolution of CAM photosynthesis similarly facilitated the colonization of new niches. It has been suggested that CAM-like physiology might promote succulence, strengthening the ecological association to arid environments in some groups (Edwards and Ogburn, 2012). Phylogenies have confirmed that CAM facilitated the transition from humid to dry habitats in some terrestrial orchids (Bone *et al.*, 2015) and *Kalanchoe* (Kluge *et al.*, 2001). The ability of many CAM plants to plastically get rid of CAM photosynthesis may also open niches in stressful, changing environments such as near the shore in lakes (Aulio, 1985), and is thought to contribute to the wide range of niches occupied by the *Clusia* genus of trees (Lüttge, 2008).

Concluding Remarks

C_4 and CAM photosynthesis are complex traits that were recurrently assembled by plants through the co-option of multiple anatomical and biochemical components. They represent evolutionary strategies to address the affinity of Rubisco for O_2, inherited from cyanobacteria, billions of years ago. Their main effects are to decrease photorespiration and increase water-use efficiency, which allow the colonization of a diversity of habitats. This includes warm, arid, saline, low-nutrient, and aquatic habitats, all of which lead to a depletion of internal CO_2 concentrations. These ecological attributes favored the spread and diversification of C_4 and CAM lineages during the Miocene, when open and arid environments expanded. In addition, the C_4 and CAM traits enable the exploration of new ecological niches when integrated with the other attributes of the organism. Overall, these properties contributed to the ecological success of C_4 and CAM plants, which nowadays cover most of the open habitats in the tropic and subtropical regions of the globe. C_4 photosynthesis alone is estimated to contribute to 25% of terrestrial primary production (Still *et al.*, 2003), and C_4 grasses have shaped multiple biomes, with an influence on major groups of herbivores, strong impacts on human evolution, and a key role in the feeding of human population (Sage and Stata, 2015).

See also: Carbon Relations, the Role in Plant Diversification of

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Carbon Relations, the Role in Plant Diversification of

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Glossary

Biogeochemistry The integrated study of processes that involve chemical exchanges between living and nonliving systems at a global scale.

C4 photosynthesis A suite of anatomical and physiological adaptations in several lineages of plants, notably about half of all grass species, to concentrate CO_2 in and limit photosynthesis to bundle sheath cells that starts by fixing atmospheric CO_2 in a 4-carbon compound.

Calcifier An organism that precipitates calcium carbonate ($CaCO_3$) compounds from carbonate (CO_3^{2-}) ions dissolved in seawater.

Carbon pool A large reservoir of carbon compounds with relatively homogeneous dynamics, connected to other such pools via fluxes of organic and inorganic carbon compounds.

Residence time A model-based estimate for the average length of time a unit of carbon resides in a particular carbon pool.

Rock weathering The temperature-dependent chemical reaction of carbonate and silicate minerals with carbon acidic carbon compounds in rainwater or soils.

Slow carbon cycle The slow and largely geological exchange of carbon compounds from minerals in the crust, to dissolved carbon in the oceans, to carbonate minerals in the seafloor, to CO_2 emitted with volcanic eruptions.

Thermohaline circulation The movement of currents driven by variation in seawater density that arises from geographic gradients in marine temperature and salinity.

Tracheophyte An evolutionary lineage, also known as vascular plants, which includes clubmosses, flowering plants and their common ancestor, that is distinguished from other plants by lignified vascular tissues among other shared derived traits.

White rot A form of wood decay mediated by peroxidase enzymes that leaves whitish residues of crystalline cellulose.

Contemporary Carbon Cycle and Roles for Organisms

From an earth system perspective, the global carbon cycle consists of an interconnected set of carbon pools. A carbon pool represents a large reservoir of carbon compounds, generally measured in petagrams (10^{15} g, Pg), which exchanges carbon with other pools via fluxes of organic and inorganic carbon compounds. Modeling carbon pool dynamics, including the average residence time of carbon in any pool, requires simplifying assumptions that vary with the balance between precision and generality demanded by a particular research question. Consequently, the delineation of carbon pools varies from study to study. For a general understanding of the contemporary carbon cycle as it relates to biodiversity, the basic dynamics are captured by four carbon pools (Figure 1).

Atmosphere

The atmospheric carbon pool is relatively small (762 Pg) but cycles quickly, having a residence time of only three years (Sabine *et al.*, 2004). The short residence time reflects huge fluxes to other carbon pools. Each year, the atmosphere loses and regains over 190 Pg of carbon largely to photosynthesis on land (54%) or in the oceans (46%) (IPCC, 2013). Almost the same quantity of carbon returns to the atmosphere with respiration from plants and the heterotrophs they support. The biological drivers of atmospheric carbon dioxide concentrations are evident in its annual cycle (Figure 2). After the autumnal equinox, as day length and temperature fall in northern forests, the relative rate of photosynthesis falls. The atmospheric concentration of CO_2 concentrations rises to a peak 6 ppm higher in May, at which point increasing productivity during the northern spring drives a steep decline in atmospheric CO_2. The amplitude of this cycle reflects the massive effect of terrestrial ecosystems, and its sinusoidal shape reflects the near match of global photosynthesis and respiration across years. However, slight differences between input and output can drive changes in the size of the atmospheric carbon pool through time, over decades to millions of years.

Just as the short-term dynamics of the atmospheric carbon cycle are driven by the biological activity, biological dynamics are subject to feedback from atmospheric carbon through its effects on global climate. The two most abundant atmospheric carbon compounds, CO_2 and CH_4, are also among the two most important greenhouse gases (IPCC, 2013). Both transmit solar radiation but absorb and re-emit infrared radiation from the Earth's surface. Without the resulting greenhouse effect, Earth's surface would freeze, precluding the existence of life as we know it. Life also responds to variation in temperature associated with changes in greenhouse gas concentrations. The effects of climate change on biodiversity are complex and depend on both the direction and rate of change (Mayhew *et al.*, 2008; Oberle and Schaal, 2011). Today, habitats in warmer regions are more biodiverse, suggesting that a warmer world may accommodate more species (Allen *et al.*, 2002; Hawkins *et al.*, 2003). While this biogeographic pattern is clear, the mechanism driving it and the validity of the prediction are much more uncertain (Currie *et al.*, 2004). Compared to our incomplete understanding of how the direction of climate change impacts biodiversity, the effect of the rate of change is unambiguous: rapid climate change has accompanied every known mass extinction (Twitchett, 2006).

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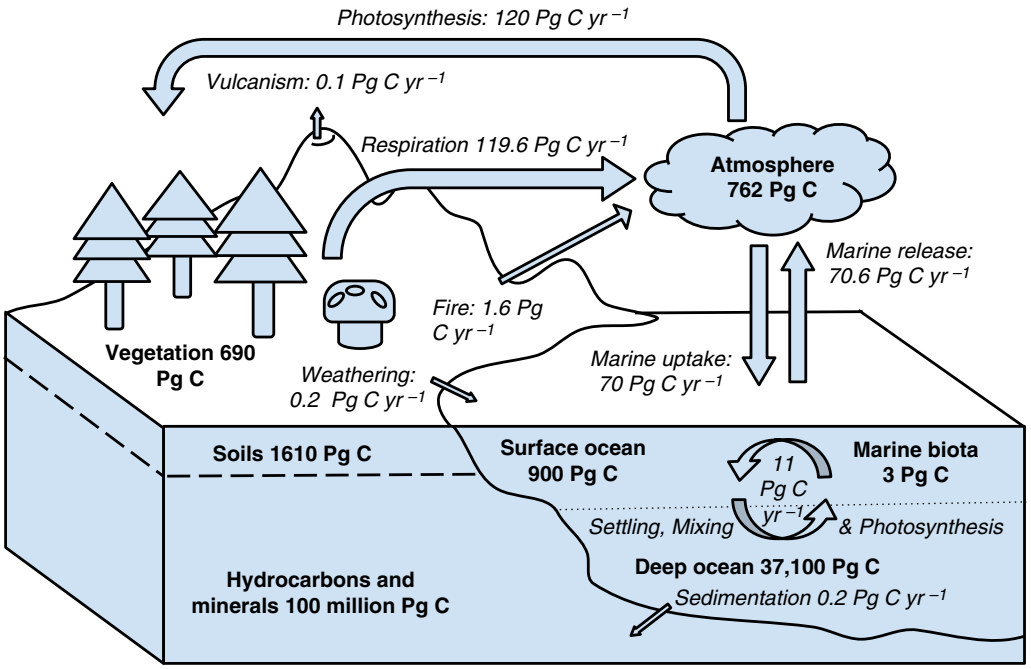

Figure 1 A representation of the natural carbon cycle in the 1990s based on estimates from IPCC (2013) with modifications from Sabine *et al.* (2004). Carbon pools are listed in bold while fluxes are in italic. Fluxes associated with human activity are not represented.

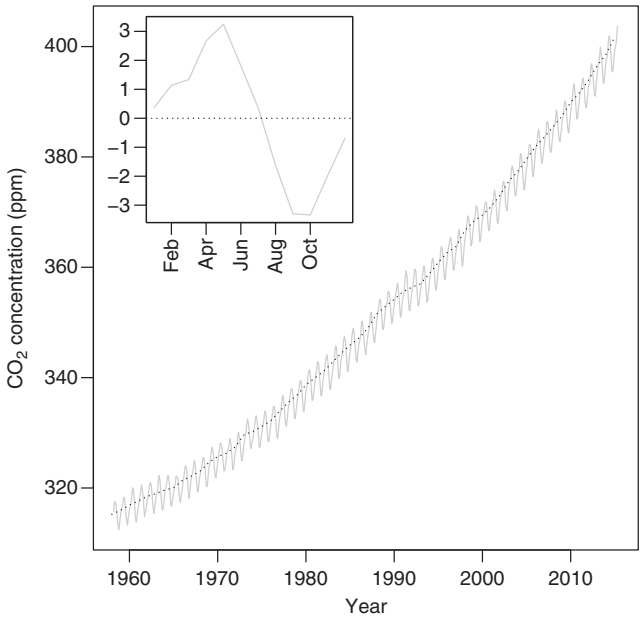

Figure 2 The instrumental record of atmospheric CO_2 concentrations as recorded at the Mauna Loa observatory curated by the Scripps Institute of Oceanography (https://scripps.ucsd.edu/). The inset shows monthly average deviations from the larger increasing trend.

Crust

While the atmosphere is the smallest and most quickly cycling major carbon pool, the largest and slowest cycling carbon pool resides in the Earth's crust. This carbon consists of two different kinds of compounds: hydrocarbons and carbonate minerals. Hydrocarbons represent approximately 4000 Pg of carbon bonded only to hydrogen that form when biological tissues sediment under anaerobic conditions. While hydrocarbons represent a huge carbon pool, 10 000 times more carbon is stored in other sedimentary rocks. The largest mineral reservoir of carbon is limestone, a sedimentary rock composed primarily of calcium carbonate ($CaCO_3$). Much of the calcium carbonate in limestone was produced by marine organisms with calcified shells, such as corals and foraminifera (Ridgwell and Zeebe, 2005). The abundance of their fossils in

many limestones, like roots of fossil trees above coal seams, attests to the biological origins of the geological carbon pool.

Exchange rates between the crust and other pools are small (0.1–0.5 Pg yr^{-1}) and residence times last millions of years. Nevertheless, this slow carbon cycle plays a critical role in regulating Earth's climate. Carbonate and silicate rocks react with atmospheric CO_2 at a rate which increases with temperature. Consequently, warm conditions tend to reduce the concentration of this key greenhouse gas, acting like a global thermostat over very long timescales. The buffering effect of the slow carbon cycle on climate is the most likely explanation for the relative stability of Earth's surface temperatures despite the gradual 40% increase in solar radiation since Earth's origins (Bonan, 2010).

Oceans

Besides the crust, Earth's oceans form the next largest carbon pool. The dynamics of oceanic carbon largely depend on depth (IPCC, 2013). The deep ocean, like earth's crust, forms a relatively large (37 100 Pg) and stable carbon pool, with a residence time near 1000 years. Over this timescale, the deep ocean gains carbon from settling detritus and loses it to the crust through sedimentation. The deep ocean also exchanges carbon via thermohaline circulation with the smaller (900 Pg) and more dynamic surface ocean. The surface ocean, which exhibits carbon residence times of months to years, directly exchanges CO_2 with the atmosphere (Cerling *et al.*, 2005). Once dissolved, CO_2 reacts with seawater to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. These forms of dissolved inorganic carbon become available to calcifiers which drive sedimentation and primary producers which transfer dissolved and particulate organic carbon to the rest of the marine food web.

Compared to the geological carbon pool, carbon stored in Earth's oceans is more dynamic and entails important feedbacks with living organisms. One such feedback involves corals and their photosynthetic symbionts (Hoegh-Guldberg *et al.*, 2007). As atmospheric CO_2 increases, surface temperatures increase and pH decreases, reducing the rate of calcification by corals and driving the rejection of their photosynthetic symbionts. Both factors may limit oceanic CO_2 absorption and generate positive feedback that amplifies warming. Because corals form the backbone of reefs, one of the most biodiverse habitats on Earth, reduced coral productivity may portend major extinctions (Carpenter *et al.*, 2008). A contrasting pattern may prevail in some seagrass communities which are less extensive and diverse but more productive than corals. Under naturally elevated CO_2, some seagrasses grow faster and appear to shelter associated calcifying organisms (Garrard *et al.*, 2014). These contrasting dynamics of coral reefs and seagrasses illustrate how the physiology of species that characterize biomes may influence how carbon cycle and climate change interact with diversity.

Land

Life dominates carbon cycling on land. The terrestrial carbon pool is intermediate in size between the atmosphere and oceans, containing 1300 Pg C. Of this, more than two-thirds is stored in soils primarily as the living and dead tissues of organisms. Most of the rest is stored in living plant biomass. Turnover rates in the terrestrial carbon pool are also intermediate between atmosphere and ocean, and vary between soil and plant biomass (IPCC, 2013). Soil carbon turns over approximately every 25 years while living plant carbon turns over every 5 years. Considering that soils form from the interaction between plants, decomposing organisms, and mineral substrates, almost all of the terrestrial carbon pool represents living organisms or their remains.

The physiological and chemical characteristics of terrestrial organisms largely determine dynamics within the terrestrial carbon pool as well as exchanges to other pools. The annual flux from atmosphere to land, at 120 Pg C yr^{-1}, is the largest in the carbon cycle and almost completely determined by the rate of land plant photosynthesis (Sabine *et al.*, 2004). Photosynthetic rates, in turn, depend on plant functional traits (e.g., leaf shape, C:N ratio), which vary among species that characterize different biomes (Bonan, 2010). Of the carbon absorbed by land plant photosynthesis, about half is respired back into the atmosphere by plants themselves, and most of the rest is transferred to the soil through root exudation, tissue senescence, and mortality (Sabine *et al.*, 2004). Once in the soil, carbon dynamics largely depend on temperature, the chemical composition of the plant tissues, and the metabolic efficiency of decomposing organisms (Allison *et al.*, 2010). Among these organisms, bacteria and fungi play especially important roles. Bacteria are numerically dominant and drive soil nitrogen cycling, a major control on carbon uptake and breakdown. Fungi represent a huge portion of soil biomass with different taxonomic groups affecting soil carbon dynamics in different ways. Symbiotic fungi can increase plant productivity by providing limiting soil nutrients, while certain decomposers are largely responsible for breaking down the most recalcitrant plant compounds (Jastrow *et al.*, 2007).

Long-Term Drivers of Change in the Carbon Cycle

The foregoing description of the carbon cycle reflects the best contemporary estimates of recent dynamics (e.g., Sabine *et al.*, 2004; IPCC, 2013). In the distant past, the carbon cycle has had very different properties (Beerling and Royer, 2011; Berner, 1998; Beerling and Woodward, 2001). Ancient changes in carbon cycling have depended primarily on two long-term drivers: plate tectonics and physiological evolution.

Plate tectonics, or the gradual movement of Earth's crust relative to its mantle, produces two effects that are critical for carbon cycling. First, plate movement closes the slow carbon cycle by gradually recycling surface rocks, into CO_2 through volcanism. Second, changes in the geographic configuration and topography of continents influence the distribution and carbon dynamics of biomes. Rates of carbon accumulation in terrestrial ecosystems depend on local climates which shift as continents move and mountains rise (Bonan, 2010). The position and size of continents also alters global circulation. As wind and water move, they redistribute heat, generating important regional climatic patterns like monsoon rainfall (Cerling *et al.*, 2005). Changes in ocean currents are associated with the origin of novel biomes and major shifts in carbon cycling within them (Keeley and Rundel, 2005).

The second major driver of long-term change in carbon cycling is the evolution of physiological traits. Ever since

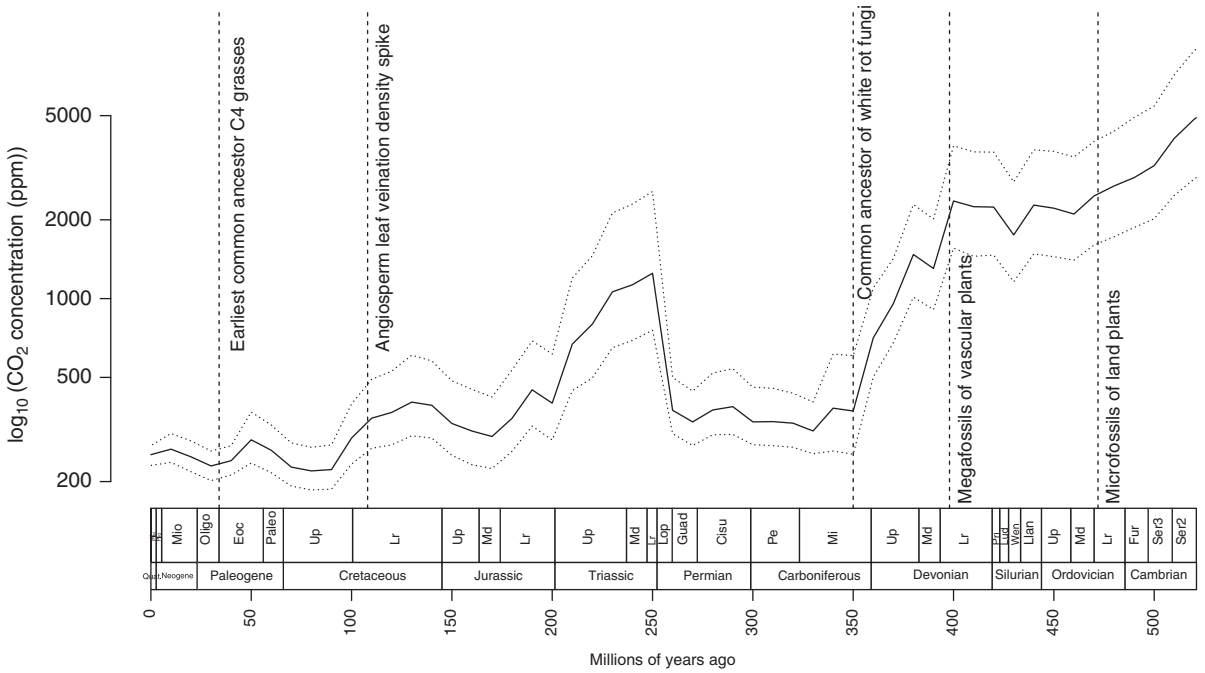

Figure 3 Timing of major events in physiological evolution with atmospheric CO_2 concentrations during the Phanerozoic based on the GEOCARBSULF model (Berner, R.A., 2006. Geochimica et Cosmochimica Acta 70, 5653–5664) as implemented using code available from D. Royer (Royer, *et al.*, 2014. American Journal of Science 314, 1259–1283). Dotted lines around the median trend line represent the 25- and 75-percentiles of simulations drawn from resampled input parameters. Vertical lines denote estimates of events described in the text. The timing of C4 origins is based on fossil calibrated phylogenies summarized in Edwards *et al.* (2010). The timing of the spike in angiosperm venation density is based on fossil evidence in Feild *et al.* (2011). The date for white rot origins is the posterior mean for the stem node of the Agaricomycetes in the fossil calibrated phylogeny in Floudas *et al.* (2012). The timing of tracheophyte origins is based on fossil evidence in Kenrick and Crane (1997). The date for the earliest fossil spore is presented in Rubinstein *et al.* (2010).

modern photosynthesis evolved in Precambrian oceans, competition for light, water, and nutrients in progressively more stressful habitats has driven physiological innovation among plants. Certain biochemical and anatomical adaptations that characterize major plant lineages influenced growth, mortality, and decay so dramatically that they shifted the distribution of carbon between land, atmosphere, and oceans with major repercussions for ancient ecosystems (Beerling and Berner, 2005; Beerling, 2005). Diverse sources of evidence, including biogeochemical markers, paleoclimate models, the fossil record, and comparative analyses bear testament to the lasting consequences of plant-driven revolutions in the global carbon cycle. Among these, three case studies illustrate the feedback between life and climate driven by physiological adaptation in plants and dependent heterotrophic organisms (**Figure 3**).

The Colonization of Land and Rock Weathering

Following the origins of chlorophyll-based photosynthesis, the next major evolutionary event that impacted both the carbon cycle and diversification was the colonization of land in the Ordovician. Prior to that time, terrestrial ecosystems were structurally and biogeochemically simple. They left few fossils, suggesting largely microbial communities of fungi, bacteria, and lichens that were physiologically incapable of forming deep soils (Quirk *et al.*, 2015). Consequently, rates of rock weathering were likely similar to those driven solely by abiotic processes, leaving considerable carbon dioxide in the atmosphere (Berner, 1998). In the interval between the origin of green plant ancestors and the spread of land plants, carbon cycling and terrestrial diversity changed dramatically, but conflicting paleontological, biogeochemical, and phylogenetic data obscure the sequence and therefore causality of major events (Kenrick *et al.*, 2012).

The earliest unambiguous fossil evidence for land plants comes in the form of microscopic spores (Rubinstein *et al.*, 2010). While only a few microns in diameter, the earliest spores are conspicuous for their preserved outer envelopes which likely contained sporopollenin, a tough polymer that resists desiccation and UV irradiation, allowing plants to spread inland (Hemsley *et al.*, 1992). For 60 million years, spores and enigmatic fragments represent some of the only fossil evidence for land plants, until 410 million years ago when abundant tissue fossils appear in the Rhynie chert (Kenrick and Crane, 1997). The variety and detail of these fossils document key adaptations that made land plants major players in the terrestrial carbon cycle. Among them are fossil stomates which mediate gas exchange and develop such that their density decreases with CO_2 concentrations, making fossil photosynthetic surfaces good proxies for CO_2 in the ancient atmosphere (Beerling and Royer, 2011). Belowground, fossilized primitive roots retain evidence of fungal ingrowths that characterize mycorrhizal symbiosis (Kenrick and Crane, 1997). Mycorrhizas today play a key role in plant nutrition, soil formation, and rock weathering.

The fossils of the Rhynie chert formed at a time when CO_2 concentrations and temperatures had been falling for millions of years, implying that land plant growth and associated increases in rock weathering had been driving changes in the carbon cycle (Berner, 1998). However, mixed evidence from more advanced models and physiological studies of basal embryophytes raises the question of whether land plants were the drivers or passengers of early Paleozoic carbon cycle changes (Quirk *et al.*, 2015). While the cause of cooling is ambiguous, it set stage for a host of subsequent changes in plant physiology, carbon cycling, and diversification.

Leaf Evolution, Plant Water-Relations and Biomes

As the organ specialized for photosynthesis in most plants today, leaves have played a key role in carbon cycling, climate, and diversification since their origin in the mid-Silurian. With plants having colonized land 60 million years earlier, the long delay before the simultaneous emergence of true leaves in unrelated lineages represents something of a paradox. Distantly related land plants with different leaf types share certain developmental pathways (Harrison *et al.*, 2005) and several fossil taxa produced leaf-like structures long before true leaves became widespread, suggesting that early land plants had the capacity to develop functional leaves (Beerling, 2005). However, prevailing high atmospheric carbon dioxide concentrations would have limited stomate density and ability of transpiration to cool large laminar surfaces. Once carbon dioxide levels began dropping in the mid-Silurian, higher stomate densities became selectively advantageous for carbon gain (Beerling *et al.*, 2001). As a side effect, increased evaporation from transpiring surfaces cooled them enough to unleash the development of leaves as key photosynthetic organs. The resulting spike in oxygen concentrations is associated with the diversification of giant insects in Paleozoic forests (Harrison *et al.*, 2010).

The evolutionary balance between carbon gain and water loss in leaves continued to influence biogeochemical cycles and diversification into the Devonian. Fossil leaves from those forests differed conspicuously from those of modern floras in their low average venation density (Feild *et al.*, 2011). Venation density, or the linear distance of vascular tissue within a fixed leaf area, strongly influences leaf function. Leaves with low venation density require water to diffuse further through parenchyma, limiting rates of photosynthesis and transpiration (Brodribb *et al.*, 2007). Higher venation densities, which occur in progressively more derived tracheophyte lineages, can gain carbon more quickly in moist habitats, where high soil moisture increases plant hydration, accelerating transpiration, canopy-level humidity, and the potential for recycling precipitation within forested habitats (Brodribb and Field, 2010). This hydrological feedback is characteristic of rainforests, which are the most diverse contemporary habitats. Falling CO_2 in the late Cretaceous is associated with a spike in angiosperm venation density that is coincident with the recruitment of this lineage into the rainforest canopy (Feild *et al.*, 2011). Several existing plant lineages took advantage of dry and low light microclimates in these emerging modern rainforests where they accumulated the majority of their current diversity (Schneider *et al.*, 2004). It is possible that hydrological feedback permits the accumulation of rainforest lineages during periods of dramatic external changes in climate forcing, reducing extinction and explaining why they are the ancestral habitat for most contemporary plant lineages (Wiens and Donoghue, 2004).

A more recent adaptation in leaf form and function that has impacted carbon cycling and diversification is the evolution of C4 photosynthesis among grasses. In most plants, atmospheric CO_2 is directly fixed into a 3-carbon compound (i.e., C3 photosynthesis) by the nonselective enzyme RUBISCO which increasingly accepts oxygen at high temperatures and low CO_2. To limit energy loss to the photorespiration the ancestors of certain grasses evolved a specialized leaf anatomy and physiology that restricts photosynthesis to cells surrounding veins where CO_2 is concentrated first into 4-carbon compounds (i.e., C4 photosynthesis, Edwards *et al.*, 2010). Because this pathway requires energy, C4 plants only have an advantage over their relatives under conditions of high temperature, periodic water stress, high light, and low atmospheric CO_2 (Osborne and Sack, 2012). These conditions arose in open habitats of the Oligocene when C3 grasses had already formed a distinctive disturbance-dependent biome characterized by high productivity, low above ground biomass, and deep soils (Strömberg, 2011). When tectonic and orbital processes exaggerated seasonality and monsoons, a shift in fire regimes prompted C4 grasses sweep to dominance (Keeley and Rundel, 2005). By the late Miocene, C4 grasses came to rival forests in terms of total net terrestrial productivity even though they account for only a small fraction of plant diversity. The greatest impact of these grasses on diversification may be mediated indirectly by humans, whose agricultural communities depend on C4 grasses and their grazers.

Stems and the Evolution of Lignin Synthesis and Degradation

The evolution of lignin metabolism played a dramatic role in Earth's carbon cycle beginning in the Devonian. At the time, short land plants were confined to wet habitats by the biophysical limitations of the structural polymer in their cell wall, cellulose (Kenrick and Crane, 1997). Cellulose can produce extremely rigid structures when dry but in water, the hydrogen bonds that hold parallel chains together are broken, leaving only the tensile strength along the β 1–4 glycosidic bonds. While these bonds resist turgor pressure in hydrated cells, supporting erect plant tissues in wet habitats, early land plants could not grow tall or away from consistent moisture.

The race for light changed radically when the common ancestor of tracheophytes evolved an accessory structural polymer, lignin. Formed from phenol groups with irregular bonding patterns, lignin surrounds cellulose microfibrils in plant cell walls, like concrete surrounding rebar, protecting the bonding arrangement of cellulose from breakdown (Weng and Chapple, 2010). With rigid cell walls, lignified plants could resist wilting and grow taller. Furthermore, lignified vascular cells are sufficiently rigid to maintain their shape under extreme negative pressures required for long-distance water transport under tension (Comstock and Sperry, 2000). With

the ability to grow taller, several tracheophyte lineages also evolved the ability to grow wider through the action of lateral meristems. These meristems deposit new vascular tissue around the stem to maintain vertical transport and structurally reinforce the canopy (Kenrick and Crane, 1997). Elevated plant canopies became more efficient, and habitats that were too dry for early land plants were quickly colonized. Across habitats, the increased demand for soil resources was met by increasingly elaborate roots and effective mycorrhizal symbioses which increased rock weathering (Stein *et al.*, 2012). Together, these factors drove a huge transfer of carbon from the atmosphere into living plant tissues and soils that continued into the Carboniferous (Mora *et al.*, 1996).

Not only did lignin revolutionize plant growth, it also transformed plant decay. The irregular bonding structure of lignin not only resists decay itself, but it shields embedded cellulose (Weng and Chapple, 2010). Consequently, much of the carbon flooding onto land stayed there, unavailable to heterotrophic organisms (Robinson, 1990). The resulting imbalance between photosynthesis and respiration, along with a continental configuration that supported extensive lowland rainforests, contributed to massive accumulation of terrestrial carbon sediments which characterize the Carboniferous. The simultaneous drawdown of CO_2 weakened the greenhouse effect. With falling temperatures and less resilient hydrological cycles, carboniferous rainforests collapsed causing over 80% local extinction of terrestrial plant species in some locations (DiMichele and Phillips, 1996).

The drop in atmospheric CO_2 and temperature that began with lignin synthesis among tracheophytes reversed with a complementary physiological innovation in the common ancestor of Agaricomycete fungi. These fungi today are generally associated with trees as mutualists, pathogens, or decomposers. A comparative analysis of 31 fungal genomes found that decomposers had many extra copies of peroxidase genes which produce enzymes and free radicals capable of degrading the complex bonding structure of lignin (Floudas *et al.*, 2012). In wood, prolonged peroxidase exposure strips lignin away to reveal a bleached mass of cellulose recognizable as 'white rot.' The common ancestor of the Agaricomycetes was likely a white rot fungus that introduced the physiological capacity for lignin degradation in the late Carboniferous, when coal deposition slowed, atmospheric CO_2 recovered, and temperatures gradually climbed (Floudas *et al.*, 2012). With this rebalancing of the Paleozoic carbon cycle came a recovery of biodiversity across a range of lineages that ended only as another physiological innovation among methanogenic Archea may have precipitated carbon cycle changes that accompanied the mass extinction at the end of the Permian (Rothman *et al.*, 2014).

General Patterns

The historical episodes of feedback between physiological evolution and carbon cycling suggest some general relationships with diversification. First, physiological innovations that persist among successful plant lineages may be more likely to arise as atmospheric carbon concentrations drop. The colonization of land, the evolution of tracheophytes, the increase in angiosperm venation density, and the origins of C4 photosynthesis all arose as carbon dioxide concentrations fell. This macroevolutionary pattern may relate to experimental results which have found that low CO_2 is a more effective agent of selection for traits related to fitness than is high CO_2 (Ward *et al.*, 2000; Collins and Bell, 2004). It is possible that the absolute decrease in atmospheric CO_2 since the Proterozoic has selected for clades with genetic architectures that moderate fitness loss with declining CO_2. On the other hand, diversification itself may have driven the long-term decline in carbon concentrations. If weathering increases with terrestrial biomass and biomass increases with species diversity, then diversification for whatever reasons may reduce atmospheric CO_2 (Rothman, 2001). In comparison to the scanty evidence linking increasing diversity with gradually declining atmospheric CO_2, the fossil record, biogeochemical models, and comparative analyses are unequivocal in showing that rapid changes in the carbon cycle are associated with major extinction events across taxonomic groups (Twitchett, 2006). With these precedents, the current spike in CO_2 may only exaggerate the ongoing mass extinction event.

See also: C_4 and CAM Photosynthesis in Land Plants, Evolution and Diversification of. Directed Evolution, History of. Evolutionary Biology, History of. Symbiosis, History of. Synthetic Theory of Evolution, History of. Water Transport, the Role in Plant Diversification of

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Cellular Behaviors Underlying Pattern Formation and Evolution

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Glossary

Morphogenetic gradients Gradients of chemicals that inform cells about their position in the body in a dosage-dependent fashion.

Pattern formation The spatial and temporal organization of cells of distinct fates during embryonic and organ development.

Pleiotropic Requirement of same genes in different developmental stages or tissue types.

Introduction

One of the greatest wonders in nature is the diversity of body shapes, sizes, textures, and color patterns seen across different species. To understand how this diversity is generated and evolves over time, it is necessary to uncover and compare the underlying cellular mechanisms in different species. This type of research is the subject of the multidisciplinary field of Evolution and Development, or Evo-Devo. This maturing field has been propelled by the establishment of emerging models that encompass different branches of the phylogenetic tree, and is providing us a new perspective about how genes pattern organisms and how they can be modified to generate the diversity seen in nature (Sommer, 2009; Müller, 2007).

This article discusses the importance of cell behaviors for the evolution of pattern formation. It first introduces the concept of morphogenetic gradients and cell fate specification during embryonic development. The focus given is on the fruit fly *Drosophila melanogaster*, a reference model that lent support to the theoretical idea that tissues are patterned by gradients of form-generating substances or morphogens, and allowed the discovery of main developmental mechanisms with broad implications to other invertebrate and vertebrate models alike (Veraksa *et al.*, 2000). There are two relevant aspects that challenge the classical notion on how morphogenetic gradients are formed and exert their function, which will be presented here. The first regards to the problem of scaling to size during evolution, and the second regards the cell proliferation and movements during morphogenetic pattern formation. Significant advances to address those problems are being achieved by a combination of comparative evolutionary studies, mathematical modeling, and by revisiting well-established morphogenetic systems in flies and vertebrates using novel tools for live-imaging and single cell analysis.

Morphogenetic Gradients and the French Flag Model

Embryonic Axis Formation Is Initiated by Maternal Information

The *D. melanogaster* embryo is an iconic textbook example for understanding how pattern formation arises from a field of undifferentiated cells. Its development begins as a syncytial blastoderm and is characterized by rapid cycles of nuclear divisions, followed by the migration of the nuclei to the embryo surface (Wolpert *et al.*, 2007). After the thirteenth cleavage cycle, about 6000 nuclei are arranged within a single layer, and cell membrane growth ensues without further nuclear divisions (Edgar *et al.*, 1986). By the end of the cellular blastoderm stage, the embryo is subdivided into several gene expression domains, prior to any major gastrulation movement. These domains specify cells into antero-posterior (AP) fates that contribute to the head, thorax, abdomen, and tail; and dorso-ventral (DV) fates that contribute to the major tissue types of the body, i.e., the mesoderm, neuroectoderm, and ectoderm (Ip *et al.*, 1992).

Several mutations that disrupt AP and DV patterning were isolated in genetic screenings in the fly (Nusslein-Volhard and Wieschaus, 1980; Nusslein-Volhard *et al.*, 1984). These screenings also identified mutations in two maternal genes, *bicoid* (*bcd*) and *dorsal* (*dl*), which encode transcription factors distributed in a graded fashion, with higher to lower levels of Bcd from anterior to posterior side, and higher to lower levels of Dl from ventral to dorsal side (**Figure 1(b)**) (Frohnhöfer and Nüsslein-Volhard, 1986; Driever and Nüsslein-Volhard, 1988a,b; Roth *et al.*, 1989). These gradients provide a system of coordinates that inform cells about their position along the axes. In an embryo mutant for Dl, for instance, ventral and lateral fates giving rise to the mesoderm and neuroectoderm are abolished. As a result, the embryo became dorsalized and assumes an ectodermal fate along the entire DV axis (Nusslein-Volhard *et al.*, 1980; Anderson *et al.*, 1985; Nusslein-Volhard *et al.*, 1984).

Studies on Bcd and Dl provided solid experimental evidence for the early theoretical ideas of pattern generation by morphogenetic gradients proposed by Alan Turing (Turing, 1952), and the French Flag model of positional information proposed by Wolpert (**Figure 1(a)**) (Wolpert, 1969). According to this model, cells can discern their relative positions by sensing different threshold levels of morphogens, and therefore acquire distinct fates. This patterning mechanism was found to be general, and shared by invertebrates and vertebrates. Additional morphogens were identified, including decapentaplegic/Bone Morphogenetic Protein (dpp/BMP-4), wingless/Wnt (wg/Wnt), and Hedgehog/Sonic Hedgehog (Hh/Shh), all of which play roles in embryonic, limb, and organ patterning (Affolter and Basler, 2007; Neumann and Cohen, 1997).

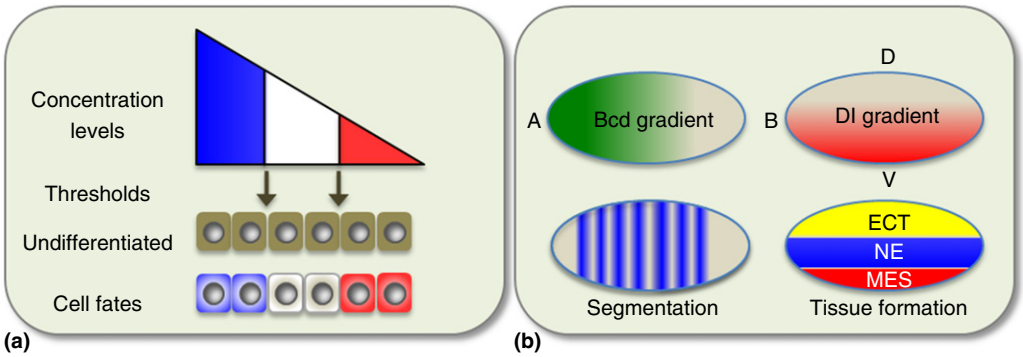

Figure 1 Morphogenetic gradients organize the embryonic axes. (a) A schematic representation of the classical French flag model. Graph shows the concentration levels of a morphogen, which establish different thresholds that are read by undifferentiated cells and cause their differentiation in three fates represented by the colors blue, white, and red. (b) Representation of the *Drosophila* maternal gradients Bicoid (Bcd) along the AP axis (left panel), and Dorsal (Dl) along the DV axis (right panel). The Bcd gradient establishes the expression patterns of segmentation genes (blue stripes), while the Dl gradient defines the main tissue types of the embryo (MES, mesoderm in red; NE, neuroectoderm in blue; ECT, ectoderm in yellow).

Over the several past years, our understanding of gradient formation and interpretation has greatly advanced. Seminal work found evidence that morphogens diffuse from localized sources (e.g., Bcd gradient (Berleth *et al.*, 1988), and the Shh and BMP gradients in vertebrate neural tube (Liem *et al.*, 2000); reviewed in (Cohen *et al.*, 2013). In *Drosophila*, gradients established by the same morphogen Dpp can be formed through distinct mechanisms in limbs and embryos. During wing formation, Dpp is secreted from an organizer region that is stabilized by a compartment boundary between the anterior and posterior regions of the developing wing (Basler and Struhl, 1994). In contrast, the Dpp gradient in the embryonic ectoderm is formed by a more unusual mechanism that involves the transportation of Dpp/BMP-4 to dorsal regions by extracellular antagonists (Eldar *et al.*, 2002; Mizutani *et al.*, 2005; Shimmi *et al.*, 2005). Furthermore, the identification of biochemical interactions involved in gradient formation and cell specification, along with improved methods in confocal microscopy and fluorescent protein tags, have allowed the acquisition of precise quantitative data, and spurred a series of mathematical models that expand our ability to test new predictions of pattern generation (Tomlin and Axelrod, 2007).

Evolutionary Modifications of Gradient Formation and Interpretation

The most general explanatory models of gradient formation and interpretation were first demonstrated in the *Drosophila* blastoderm, owing to its simple two-dimensional organization of a single cell layer and the genetic identification of Bcd and its target genes (Driever and Nüsslein-Volhard, 1988a,b). In the source–sink model (Crick, 1970), a morphogen is released from a localized source, and is asymmetrically distributed as a function of diffusion and degradation away from its source (sink). In the affinity-binding model, the interpretation of the gradient by cells is explained as a result of a differential sensitivity in Bcd DNA binding sites in the regulatory regions of its target genes (Driever *et al.*, 1989).

Although useful to define the basic mechanism of morphogen action, the models above are simplifications that do not fully account for the observed temporal and spatial scales in which gradients are formed. They also do not explain the formation of very precise and sharp borders of gene expression domains (Coppey *et al.*, 2007). Follow-up studies suggested alternative mechanisms to explain morphogen diffusion rather than simple passive diffusion (Müller *et al.*, 2013), including facilitated transport (Eldar *et al.*, 2002; Mizutani *et al.*, 2005; Shimmi *et al.*, 2005), or cell-based mechanisms such as dispersal through filopodial extensions called cytonemes (Fairchild and Barna, 2014). In addition, regulatory gene networks of target genes are necessary to refine and reinforce the expression borders initially established by gradients (Balaskas *et al.*, 2012; Stathopoulos and Levine, 2005).

The gradient mechanics also raise important issues of relevance to evolutionary biology. While direct experimentation *in D. melanogaster* has provided valuable insights into the mechanics of gradient formation and interpretation, this single species approach usually falls short of explaining how gradients define robust responses during evolution, or are reshaped to generate novel patterns. To address these issues and gain a more complete understanding of morphogenetic patterning, it is necessary to investigate other species. These species comparisons, coupled with experimentation in *D. melanogaster*, allow us to ask and test ideas that cannot be done in a single species. One example is how gradients can be established over variable distances to specify cell fates in relatively similar positions in embryos from related species that vary in egg size (**Figure 2**) (Gregor *et al.*, 2005; Chahda *et al.*, 2013).

Another fascinating issue regarding the mechanism by which morphogens instruct cells about their fate is the fact that they act on a field where the position of cells changes as a function of time. This observation challenges the positional information models, which assume that cells remain in a fixed position in relation to the gradient in order to acquire a given fate. Although this has been known for a long time, it is difficult to reconcile this observation with the current models of morphogenetic pattern (Affolter and Basler, 2007; Cayuso and Martí, 2005). It is possible that evolutionary changes in cell proliferation and movements could refine or even modify the

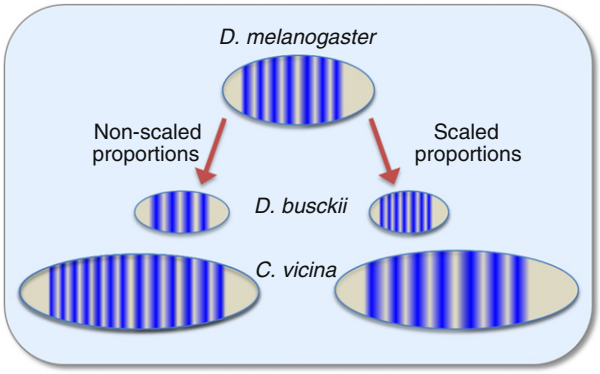

Figure 2 Hypothetical scenarios of scaled and non-scaled patterns of segmentation genes during the evolution of Diptera. An embryo from *Drosophila melanogaster* expressing a pair-rule gene is represented on top. The left panel represents the lack of segmentation scaling in small eggs (*Drosophila busckii*) and large eggs (*Calliphora vicina*). In this case, *D. busckii* would miss some stripes, while *C. vicina* would have additional stripes. The right panel represents the presence of scaling to size, as observed in actual experiments. The numbers and relative positions of the segmentation stripes would be preserved in all species.

overall pattern information provided by gradients, providing an alternative source for pattern changes across species. However, these possibilities remain largely untested.

On a broader view, the fact that tissue patterning arises in a dynamic field, and that different species can pattern tissues differently, has direct relevance to regenerative medicine as it may provide important insights into the scaling of organs during growth. Recent advances in applying quantitative methods to address the problem of scaling of gradients in a variety of system has been discussed elsewhere (Umulis and Othmer, 2013; Ben-Zvi *et al.*, 2011).

Scaling of Morphogenetic Gradients during Evolution

The AP System Employs Different Scaling Strategies within and across Diptera Species

Despite extensive work on maternal gradients in *D. melanogaster*, some questions regarding gradient dynamics can be best addressed using a combination of studies within and across species. One fascinating observation is the relatively proportional body pattern seen across different fly species. This scaling can be best illustrated by the embryonic position of stripes of various segmentation genes that respond to Bcd gradient, such as *hunchback*, *giant*, *paired*, and *even-skipped*. In embryos that significantly vary in size, not only the same number of stripes of these genes is conserved, but each individual stripe is located at relatively similar positions (**Figure 2**) (Gregor *et al.*, 2005a; Lott *et al.*, 2007; Hare *et al.*, 2008; Fowlkes *et al.*, 2011; Blechert *et al.*, 2011). This remarkable finding could be either explained by the ability of the Bcd gradient to scale to embryo length or by a differential sensitivity to Bcd levels caused by changes in the cis-regulatory sequences of target genes.

The quantitative measurement of the Bcd gradient in *Drosophila busckii*, a species with eggs twice as small as *D. melanogaster*, and in *Calliphora vicina*, a species with eggs three times larger than *D. melanogaster*, revealed that the Bcd gradient profile is similar in all species after normalization by embryo length (Gregor *et al.*, 2005). In this study, Gregor *et al.* show that the amplitude of the gradient scales with its constant length and reach farther distances, suggesting that the gradient threshold information is adjusted to size and that the generation of conserved expression domains may not necessarily involve the evolution in cis-regulation of Bcd target genes (Gregor *et al.*, 2005).

Considering one of the gradient formation models that involves the diffusion of Bcd from the anterior region, the scaling of the Bcd gradient in the different species could be explained by either changes in the Bcd effective diffusion constant or a modified time scale for the gradient formation, or alternatively, through an increased stability of the protein. The first two possibilities were ruled out given that all species tested display similar developmental rates, and the measured passive diffusion of an inert molecule injected in small and large embryos reveals no significant difference (Gregor *et al.*, 2005). The possibility that Bcd may have acquired distinct lifetimes during evolution remains open. In experiments in which the Bcd proteins from *C. vicina* and *Lucilia sericata* (two species with large embryos) were expressed in *D. melanogaster* revealed that the exogenous protein gradients assume the same shape of the host species gradient (Gregor *et al.*, 2008). Although these experiments indicate that the degradation of Bcd from *C. vicina* and *L. sericata* is the same as in *D. melanogaster*, an exhaustive test of Bcd degradation in species-specific environment has not been performed. In any case, it appears that the scaling of Bcd gradient is likely to depend on other species-specific factors that affect the gradient formation.

Another relevant question is whether scaling is achieved by similar or different mechanisms within same species. To answer this question, Cheung *et al.* used artificially selected strains of *D. melanogaster* with significantly different egg sizes (Cheung *et al.*, 2011; Miles *et al.*, 2011). They found that the Bcd gradients in strains with large eggs reach larger distances by having the production rate of Bcd scaled with embryo volume. The authors confirm that net amounts of maternally deposited Bcd RNA in the ovary increases in the strains with large eggs (Cheung *et al.*, 2011). Given that variation in embryo size is a fast evolving trait determined by multiple independent *loci* (Markow *et al.*, 2009; Warren, 1924), the increase of *bcd* RNA deposit provides a simple solution to buffer variations in embryo size and achieve gradient scaling within the same species, without requiring modifications in the Bcd protein sequence or its degradation pathways that would not have sufficient time to undergo selection.

The DV Axis Is Patterned by Two Opposing Gradients: One Regulated by a Fast Evolving Pathway, and Another That Is Highly Conserved across Evolution

The DV patterning system has also provided insights about how tissues scale during evolution. As mentioned before, the main tissue types are initially established along the DV axis in

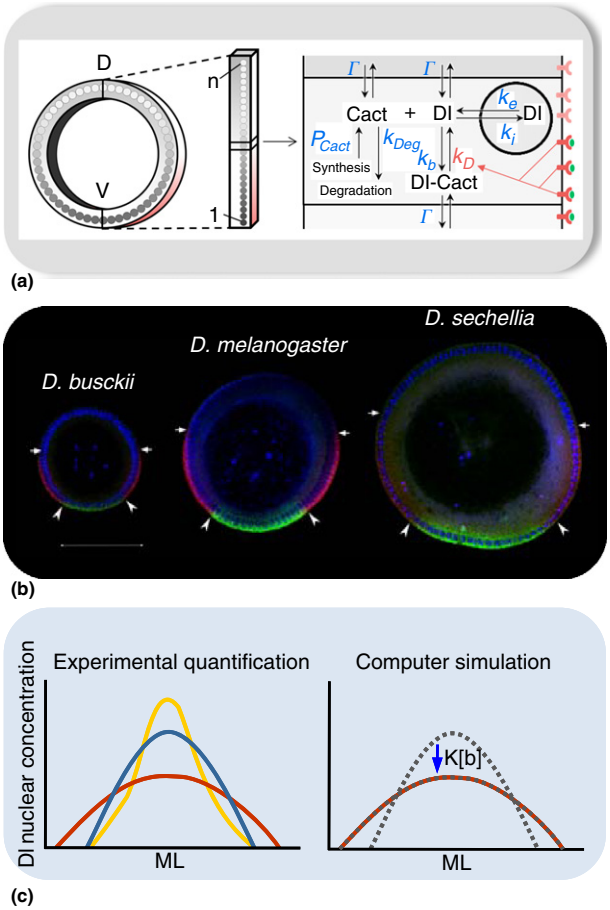

Figure 3 Scaling properties of the DV system. (a) Representation of a DV cross-section of a *Drosophila* embryo (left panel). The nuclei from ventral to dorsal side can be modeled as a single strip of cells. Right panel shows the Toll (Tl) signaling pathway. Partial differential equations representing the relationships between the cytoplasmic components of this pathway were used in a mathematical model to test the formation of the Dl gradients, and its scaling properties across species (see text for details). (b) Cross-section of related *Drosophila* embryos that vary in size, stained for a nuclear dye (blue), and markers for the mesoderm (*snail*, in green), and neuroectoderm (*short gastrulation*, red). Note variation in the mesodermal domains, indicated by arrowheads. (c) Computational modeling of the Dl gradient. Representation of Dl nuclear concentration levels quantified from different *Drosophila* species (left panel). Blue line, *D. melanogaster* and *D. sechellia* gradients; yellow line, *D. busckii* gradient; red line, *D. simulans* gradient. By adjusting parameters of the Tl pathway (e.g., K[b], binding rate between Dl and Cactus), it is possible to reproduce the flattened gradient of *D. simulans* species. Panel shown in (a) is reproduced from Ambrosi, P., Chahda, J.S., Koslen, H., Chiel, H.J., Mizutani, C.M., 2014. Modeling of the dorsal gradient across species reveals interaction between embryo morphology and Toll signaling pathway during evolution. PLoS Computational Biology 10 (8), e1003807. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4148200&tool=pmcentrez&rendertype=abstract (accessed 27.05.15); Panel in (b) is reproduced from Belu, M., Mizutani, C.M., 2011. Variation in mesoderm specification across Drosophilids is compensated by different rates of myoblast fusion during body wall musculature development. PLoS One 6 (12), e28970. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3237579&tool=pmcentrez&rendertype=abstract (accessed 21.12.11).

response to the maternal Dl gradient. Dl is an NFκ-B transcription factor that is transported into the nucleus following the activation of Toll signaling pathway (**Figure 3(a)**) (Anderson *et al.*, 1985). Unlike the Bcd gradient, which is thought to be established by the protein diffusion from tightly localized RNAs in the anterior region of the embryo (Gregor *et al.*, 2007) or arise as a result of a RNA gradient (Spirov *et al.*, 2009), both Dl and Toll (Tl) receptor are deposited ubiquitously in the embryo. The asymmetric distribution of nuclear Dl along the DV axis is achieved through a localized ventral source of the ligand Spätzle, which activates Tl in a graded fashion along the DV axis. The activation of Tl triggers the degradation Cactus, a cytoplasmic inhibitor of Dl. Once Dl is free from binding to Cactus, it is transported into the nucleus (Belvin *et al.*, 1995). High nuclear levels of Dl in the ventral side of the embryo activate mesodermal genes, whereas moderate nuclear levels activate neuroectodermal genes (Hong *et al.*, 2008). Finally, in the dorsal side of the embryo, the absence or low levels of nuclear Dl are insufficient to repress the expression of the secreted morphogen *dpp*/BMP-4, which in turn establishes an opposing dorsal-to-ventral gradient that induces the amnioserosa and ectodermal fates, and represses the expression of neural genes (Mizutani and Bier, 2008).

It is noteworthy that the Tl pathway, which ancestrally played a role in innate immune response against pathogens in both invertebrates and vertebrates (Hoffmann and Reichhart, 2002), became co-opted for DV patterning in derived dipterans like *Drosophila* (Lemaitre, 2004; Hoffmann and Reichhart, 2002; Belvin and Anderson, 1996). Components of the Tl pathway are fast evolving, likely due to the constant contact to new pathogens in the environment (Clark *et al.*, 2007; Jiggins and Kim, 2007; Sousa-Neves and Rosas, 2010; Obbard *et al.*, 2009; Schlenke and Begun, 2003). As discussed later, the fact that the DV patterning is regulated by some fast evolving genes shared by the Tl pathway appears to produce a high rate of variability capable to create diverse Dl gradient shapes, which might have been an important feature to adapt to the fast evolution of egg size.

In contrast to the newly acquired role of Dl in DV patterning, the *dpp*/BMP-4 is the most ancestral signaling pathway responsible for DV patterning among metazoans (Mizutani and Bier, 2008). This pathway plays a pivotal and conserved role in the formation of a centralized nervous system and in the establishment of neural domains of equivalent neural cell types within the neuroectoderm of organisms from the major branches of invertebrates (ecdyzoa and lophotrocozoa) and in the neural tube of vertebrates (Liem *et al.*, 2000; Mizutani *et al.*, 2006; Denes *et al.*, 2007). Thus, in *Drosophila*, cells along the DV axis receive information from a highly conserved DV signaling pathway (Dpp/BMP), and from a newly co-opted fast evolving pathway (Dl/NF-κB).

The Domains of Neural Identity Genes along the DV Axis Are Highly Constant during Evolution

One striking feature regarding the neuroectoderm described several years ago is the highly conserved numbers and types of neuroblasts (neural stem cell-like cells) across divergent

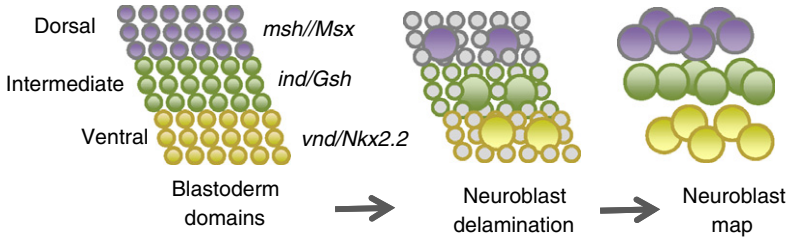

Figure 4 Developmental stages leading to the formation of a conserved neuroblast map. During blastoderm stage, three non-overlapping domains of neural identity genes subdivide the DV axis of the *Drosophila* neuroectoderm. Later in development, neuroblasts delaminate from the neuroectoderm. About 30 neuroblasts of unique identity are formed per hemisegment. This neuroblast map is highly conserved among insects.

insects. Early studies that defined the 'neuroblast map' in divergent insects, including grasshopper and *D. melanogaster*, found that each hemisegment generates about 30 neuroblasts of unique identity in these species (**Figure 4**) (Doe, 1992; Whitington, 1996; Bate, 1976). These neuroblasts give rise to homologous neuronal lineages that establish connectivity patterns of remarkable similarity in these species (Thomas *et al.*, 1984). The degree of conservation of neuroblast identity can even be observed to a certain extent in the crustacean branch (Ungerer and Scholtz, 2008).

After the description of neuroblast maps, studies in *D. melanogaster* identified three key homeobox transcription factors, *ventral nervous system defective* (*vnd*)/Nkx2.2, *intermediate neuroblast defective* (*ind*), and *muscle segment homeobox/Drop* (*msh*)/*Msx*, which are expressed in three non-overlapping domains in the ventral, intermediate, and dorsal regions of the neuroectoderm, respectively (McDonald *et al.*, 1998; Weiss *et al.*, 1998; Chu *et al.*, 1998; Isshiki *et al.*, 1997; Jimenez *et al.*, 1995). These genes were called neural identity genes because they define the types of neurons that are formed at distinct DV positions, such as motorneurons, serotonergic and secretory neurons (**Figure 4**).

The neural identity genes and mesodermal genes are part of a DV self-regulatory gene network, in which genes expressed ventrally repress genes expressed more dorsally in what is referred to as 'ventral dominance' (Cowden and Levine, 2003). In the ventral dominance model, the initial DV gene expression domains established by the Dl gradient are reinforced by the repression of neural genes by *snail* (*sna*), a mesodermal gene expressed in the ventral most region of the embryo, followed by the repression of *ind* and *msh* by *vnd*, and repression of *msh* by *ind*. This cross-regulation assures that the borders of neuroectodermal genes are reinforced.

In experiments that either induced expansions or retractions of the blastoderm domains of neural identity genes, there is a shift in ventral to dorsal neuroblast identity, or vice-versa, caused by an invasion of expression of the adjacent genes (McDonald *et al.*, 1998; Cornell and Ohlen, 2000; Weiss *et al.*, 1998; Isshiki *et al.*, 1997; Jimenez *et al.*, 1995). As a consequence, after neuroblast delamination from these altered domains, entire neural lineages are duplicated at the expense of other lineages that are eliminated altogether. These experiments, along with the observation of neuroblast map conservation, suggest that there is a strong selective pressure to precisely maintain the neuroectodermal domains in the early blastoderm. However, the underlying molecular mechanism that imposes this selective pressure has only recently begun to be understood.

The Germ Layers Formed along the DV Axis Are Unequally Scaled during Evolution

The remarkable constancy in the neuroectodermal expression domains occurs despite significant changes in embryo size in insect species that diverged over 300 MYA (i.e., grasshopper and *Drosophila*), and raises the outstanding question of how the remaining DV germ layers are specified. It turns out that while the neuroectoderm remains constant in size, the mesodermal and ectodermal domains can either retract or expand across *Drosophila* species (Belu and Mizutani, 2011). For instance, there is a significant reduction of the mesoderm in small eggs from *D. busckii*, and an expansion in large eggs from *Drosophila sechellia* (**Figure 3(b)**). The variation in mesoderm is not always correlated to egg size, since *Drosophila simulans*, a species more closely related to *D. sechellia*, but with similar egg size as *D. melanogaster*, also display an expansion in the mesoderm (Belu and Mizutani, 2011). The variation in the mesodermal size versus a more conserved neural patterning was also verified in the artificially selected *D. melanogaster* strains with variable egg sizes described above (Garcia *et al.*, 2013; Miles *et al.*, 2011).

The changes in the mesodermal domain across species appear to be directly caused by a lack of scaling in the Dl gradient since the quantification of the nuclear Dl distribution across species revealed striking modifications in the shape of the gradient varying from sharp to flat profiles (Chahda *et al.*, 2013). Furthermore, in experiments using hybrid embryos between sibling species *D. sechellia*, *D. simulans*, and *D. melanogaster*, it was possible to show that the mesodermal genes are activated by same Dl threshold levels, ruling out the possibility that the changes in their mesodermal domains are due to evolution in cis-regulation (Chahda *et al.*, 2013). This result is significant because it provides clear alternatives to affinity-binding models of pattern evolution and also reveals how the requirement of a signaling pathway that participates in both immune response and DV patterning can lead to extremely fast changes in the embryonic organization.

The experiments above also reveal the underlying mechanisms that restrict the neuroectoderm layer. Unlike what is seen with the Bcd gradient, the Dl gradient does not scale to size in the different species (Chahda *et al.*, 2013; Gregor *et al.*, 2008). Since mesodermal genes maintained their sensitivity to Dl activation, the ventral border between the mesoderm and neuroectoderm, and the dorsal border between the neuroectoderm and the ectoderm, are expected to be determined by a relatively constant slope of the Dorsal gradient across

species. Thus, the neuroectoderm can be shifted as a single block to a new DV position in response to novel Dl gradient shapes. The ability to shift the position of the neuroectoderm as a single block would ensure that correct numbers of neuroectodermal cells are formed in small and large embryos (Chahda *et al.*, 2013).

One might ask whether the expansions and retractions of the mesoderm could lead to a loss or gain of muscles in larvae. In experiments that addressed this question, it was shown that variations in mesodermal size are corrected during later developmental stages. The stereotyped pattern of the muscle body wall, for instance, is preserved due to a modified regulation in myoblast fusion during formation of individual muscle fibers (Belu and Mizutani, 2011).

Together, these results highlight the extreme versatility of the DV self-organizing system. This appears to be a very robust system capable of adjusting itself to a significant number of challenges imposed by embryo size and pleiotropic effects of the Tl pathway to produce fully viable individuals.

A Mathematical Modeling of Dl Gradient Indicates that Scaling Is Achieved by Changes in the Embryo Anatomy and Tl Signaling Pathway

Different factors could influence the modified shapes of the Dl gradient during evolution. One of them is the evolution of the Tl signaling pathway. Indeed, analyses of different species reveal that the ranges Tl signaling are modified and these changes are accompanied by variations in the size of mesodermal domains (**Figure 3(b)**). However, there are noticeable changes in embryo length and circumference, number and size of nuclei, and in nuclear density patterns across species that could in principle modify this gradient. This possibility was tested in experiments that manipulated the number and density of nuclei in *D. melanogaster*. Interestingly, these manipulations cause the sharpening or flattening of the Dl gradient as predicted, indicating that nuclear density and size contribute to the shape of the gradient (Chahda *et al.*, 2013).

To tease apart the relative contributions of anatomical changes and Tl signaling pathway evolution, a mathematical model originally developed for the Dl gradient formation in *D. melanogaster* (Kanodia *et al.*, 2009) was modified in order to identify which parameters are required to reproduce the different gradients observed from related *Drosophila* species (Ambrosi *et al.*, 2014). This model is based on partial differential equations that represent the interactions of the Tl pathway components (**Figure 3(a)**) (Belvin *et al.*, 1995; Kanodia *et al.*, 2009). The model also takes into consideration the anatomical features of the embryo, and was shown to correctly reproduce the dynamics of gradient formation in live-embryos expressing Dl tagged with green fluorescent protein (Kanodia *et al.*, 2009; DeLotto *et al.*, 2007). By testing different parameter sets, the model indicates that changes in anatomy alone are not sufficient to recreate the gradients from the species, and it is necessary to include modifications in the Tl pathway (**Figure 3(c)**) (Ambrosi *et al.*, 2014). This modeling experiment led to three important conclusions. First, pairs of very closely related species that diverged only ~0.5 MYA and produce similar changes in the mesoderm, but underwent separate speciation events (i.e., *D. sechellia* and *D. simulans*; and *Drosoplila santomea* and *Drosophila yakuba*), share similar modifications in the Tl pathway that correctly reproduce their gradients in model simulations. For instance, *D. simulans* has a flattened gradient in relation to the gradient of *D. sechellia*. Yet, the model can simulate these very different shapes after similar manipulations of Tl signaling parameters, such as increasing the rates of Cact degradation or decreasing the binding rate between Cactus and Dl (**Figure 3(c)**) (Ambrosi *et al.*, 2014). Furthermore, the modifications in the Tl pathway predicted by the model are supported by changes in amino acid sequences of Dl and Cactus protein domains related to these functions in these species. The second conclusion that can be drawn from this modeling is that the changes in anatomy and Tl pathway interact with one another. In order words, changes in Tl pathway are exacerbated by the changes in anatomy. Finally, the model also predicts that small additive changes in the Tl pathway are more likely to occur in nature, since more drastic changes of a single parameter affecting this pathway renders the model more unstable. This prediction is supported by the amino acid changes seen in many Tl pathway components from these species (Sousa-Neves and Rosas, 2010; Clark *et al.*, 2007; Jiggins and Kim, 2007; Obbard *et al.*, 2009).

Contribution of Cell Migration during Morphogenetic Gradient Patterning

The information for cell specification provided by gradients rarely occurs in a stable environment, and it is complicated by the constant proliferation and migration of cells that takes place during growth, such what is seen during the patterning of the vertebrate neural tube (Gouti *et al.*, 2015). As mentioned at the beginning of this article, the *Drosophila* blastoderm has been a highly successful model to understand pattern formation due to its simple arrangement of cells. Furthermore, the blastoderm cells seemed to be a close approximation to static fields of cells as they receive morphogen signals, since they are no longer dividing and appear mostly immobile (Edgar *et al.*, 1986). However, recent studies show that the nuclei actually move from one to three nuclear diameters in distance during blastoderm stage, and follow stereotyped trajectories that ultimately lead to species-specific nuclear densities that are polarized along the AP and DV axis (Keranen *et al.*, 2006; Meyer *et al.*, 2010; Fowlkes *et al.*, 2011). These stereotyped movements are abrogated in mutant embryos that eliminate either the Bcd or Dl gradients, and appear to contribute to a shift in the position of the stripes of the segmentation gene *fushi tarazu* in mutant versus wildtype embryos (**Figure 5(a)**) (Keranen *et al.*, 2006).

These novel observations are fascinating at several levels. First, they indicate that *Drosophila* might have remnants of the ancestral and more dramatic nuclear blastoderm movements that occur in the embryogenesis of short-germ band insects, as it has been shown in crickets (Nakamura *et al.*, 2010). Second, evidence mounts that species-specific cell movements in *Drosophilids* may produce some slight differences observed in the positioning of stripes of segmentation genes along the AP axis (Keranen *et al.*, 2006; Lott *et al.*, 2007; Gregor *et al.*, 2008; Fowlkes *et al.*, 2008; Meyer *et al.*, 2010; Blechert *et al.*, 2011). It

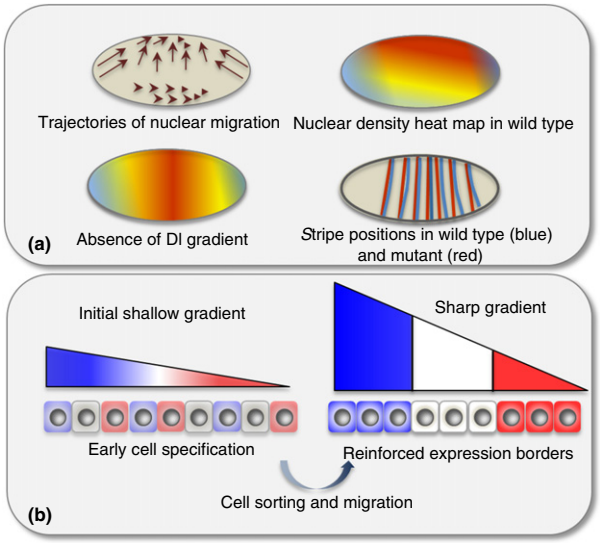

Figure 5 Contribution of cell sorting to pattern formation. (a) The *Drosophila* blastoderm embryo displays stereotyped nuclear movements. Top panel: Trajectories of nuclear migration are represented by arrows, which show a nuclear flow toward the dorsal midline. By the end of the blastoderm stage, the nuclear density becomes polarized, with high density levels in the dorsal side (red to yellow shades) and low density levels in the ventral side (green to blue shades). Bottom panel: Nuclear density patterns (left) and gene expression patterns (left) are disrupted in mutants that eliminate the Dl gradient. The new locations of the segmentation stripes (red) in relation to wild type stripes (blue) is predicted to be partly caused by disruptions in nuclear flow. (b) Revised version of the French flag model according to findings reported by Xiong *et al.*, 2013. The authors found that cells of the neural tube of zebrafish first acquire fate in a salt and pepper fashion while the Shh gradient is noisy. Over time, the gradient becomes sharp, and these cells maintain their fate and correctly sort to their final positions.

has also been reported that within the same species *D. melanogaster*, there is a dynamic shift in the positioning of stripes that occurs over time during the blastoderm stage, which was initially thought to be due to gene regulation (Jaeger *et al.*, 2004). However, in experiments that identify all individual nuclei in cohorts of fixed embryos of different stages, it was possible to develop a model for the observed nuclear flow and predict that the nuclear flow contributes to the shift in stripe positions (Keranen *et al.*, 2006). Together, these findings suggest cell sorting as a significant patterning mechanism that can sharpen the borders of AP segmentation, and that is most likely linked to morphogen regulation and distribution.

Another significant role for cell sorting was uncovered in a recent study that revisited the DV patterning formation of the neural tube in zebrafish. This well-established system is patterned similarly to the *Drosophila* neuroectoderm, and is subdivided by homologous DV neural patterning genes that cross-regulate each other (Cohen *et al.*, 2013). These neural genes are regulated by the *dpp*/BMP-4 gradient that emanates from the dorsal neural plate and by a ventral source of Shh gradient from the notochord, instead of the Dl gradient as seen in flies (reviewed in Mizutani and Bier (2008)). By tracking the formation of early ventral neural progenitor cells, Xiong *et al.* discovered that cell specification occurs in a salt and pepper pattern early in development, while the Shh gradient is still too shallow and noisy to provide reliable positional information (Xiong *et al.*, 2013). Unexpectedly, these specified cells apparently maintain their fate, and migrate to their final positions as the Shh gradient sharpens (**Figure 5(b)**). The directional movement of these cells is disrupted by the removal of the cell-adhesion molecule Cadherin-2, but it is not influenced by Shh (Xiong *et al.*, 2013). Although the exact molecular mechanisms for this cell sorting remain unclear, it is plausible that these progenitor cells respond to cues provided by different cell adhesion molecules that are asymmetrically distributed along the DV axis, which may in turn be regulated by other gradients. A candidate is the BMP-4 gradient, which has been shown to regulate the expression of Cadherin-2 and coordinate cell movements during dorsal convergence during early embryonic development of zebrafish (von der Hardt *et al.*, 2007). Models for cell sorting mechanisms that integrate morphogens to generate patterns from initially scattered cell fates have been proposed during the patterning of the slime mold Dictyostelium (Vasiev and Weijer, 1999; Kay and Thompson, 2009).

While there is no evidence that the *Drosophila* blastoderm development involves any massive salt and pepper patterns of specified cells that migrate to their final domains, there is evidence for a more subtle, but quantifiable, cell sorting mechanism that can contribute to the formation of gene expression borders (Keranen *et al.*, 2006). This mechanism could be relevant to achieving the final numbers of cells within the neuroectoderm across *Drosophila* species, since the nuclei within the lateral regions of the embryo that give rise to the neuroectoderm actively move toward the dorsal midline (**Figure 5(a)**) (Keranen *et al.*, 2006). Using *Drosophila* genetics, it may be possible to isolate candidate genes such as cell adhesion molecules that may be involved in cell sorting and test how this mechanism is integrated with morphogenetic gradients.

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See also: Developmental Mechanisms Controlling Cell Fate, Evolution of. Gene Networks Driving Development, Conservation and Evolution of. Novel Structures in Animals, Developmental Evolution of. Robustness and Evolvability in Molecular Evolution

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Climate Change, Quantitative Genetics and

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Glossary

Adaptive evolution Enhanced population fitness due to the increase in frequency of advantageous alleles through positive selection or elimination of disadvantageous alleles through negative selection.

Additive genetic variation A measure of heritable variation caused by the predictable effects of alleles at the current allele frequencies.

Artificial selection Human-mediated reproduction between individuals in a population to propagate desirable traits.

Aster models Maximum likelihood models for estimating fitness-based traits at different stages of the life cycle (e.g., survival and fecundity) and that have different underlying statistical distributions (e.g., binomial and Poisson).

Breeding values The expected phenotype of an individual's offspring (measured as the deviation of an individual's offspring from the population mean). It provides a measure of the heritable contribution of the genotype.

Dominance variance Phenotypic variation attributable to interactions between alleles at a locus.

Epistatic variance Phenotypic variation attributable to interactions between alleles at different loci.

Evolutionary response The difference in phenotype between generations that occurs when selection favors heritable traits in the parental generation.

Fitness The ability of an organism to survive and reproduce in a particular environment.

G-matrix The pattern of additive genetic variance and covariance for multiple traits or, in some cases, the same trait or character states in different environments.

Genetic architecture The distribution and nature of genetic variation at multiple scales including variation at the levels of species, region, population, family, and individual.

Genetic correlation A standardized estimate of the additive genetic effect shared between pair of traits.

Genetic linkage The tendency for alleles in close proximity on a chromosome to be inherited together.

Heritability The fraction of that variance in phenotype that can be attributed to genetic variation that is inherited directly from parents.

Natural selection Differences in fitness between individuals in a population in a single generation due to the traits that they possess.

Pleiotropic gene A gene that influences more than one phenotypic characteristic.

Polygenic trait A phenotypic trait that is influenced by more than one gene.

Relative fitness Individual fitness relative to the average fitness of other genotypes in the populations. Typically expressed as individual fitness divided by population mean fitness.

Resurrection ecology A technique that allows direct observation of evolutionary change rearing revived dormant propagules (e.g., eggs, seeds) that represent ancestors in a common environment with contemporary descendants.

Selection differential A composite measurement of direct and indirect selection on a trait.

Selection gradient A measurement of direct selection on a trait, measured as the linear relationship between a trait and relative fitness.

Introduction

Earth's biota is currently being challenged by an uncontrolled experiment imposed by climate change and other anthropogenic stressors. Natural populations will either survive these challenges through range shifts, adaptive plasticity and evolution, or face extinction (Davis and Shaw, 2001; Davis *et al.*, 2005). Extinction has occurred for some plant (Jackson and Weng, 1999) and animal species (Martin and Klein, 1989) during historical periods of climate change and continues to be a serious threat to biodiversity (Thomas *et al.*, 2004). However, there are also many taxa that have persisted in spite of climate change by range expansions and contractions (Mitton *et al.*, 2000), adaptation *in situ* (Mcgraw, Fetcher, 1992), and simultaneous evolution and range shifts (Cwynar Macdonald, 1987).

Biotic Response to Climate Change Is Already Happening

We have witnessed responses of native organisms to climate warming as increasing earliness of budburst (Nordli *et al.*, 2008), flowering (Miller-Rushing and Primack, 2008), and the spring phenology of insects and birds (Roy and Sparks, 2000; Gordo and Sanz, 2006). However, in only a few cases can phenotypic responses to longer growing seasons and warmer temperatures (i.e., plasticity) be distinguished from genetically based evolutionary change in response to changes in natural selection. Even in cases where there is evidence strongly implicating an evolutionary response, we lack understanding of the genetic basis of these changes. In spite of many studies documenting the strength of selection in natural populations (Kingsolver *et al.*, 2001), we have little information on one of the most basic aspects of ecological genetics: the rate of

evolution in the wild. Filling these gaps in our knowledge will advance understanding of the mechanisms of evolution, strengthen prediction of the limits to adaptation to anthropogenic changes in the environment, and provide baseline information to develop management approaches that reduce the extinction risk.

The goal of this encyclopedia article is to explain how we can use the tools of quantitative genetics to understand the extent to which populations have already adapted to climate change and the potential for ongoing evolution in response to anthropogenic and natural changes that are happening concurrent with climate change.

Evolution in Response to Climate Change Is Multidimensional

It is important to bear in mind that climate change is only a subset of anthropogenic factors that influences the evolutionary dynamics of native organisms. For example, fragmented populations embedded in a matrix of altered habitat may have reduced opportunities for range shifts and may be cut off from gene flow lowering the influx genetic variation (Swindell and Bouzat, 2006). For many species, contemporary population sizes are reduced, which may cause genetic diversity to be lost by drift and inbreeding and increase susceptibility to extinction by stochastic environmental events (Heschel and Paige, 1995). Increasingly, wild populations have been restored, which can alter population size and genetic composition (Fenster and Dudash, 1994). Habitat degradation may also allow invasion of exotic species including diseases, pests, and competitors that may compound the stress of climate change (Strauss *et al.*, 2006). Furthermore, positive interactions between organisms, such as between plants and pollinators, may become decoupled as species respond to climate change in different ways (Mccarty, 2001). Thus the persistence of organisms will depend upon a multiplicity of interacting factors. To the extent possible, other interacting biotic and abiotic factors should be taken into consideration in the design of quantitative genetic studies.

Why Use Quantitative Genetics?

Classical quantitative genetics is a powerful tool for investigating evolution in response to climate change. This discipline provides the tools to measure key components of the evolutionary process including the strength and direction of natural selection on traits, phenotypic plasticity and its potential for ongoing evolution, and genetic variability and correlation structure for traits that are targets of selection. These approaches can be used to quantify evolutionary change that has already occurred and predict ongoing evolution into the future. Quantitative genetics is largely a 'black box' approach where phenotypic variation is partitioned into genetic and environmental causal components but the underlying genetic details are not explicitly investigated.

Considering advances in our understanding of traits important for climate response at the molecular level (Stinchcombe *et al.*, 2004; Wilczek *et al.*, 2009), one may wonder why quantitative genetics is still a relevant approach? The answer is that despite the discovery of molecular mechanisms that underlie a few specific traits (e.g., Fangue *et al.*, 2006), our understanding is still woefully inadequate to describe more complex polygenic phenotypes as they interact with the environment in the wild. For example, timing of life history events, dispersal ability, thermal and drought tolerance, and competitive ability are traits with complex genetic underpinnings that will likely be the direct targets of selection with climate change. Although some of these traits are influenced by a few regions of chromosomes with major effects, called quantitative trait loci (QTLs), ongoing research has yet to resolve how many genes are represented within these regions and whether these genes are relevant under field conditions (e.g., Weinig *et al.*, 2002). Quantitative genetics offers alternative measurements of genetic variation for single traits (additive genetic variance and heritability) or multiple traits (G-matrix). Models have been developed that can accommodate genes of major effect into a quantitative genetic framework (Walsh, 2001).

Questions That Can Be Addressed Using Quantitative Genetic Approaches

Quantitative genetics has been used for decades to explore the underlying genetic architecture of natural populations, elucidate patterns of natural selection, and predict and measure evolutionary change. More recently, the scope of questions addressed in wild populations has expanded (reviewed in Kruuk *et al.*, 2008), and we expect this trend to continue into the future, given that many traits that contribute to fitness in nature have a genetic basis too complex to be fully understood at the molecular level (Buchanan *et al.*, 2006). Questions that are suitable for quantitative genetic approaches include:

1. How will patterns of selection on traits be altered with climate change?
2. What is the range of conditions that populations can tolerate through phenotypic plasticity?
3. To what extent are populations locally adapted to the environment they currently experience?
4. How fast can populations evolve in response to climate change?
5. What is the potential for evolution of a different climate optimum and/or a broader range of tolerance?
6. Has the population already evolved in to response to climate change?

In this article, the author will first explain basic quantitative genetic concepts with a focus on genetic architecture and natural selection. Second, the author will briefly review examples of how quantitative genetics has been used to predict evolutionary change, test the rate of evolution, and observe evolution in action.

Basic Quantitative Genetic Concepts

The potential for adaptive evolution depends upon both genetic and ecological factors including the genetic (co)variation

of traits and the strength and direction of natural selection. The relationship between these key components of the evolutionary process is expressed by the 'breeder's equation'

$$R = h^2 S$$

where the amount of phenotypic change expected in response to selection per generation (R) for a single trait is a function of narrow-sense heritability (h^2) and the strength of selection as measured by the selection differential (S). This simple expression shows that the magnitude and direction of an evolutionary response is dependent upon the extent to which traits have a heritable genetic basis and upon the strength and direction of natural selection. It is important to realize that this expression only predicts evolutionary response for the subsequent generation for several reasons. First, genetic variation is reduced by directional selection in each generation and this can reduce heritability, and thus evolutionary potential, over time. Second, heritability depends upon the environment in which the population was measured and this is likely to differ between generations. Finally, natural selection is rarely consistent between generations but fluctuates according to prevailing conditions, such as climate. In general, we expect a large response to selection to occur if natural selection is consistently and strongly targeting traits that are heritable and are not genetically correlated with other traits under selection. The following sections explain these fundamental quantitative genetic concepts in more detail.

Genetic Architecture

Genetic architecture is a general term that refers to genetic structuring at the level of species, regions, populations, families, and individuals. For the purposes of predicting evolutionary change, genetic architecture at the population level is of primary interest because this is the scale where evolution occurs. The genetic architecture of a population includes genetic variation that can be transmitted across generations (heritability), genetic correlations among traits, and variation in trait expression in different environments (plasticity).

Genetic variation

The most fundamental requirement for adaptive evolution is that populations harbor genetic diversity at loci that underlie traits that are the targets of selection. Narrow-sense heritability is an estimate of the fraction of that variance in phenotypic that can be attributed to additive genetic variation (V_A) that is directly inherited by offspring from parents. Phenotypic variation may also arise from dominance interactions between alleles at a single loci (V_D), and environmental variation (V_E), but variation due to genetic interactions are not typically inherited because offspring genotypes are created anew with each generation of reproduction. This discussion is restricted to the most basic quantitative parameters although experimental designs are available to estimate other sources of variance such as epistasis (V_I) and parental effects (V_M) (for a complete treatment see Lynch and Walsh, 1998).

Heritability

Heritability can be estimated by measuring individuals in families that are related to each other to some degree. Because relatives are expected to share a certain proportion of their genes, we can use this information to partition resemblance among relatives into genetic and environmental causal factors. The precision of heritability estimates is influenced by the type of relatives under consideration.

Formal crossing designs, such as combinations of full- and half-siblings or parents and their offspring, allows additive genetic variation to be partitioned from other effects (see Lynch and Walsh, 1998 for a discussion of design options). Natural family structure in wild populations that has been observed over several generations can also be used to build a pedigreed and estimate heritability (Kruuk, 2004; Shaw, 1987). The use of either experimental or natural pedigrees permits a more precise measurement which is referred to as 'narrow-sense heritability' (h^2):

$$h^2 = V_A/(V_A + V_D + V_I + V_E)$$

Although narrow-sense heritabilities are preferable, they are more difficult to obtain because some type of pedigree information for the offspring is required. The most common approaches to obtaining narrow-sense estimates include: (1) measuring traits in parents and offspring in the wild or in lab populations over several generations (e.g., Réale *et al.*, 2003), (2) measuring offspring that were produced by experimental matings between known parents according to a specific design (e.g., Etterson and Shaw, 2001), or (3) from response to artificial selection measured over several generations (e.g., Lenski, 2001).

If replicated genotypes (e.g., clones) or broods obtained from natural matings (e.g., nestlings or seed collected by maternal plant) are measured, a coarser heritability estimate, referred to as 'broad-sense heritability' (H^2), can be estimated. Because clones and siblings share parents, they are also expected to share some variance arising from dominance and epistatic interactions. These effects, however, are not transmitted to subsequent generations and therefore will not generally contribute to evolutionary change. Consequently, broad-sense heritability is a coarser upper-bound estimate of heritability that is confounded with dominance variance:

$$H^2 = (V_A + V_D + V_I)/(V_A + V_D + V_I + V_E)$$

Breeding values are the expected phenotypic value of an individual's offspring (measured as the deviation of an individual's offspring from the population mean). It provides a measure of the heritable contribution of the genotype. The variance in breeding values is the additive genetic variance, V_A. Breeding values are not confounded by other sources of variation (dominance, epistasis, parental effects), in contrast to a simple family mean. Thus, the difference between a breeding value and a family mean is analogous to the difference between narrow-sense and broad-sense heritability (**Figure 1**).

Genetic correlations

Genetic correlations arise between traits because single genes affect more than one trait (pleiotropy) or because alleles present at different genes (each of which may only affect a

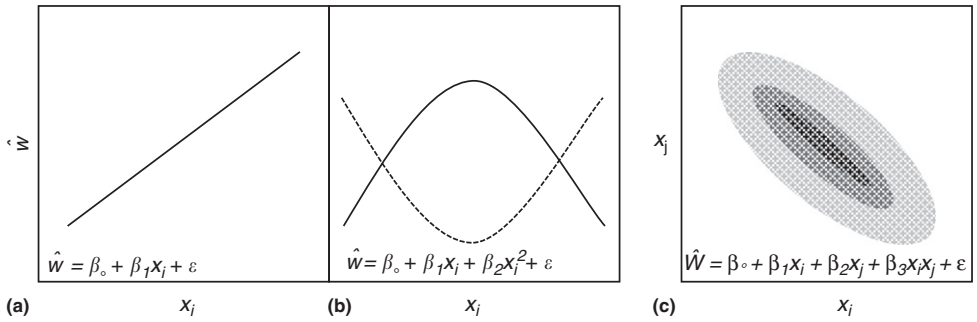

Figure 1 Illustration of some possible forms of selection obtained from regressing relative fitness onto values of trait x_i. (a) An example of linear selection where β_1 is the selection gradient. (b) The solid line provides an example of stabilizing selection where γ_2 is negative. The dotted line represents disruptive selection where the sign of γ_2 is positive. (c) An example of joint selection operating on a pair of traits x_i and x_j where the cross product term γ_3 is significant.

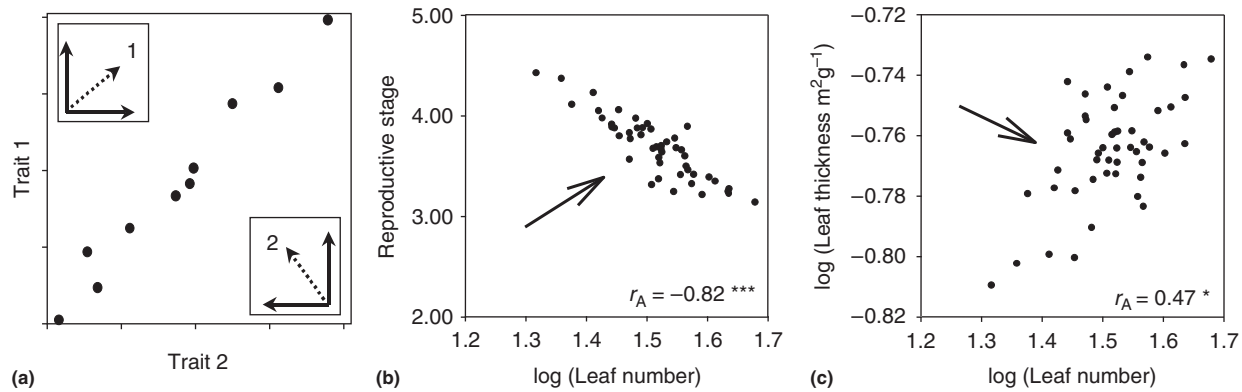

Figure 2 Examples of genetic correlations between traits. (a) Illustrates a hypothetical positive genetic correlation. The arrows in the inset box 2 shows a joint vector of selection on trait 1 and trait 2 that is in accord with the genetic correlation and will facilitate evolutionary response. Box 2 shows a joint vector of selection that is antagonistic to the direction of the correlation and will slow evolutionary response. (b) An example of a negative genetic correlation between plant phenology (reproductive stage) and leaf production that is antagonistic to the joint vector of selection show with the arrow. (c) An example of a positive genetic correlation between leaf thickness and leaf production is also antagonistic to joint vector of selection on these two traits, shown with the arrow.

single trait) tend to be inherited together as a unit (linkage disequilibrium). If traits are genetically correlated, it means that they are not inherited independently. This means that if natural selection exerts a change in one trait, a concomitant change will occur in the trait with which it is genetically correlated. A G-matrix describes the pattern of additive genetic variance and covariance for multiple traits (covariance is the unscaled version of the genetic correlation). The G-matrix is symmetrical with the additive genetic variances on the diagonal and additive genetic covariances on the off-diagonal as shown below for the three traits, *i*, *j*, and *k*.

$$G = \begin{pmatrix} V_{A_i} & \mathrm{cov}_{A_{ij}} & \mathrm{cov}_{A_{ik}} \\ \mathrm{cov}_{A_{ij}} & V_{A_j} & \mathrm{cov}_{A_{jk}} \\ \mathrm{cov}_{A_{ik}} & \mathrm{cov}_{A_{jk}} & V_{A_k} \end{pmatrix}$$

Two kinds of genetic correlations are relevant in the context of climate change: genetic correlations among traits and genetic correlations across environments.

Genetic correlations among traits

Additive genetic correlations among traits are important because they can enhance or retard selection response depending upon whether the direction of the correlation is in accord or antagonistic to the direction of selection (Etterson and Shaw, 2001). For example, if two traits are positively genetically correlated and selection is favoring high values for both traits, then the joint vector of selection on these two traits matches the direction of the correlation which 'reinforces' evolutionary response (**Figure 2(a)**, arrow 1). In contrast, if selection is favoring high values for one trait but low values for the other, then the joint vector of selection on these two traits is 'antagonistic' to the direction of the genetic correlation which may slow evolutionary response (**Figure 2(a)**, arrow 2). Adaptive evolution may be slowed and/or maladaptive evolution may occur if genetic correlations among traits are antagonistic to the direction of selection (**Figure 2(b,c)**). In addition, evolutionary change may occur for traits that are not directly under selection due to their genetic correlations with other traits that are targets of selection.

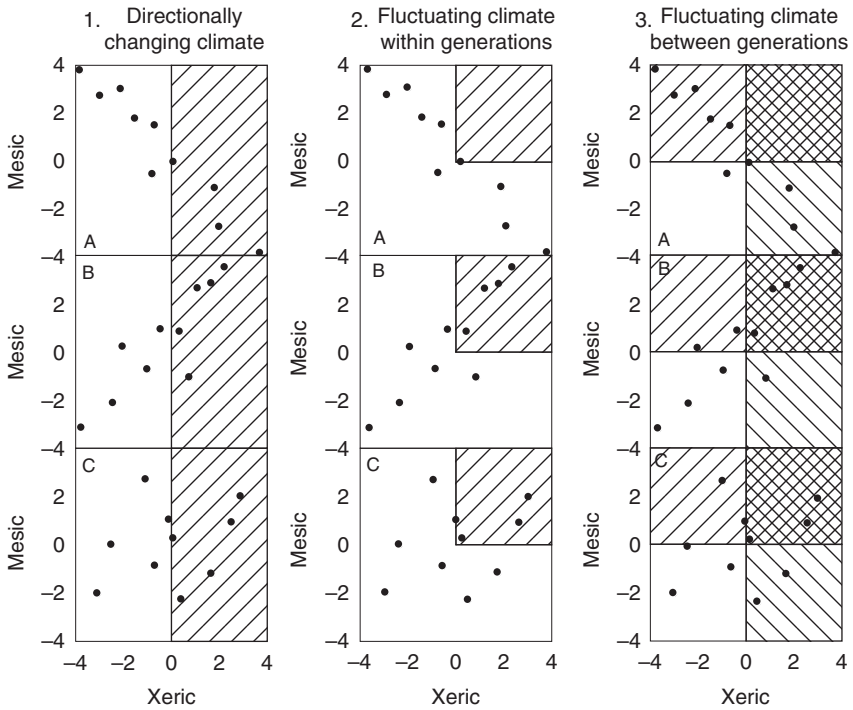

Figure 3 Illustration of the influence of the cross-environment genetic correlation, r_{Aij}, on evolutionary change given three different climate change scenarios: Column 1. A directionally changing climate becoming progressive drier. Colum 2. A climate that fluctuates between xeric and mesic conditions within the lifetime of the organism. Column 3. A climate that fluctuates between xeric and mesic conditions between generations. Hypothetical breeding values are plotted for a single population estimated in two different environments (*y* axis: mesic; *x* axis: xeric). Breeding values for each family reflect the deviation of that group from population mean fitness in one environment ($\bar{x}=0$). Panels A show a negative cross-environment genetic correlation and indicate a genetic tradeoff in performance. Panels B show a positive cross-environment genetic correlation and suggest preadaptation to a drier climate. Panels C show zero cross-environment genetic correlation and indicate potential for independent evolution toward high fitness in either or both environments. Column 1. If selection is consistently in the same direction, as depicted here, the genetic correlation across environments does not influence selection response. In each panel (A-C) there are genotypes with high fitness that can be selected under more xeric conditions (hatched rectangle). Column 2. When selection fluctuates within the lifetime of an organism, selection will favor genotypes that can obtain high fitness in both environments (hatched square). With this hypothetical data, selection response is least likely to occur when the genetic correlation is negative (A) than in other cases (B, C). Column 3. When selection fluctuates between generations, genotypes with high fitness in both environments are always favored (double hatched square). In addition to these genotypes, a distinct subset of genotypes is selected in alternate years (single-hatched square one year, alternative single-hatched square in the next year). Selection will be most efficient with a positive genetic correlation (B) than the other two cases (A, C) because fewer maladaptive genotypes are selected in the alternate years.

Genetic correlations across environments

The expression of genetic variation typically changes when genotypes are exposed to contrasting environments (e.g., mesic and dry conditions) and this can result in the production in different phenotypes, or character states (e.g., thin and thick leaves). This is commonly referred to phenotypic plasticity. Phenotypic plasticity itself may be genetically based and, therefore, can evolve in response to natural selection to contribute to adaptive evolution. When genotypes rank differently in contrasting environments, the relationship between their performance across environments can be described as a genetic correlation (Figure 3). This type of genetic correlation is important for climate change studies because it can influence evolutionary rates and is especially important if selection fluctuates over time (Etterson, 2004b). If climate change is consistent and directional (e.g., becoming monotonically warmer), then natural selection may favor adaptive evolution via fixed genetic changes such as greater thermal tolerance (Figure 3, column 1) and plasticity is not important. However, if natural selection fluctuates, such as is expected with increasing incidence of extreme weather events, then natural selection may favor adaptive solutions involving plasticity in traits that permit high fitness across a range of environments (Figure 3, columns 2, 3).

Application of Quantitative Genetics to Climate Change Questions

In the following sections, the author briefly reviews studies that have used quantitative genetic approaches to predict changes in natural selection and consequent evolutionary change, test evolutionary rates, and interpret evolutionary change over time.

Natural Selection

One of the most fundamental questions that must be answered to predict evolutionary change is how natural selection

changes with climate. The most direct way to estimate changes in climate and selection is to collect data on fitness and phenotype in natural populations over time. However, datasets that are complete enough to document such temporal changes in selection on wild populations are rare (but see Grant and Grant, 1995). An alternative approach is to compare natural selection in current environments to selection in experimental conditions that mimic those predicted for the future. However, most studies that have manipulated environmental conditions, such as temperature, precipitation, and CO_2, have focused on changes in species composition rather than on patterns of natural selection, though there are a few exceptions (Totland, 1999). Insight into temporal changes in selection may also be obtained by characterizing spatial changes in selection along gradients that encompass a range of environments similar to those predicted for the future (Etterson, 2004b) with the expectation that selection regimes will shift to higher elevations or latitudes with climate warming. However, an extensive study comparing trait variation along replicated latitudinal and elevational gradients did not reveal fully parallel patterns along these two types of environmental clines, which suggests that they are not interchangeable and may produce inconsistent results if they are used as a proxy for climate change (Jonas and Geber, 1999).

Substituting spatial variation for temporal change may provide an incomplete picture of future selection because community composition will also be altered by climate change and invasive species, pests, and diseases that expand their ranges add further changes to patterns of selection. Furthermore, natural selection may become increasingly erratic in the future if climates become more prone to extreme events such as drought, heavy precipitation, heat waves, and intense tropical cyclones. A meta-analysis of 5519 estimates of selection from 89 studies supports this assertion (Siepielski *et al.*, 2009) and reports that selection varies considerably between years, directional changes, and possibly the form of selection, are frequent, and that these shifts in selection are commonly associated with climate.

Local Adaptation

To determine whether or not evolutionary change is required for organisms to adapt to a changing climate, it is necessary to understand the extent to which populations are locally adapted to the environment that they currently experience. Species that occur across environmental gradients frequently express clinal patterns in phenotype that may suggest underlying local adaptation. However, these patterns do not necessarily reflect genetic differentiation, but may be a product of environmental responses or 'phenotypic plasticity,' which may or may not be adaptive. The extent to which clinal patterns in phenotype can be attributed to genetic differentiation, phenotypic plasticity, or some combination of these can be ascertained through 'common garden' experiments in which populations sampled from an environmental gradient and reared in common conditions. Because the populations experience the same environment, differences detected among them in morphology, physiology, and phenology must be genetically based. Furthermore, if this differentiation is related to the gradient from which they were sampled, they will retain clinal patterns in a common environment (e.g., Etterson, 2004a). Clinal patterns that disappear in a common garden can be attributed to phenotypic plasticity (e.g., Maherali *et al.*, 2002).

Local adaptation is most commonly tested with reciprocal transplant or 'provenance' experiments that compare the fitness of populations in their native environment to their fitness when they are reared in an alternative environment(s). Local adaptation is inferred if populations obtain higher fitness in their native site and this finding is common. A meta-analaysis showed that 71% of studies (published prior to 2005) yielded evidence of local adaptation and that the local population had a 45% fitness advantage of the nonlocal population (Hereford, 2009). Interestingly, the extent of local adaptation was positively associated with environmental divergence between sites but not with phenotypic divergence between populations. Reciprocal transplant experiments are particularly powerful if populations have been moved in a direction that is concordant with predictions of climate change. Such experiments provide insight into the extent and scale of local adaptation as well as decrements in fitness that may occur with climate change assuming no range shifts or adaptive evolution. This kind of experimental design was employed for large-scale provenance experiments of commercially important tree species that have been established for more than 50 years. Data from these experiments are now being reexamined in the context of climate change and show that populations have evolved different climate optima and breadth of tolerance and suggest that these populations have substantially reduced survival and growth in the future if climate changes as predicted (Rehfeldt *et al.*, 1999).

Phenotypic Plasticity

To understand the role of phenotypic plasticity in climate change response, common garden experiments can be enhanced by exposing populations sampled from contrasting environments to different environmental treatments, such as temperature, precipitation, CO_2, and/or competition. If a specific environmental factor, such as temperature, has been important in trait divergence, then populations should respond according to the conditions in their native habitats (i.e., populations from hotter environments perform better in the warmer treatment). The adaptive value of plastic responses can also be evaluated with this experimental design. For example, adaptive plasticity would be inferred if a plant maintained fitness across water-availability conditions by producing thicker leaves in more drought-prone environments to conserve water. Although instances of adaptive plasticity have been published, it often found that plasticity is passive and does not maintain fitness across environments (reviewed in Van Kleunen and Fischer, 2005). In at least one study in plants, the failure of plasticity to evolve was shown to related to the cross-environment genetic correlation structure that was antagonistic to the direction of selection (such as in **Figure 3**, panel 2A, see also Etterson, 2004b). In a 47-year study in birds, plasticity was shown to allow individuals to track climate but ongoing evolution of plasticity is not likely to occur because the genetic variation has already been exhausted (Charmantier *et al.*, 2008).

Response to Natural Selection

Do quantitative genetic studies predict that populations evolve rapidly enough to keep pace with climate change? A long-term study in Darwin's finches showed that theoretical predictions of evolutionary response based estimates of selection and heritability closed matched evolutionary outcomes in field populations (Grant and Grant, 1995). However, in a study of an annual prairie species the potential rate of evolutionary response was predicted to be slower than the rate of climate change and this was largely attributed to antagonistic genetic correlations (Etterson and Shaw, 2001). In contrast, in a study where phenological synchrony between and insect egg hatch and tree bud opening was disrupted, rapid evolutionary response of the insect species was predicted to restore the phenological match (Van Asch *et al.*, 2007). In general, too few studies of this kind have been conducted to draw strong generalizations probably because of the laborious and long-term nature of this kind of research.

Response to Artificial Selection

Rates and patterns of evolution can be directly characterized by comparing a population at one point in time with the same population at a later point in time. Artificial selection (Falconer and Mackay, 1996; Conner, 2003) and experimental evolution (Bennett *et al.*, 1992) are well-established examples of this approach that yielded tremendous insight into the evolutionary process in the lab and greenhouse. Evidence from artificial selection and experimental evolution studies suggest that it is at least plausible that adaptive evolution is contributing to observed changes in wild organisms. For example, a native herbaceous species flowered 13 days earlier after three generations of artificial selection (Burgess *et al.*, 2007) which is a larger than the 3–8-day change observed for plant species in nature. Likewise, artificial selection in the lab for over 2000 generations in the bacterium, *Escherichia coli*, showed that thermal tolerance can undergo adaptive evolution, even under conditions of fluctuating selection (Lenski *et al.*, 1991). Experimental evolution in fruit flies also showed that long-term natural selection for thermal tolerance led to evolution for some but not all traits and the evolutionary response was not consistent between life history stages (Gilchrist *et al.*, 1997). Other studies have shown that plasticity is the primary response to artificial selection under conditions that mimic future conditions (Potvin and Tousignant, 1996).

Observations of Evolutionary Change

Under rare circumstances, ancestor-descendant comparisons have been conducted in wild populations that have been the subject of long-term monitoring (reviewed in Bradshaw and Holzapfel, 2008). For example, over the last 30 years mosquitoes that breed in pitcher plants in the eastern US have evolved different genetically based photoperiodic cues for breaking dormancy that correspond to increases in the length

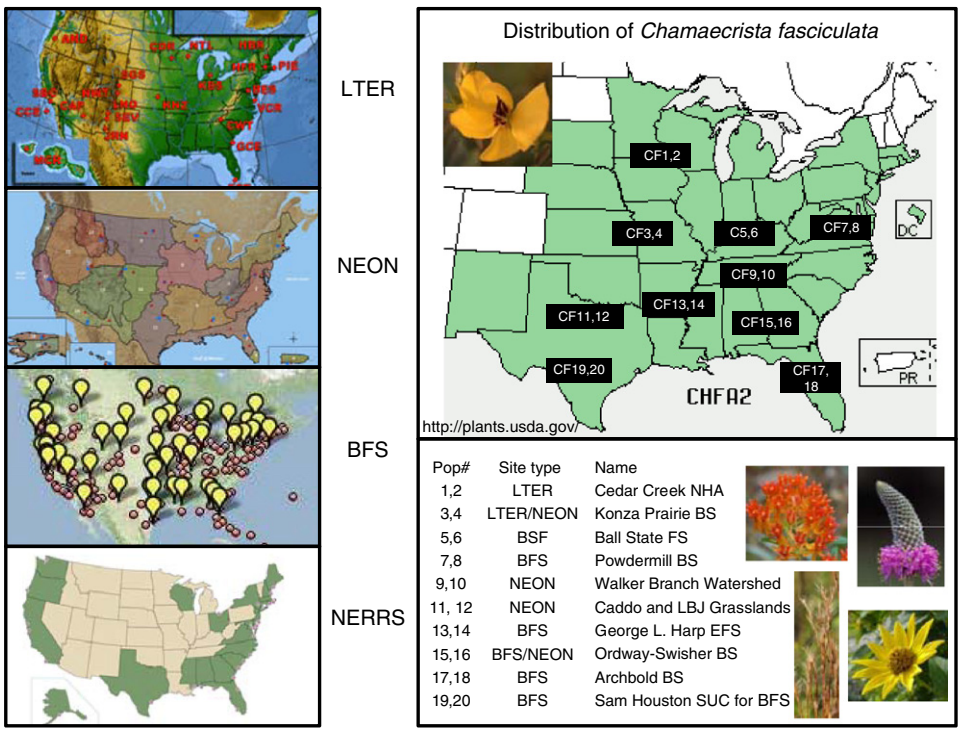

Figure 4 Illustration of how population are sampled for the Project Baseline collection according to two collection strategies. In sampling strategy 1, 20 populations (100–200 maternal liens) of each target species are identified for seed collection at sites that will be preserved into the future (e.g., LTER, NEON, BFS, NERRS, among others). In sampling strategy 2, species are selected that co-occur with the target species at as many sites as possible.

of the growing season in recent decades (Bradshaw and Holzapfel, 2001). These researchers were able to infer that these changes were attributable to evolution as opposed to plasticity because the cue to break dormancy is based on day length, a factor that has not changed over time with climate warming. In recent decades, changes in breeding phenology in red squirrels in Canada (Réale *et al.*, 2003) and migratory patterns in European blackcaps in Europe (Bearhop *et al.*, 2005) have also been observed based on long-term monitoring of wild populations. In the latter two examples, it was possible to estimate quantitative genetic parameters, such as heritability and plasticity, based on extensive records of the mating structure in the population over many years. By reconstructing a pedigree based on field observations of mating pairs on marked individuals, these researchers were able to distinguish genetic change from phenotypic plasticity on phenological traits and ultimately attributed the observed changes to both. The few studies of this kind that have been conducted suggest that the evolution of the timing of life history events may be a more important mechanism of adaptation to climate change in temperate regions than the evolution of thermal tolerance per se (Bradshaw and Holzapfel, 2001). However, this approach is rarely feasible and has only been accomplished in a few systems where individual animals in the population are marked. This approach is not tractable in plants because of wind or animal pollination and seed dispersal prior to germination.

Examining Evolution Using the Resurrection Approach

A powerful alternative approach to study evolution that is possible for organisms with dormant propagules, such as seeds, eggs, and bacteria, is often referred to as the 'resurrection approach' (reviewed in Davis *et al.*, 2005; Franks *et al.*, 2008; Angeler, 2007). In a few cases, direct demonstration of the nature of contemporary change in wild organisms has been possible because propagules were recoverable (Vavrek *et al.*, 1991; Bennington *et al.*, 1991; Hairston *et al.*, 1999; Mcgraw *et al.*, 1991; Kerfoot and Weider, 2004), fortuitously available in storage (Franks *et al.*, 2007; Bustos-Segura *et al.*, 2014; Nevo *et al.*, 2012) such that ancestors could be revived and compared side-by-side with their contemporary descendants in a common environment. This approach has also been applied in long-term experimental evolution studies in bacteria and has yielded the most detailed picture of evolution in action to date (Lenski *et al.*, 1991; Lenski and Travisano, 1994). A fundamental problem with resurrection studies based on wild-collected materials is that surviving propagules are not likely to represent an unbiased sample of the ancestral gene pool. This problem can be minimized if propagules are systematically collected and stored using best practices rather than fortuitously recovered from nature.

The use of resurrection ecology in plants will be much more feasible in future decades due to a new research seed bank, called Project Baseline. The goal of this living seed bank

Table 1 Predictions that can be tested with genetic material collected by *Project Baseline*

Trait evolution
- Longer growing season → Later flowering, altered seed dormancy
- Earlier spring but earlier summer drought → Earlier flowering and potentially compressed life cycle
- Increased temperature → Change in temperature optima for photosynthesis
- Frequent drought/elevated CO_2 → Changes in water use efficiency and specific leaf mass
- Altered pollinator communities → Changes in floral morphology, breeding system
- Altered herbivore communities → Changes in defensive traits
- Altered soil microbial community → Change in nutrient requirements/uptake ability
- Shifting climate envelope → Increased dispersal ability at leading range edge
- Reduced pollinator services due to phenological mismatches → Floral traits that promote selfing

Evolution of genetic architecture
- Selection response due to frequency shifts at a few loci of large effect, vs. many of small effect
- Strong selection responses → Reduced genetic variance
- Reduced pollinator availability due to phenological mismatches → Increased selfing and inbreeding depression
- Increased climate variance → Increased phenotypic plasticity
- Selection responses constrained by genetic correlations
- Phenotypic evolution through fixation of alleles with positive epistatic interaction
- Differential evolutionary responses among species within a ploidy series

Genomic evolution
- Selection response from novel mutations vs. standing variation
- Strong selection on locus → Reduced variation at linked neutral loci (selective sweeps)
- Selection response due to functional divergence of duplicate genes

Phylogeography/population genetic structure/extinction
- Low genetic variability → Higher extinction probability
- Extinction rates correlated to rate of climate change
- Dwindling population size at leading and trailing edge of range → Increased drift
- Neutral markers indicate net direction of dispersal

Source: Reproduced from Franks, S.J., Avise, J.C., Bradshaw, W.E., *et al.*, 2008. The resurrection initiative: Storing ancestral genotypes to capture evolution in action. BioScience 58, 870–873.

is to systematically collect, preserve, and archive seeds to be made available to future biologists for studies of evolutionary responses to anthropogenic and natural changes in the environment in the coming decades (Etterson *et al.*, 2016; Franks *et al.*, 2008). Seeds are being collected from multiple populations of ~125 species with diverse life history attributes in different habitats and climates across the species' geographic range and from numerous individuals per population (**Figure 4**). When seeds are collected, they are separated by maternal line (full or half-sib families) which will permit future quantitative genetic studies on these ancestral populations. Overall, the sampling scheme is designed to capture both variation differentiating populations and genetic variation within populations. Seeds are stored at the USDA National Center for Genetic Resources Preservation, a world-renowned germplasm repository. This project will provide seed collections that can be accessed and germinated by future evolutionary biologists for studies that address a wealth of questions and test a range of predictions in evolutionary biology (**Table 1**). Undoubtedly, advances in evolutionary theory and genetic techniques over the next few decades will expand the realm of questions that can be addressed using this collection to probe the genetic architecture of evolutionary change.

See also: Genotype-by-Environment Interaction. Modularity and Integration. Responses to Climate Change, Evolution and

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Coalescent and Models of Identity by Descent

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Glossary

Coalescence The occurrence, looking backward in time, of common ancestors of two or more alleles.
Effective population size A standardized measure of population size based on the rate of change of genetic variation as a result of reproduction but not selection.
Gamete Haploid germ cell, for example, sperm or egg.
Gene conversion A recombinational process during meiosis in which a tract of sequence is replaced by corresponding sequence from a different chromatid.
Haplotype Any haploid set of multi-locus genetic variations; more specifically, here, any particular segment of DNA sequence in a genome.
Markov process A statistical model of successive changes in the state of a system in which transition rates or probabilities depend only on the current state.
Monecious Having a single mating type; hermaphroditic.
Path coefficients Weights of factors in linear statistical models, used to assess causation.
Phenotype The physical characteristics of an individual organism.
Poisson process A random process that counts numbers of events, usually as they occur in time, in which the times between events are exponentially distributed.

Identity by descent (IBD) is a property of genetic material in related individuals. Specifically, two alleles – i.e., two pieces of DNA from the same genetic locus, and therefore segregating at meiosis in a diploid organism – are identical by descent if one of them is descended from the other or if both are descended from a third allele which existed at some time in the past. Descent, here, means DNA replication and the transmission of genetic material from parents to offspring. The voluminous literature on IBD diverges as to whether mutations are allowed in descent between IBD alleles, though it is probably more commonly allowed than not. In addition, although it usually denotes pairwise identity, IBD can be extended to more than two alleles. For example, the common ancestral, third allele mentioned above is identical by descent with both of its descendant alleles.

IBD describes an important type of sameness which arises in biological systems. Cotterman (1940) took pains to define IBD clearly along with two other kinds of genetic identity: allelic identity, meaning from the same locus, as above, and functional identity, meaning alleles that are interchangeable without consequence for function or phenotype. Crow (1954) coined the term identical by descent (Cotterman had used 'derivative') and contrasted it with a specific kind of functional identity, namely identity in state, which means having the same DNA sequence. When mutation is understood to preclude IBD, then IBD may be viewed as a special case of identity in state. Similarly, it should be noted that 'alleles' and 'allelic' are used confusingly in the literature, to refer either to sequences from the same locus only, as here, or to functionally different sequences at a locus.

The concept of IBD is general, but it is specifically used to indicate close genetic relationship, in excess of background levels in a population. Because any two individuals, even from different species, may be said to be related if a sufficiently long time frame is considered, detailed definitions of IBD depend on what is chosen to measure close relationship. Notions of IBD have been developed for (1) individuals related by a known family structure or pedigree; (2) alleles descended from a common ancestral allele within a specified time and/or without any mutations between them; and (3) genomic tracts of strong genetic similarity demarcated by recombination events. Here, these are referred to, respectively, as pedigree definitions of IBD, coalescent definitions of IBD, and ancestral-segment definitions of IBD.

The ultimate utility of any population-genetic concept, including IBD, is in the interpretation of genetic variation. Theoretical treatments of IBD have guided analyses of genetic and genomic data, and provided avenues for inference by making connections between patterns of variation and key population-genetic parameters or sources of shared common ancestry.

Pedigree Identity by Descent

Studies of what later became known as IBD began early in population genetics, with efforts to allow some non-independence of alleles in the context of the Hardy–Weinberg Law (Hardy, 1908; Weinberg, 1908). The Hardy–Weinberg Law by itself leaves little room for relatedness. In it each individual receives two alleles independently at random from an essentially infinite population. Alleles are either identical (in state) or they are different. Only if a particular relationship or set of relationships is specified and embedded within the population does it become possible to consider IBD. We may think, for example, of a single parent and its offspring, which share exactly one pair of alleles that are identical by descent, while the other two alleles possessed by these two individuals would represent independent random samples from the population.

Early notions of IBD trace back to Wright's work on inbreeding coefficients, which may be interpreted in terms of IBD. Wright applied his general method of path coefficients for decomposing correlations to compute inbreeding coefficients

given a pedigree (Wright, 1921a,b, 1922). Specifically, for a pair of individuals (a possible mating pair) who are connected to common ancestors through K different paths, or 'loops' (Cotterman, 1940) in the pedigree, the inbreeding coefficient of an offspring of the pair is

$$f_o = \sum_{i=1}^{K} \left(\frac{1}{2}\right)^{n_i + n'_i + 1} \quad [1]$$

in which n_i and n'_i are the numbers of generations on path i from each individual back to a common ancestor. Equation [1] neglects the possibility that common ancestors might themselves be inbred, but this is easily remedied if f_a is known for each ancestor (Wright, 1922).

As an illustration, it is well known that Charles Darwin married his first cousin, Emma Wedgewood. Together they had eight children, plus an additional two who did not survive infancy. The relevant family tree, genealogy, or pedigree, is depicted in **Figure 1**. Two paths must be considered in computing f_o: one in which the two alleles both came from Josiah Wedgewood and one in which they came from Sarah Wedgewood. For each path connecting Charles and Emma Darwin to their grandparents, $n_i = n'_i = 2$. Applying [1] gives

$$f_o = 2\left(\frac{1}{2}\right)^{2+2+1} = \frac{1}{16} = 0.0625 \quad [2]$$

as the inbreeding coefficient of any child of Charles and Emma Darwin.

Although Wright (1921a,b) derived [1] by considering the decomposition of the correlation of uniting gametes, it can also be interpreted in terms of IBD. Specifically, 1/2 is the probability that a randomly selected allele in an individual came from a particular one of its parents. The $n_i + n'_i$ factors of 1/2 in [1] give the chance that the two alleles which unite to form a gamete came from a given common ancestor. Another factor of 1/2 gives the chance that the two alleles traced back to that common ancestor are derived from the same allele. Finally the sum is taken over all paths.

For Charles and Emma Darwin's children, there are two paths with $n_i = n'_i = 2$. Thus, [2] gives the probability that the two alleles at a locus in one of Charles and Emma Darwin's children are identical by descent. This sort of thinking underlies the general likelihood calculations on pedigrees that have been of crucial importance in human genetics (Cannings *et al.*, 1978).

Wright (1921b) also used the method of path coefficients, later switching from f to F (Wright, 1931), to establish recursive equations of the type discussed in Section 'Coalescent Identity by Descent,' which relate inbreeding coefficients in the current generation to those in previous generations, and to quantify deviations from Hardy–Weinberg genotype proportions. For two alleles A_1 and A_2 with relative frequencies p and $q = 1 - p$ in the population, Wright described deviations from Hardy–Weinberg proportions using

$$\begin{aligned} A_1A_1 &: \quad p^2(1-F) + pF \\ A_1A_2 &: \quad 2pq(1-F) \\ A_2A_2 &: \quad q^2(1-F) + qF \end{aligned} \quad [3]$$

Then F is the correlation in allelic state of gametes that unite to form a diploid zygote, and ranges from -1 to $+1$. If inbreeding is the source of the correlation, F ranges between 0 and 1 and may be interpreted as the probability of IBD. Equation [3] says that an individual is formed either by sampling two alleles at random (with probability $1 - F$) or by sampling one allele and duplicating it to make a diploid individual (with probability F). Implicit in this interpretation, there is a 'separation of time scales' between a slow population-level process and a fast individual-level or sub-population-level process (Rousset, 2004, p. 57).

The pedigree concept of IBD has proven useful in a number of settings. Malécot (1948) and Kempthorne (1955) employed it to re-derive and extend the calculations of correlations in trait values between relatives which form the basis of quantitative genetics (Fisher, 1918; Falconer and and MacKay, 1996). Another important class of applications uses 'gene dropping' simulations (MacCluer *et al.*, 1986) to account for IBD within a pedigree in generating null distributions of genotypes under selection; for a recent example, see Gao *et al.* (2015).

Coalescent Identity by Descent

Wright (1931, p. 107) referred to F as the 'correlation between uniting egg and sperm' and the 'total proportional change of heterozygosis.' He also used path coefficients to study the rate of increase of F over time under various mating schemes,

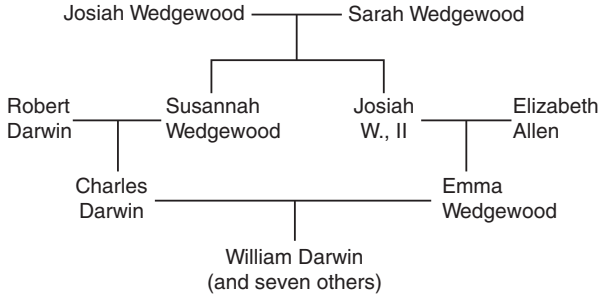

Figure 1 Part of the genealogy of Charles Darwin and Emma Darwin (*née* Wedgewood), who were first cousins. Their common grandparents, Josiah Wedgewood and Sarah Wedgewood, were also third cousins. Using a more complete pedigree, Berra *et al.* (2010) estimate that Charles and Emma Darwin's children had an inbreeding coefficient of 0.0630, which is only a little larger than the 0.0625 computed here, based only on the relationships above.

including what is now well known as the Wright–Fisher model of random mating in a finite population (Fisher, 1930; Wright, 1931). In the simple case of a diploid, monecious organism, over one generation

$$F_g = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_{g-1} \qquad [4]$$

in which g refers to the current generation and $g-1$ to the previous generation. Equation [4] can be rearranged and applied iteratively to obtain

$$1 - F_g = (1 - F_0)\left(1 - \frac{1}{2N}\right)^g \qquad [5]$$

which, with reference to the second line of [3], shows that heterozygosity is lost at rate $1/2N$ in a population of constant size N diploid individuals. More complicated populations behave similarly if they are large, and are described in relation to this monecious case using the concept of effective population size (Wright, 1931).

Malécot (1941, 1946, 1948) called F the 'average coefficient of consanguinity' and clearly understood it as the probability of IBD for two alleles in an individual. He derived recursive equations like [4] and [5] by explicitly considering the occurrence of common ancestors. In the simple example given by [4], which includes the possibility that the individual is produced by self-fertilization, $1/2N$ is the product of the probability that two alleles came from the same parent ($1/N$) and the probability they are descended from the same allele in that parent ($1/2$). When the parents are distinct, the two alleles in an individual descend from two alleles in different individuals in the previous generation, and here Malécot used 'average coefficient of kinship' to refer to the probability of IBD. Because he introduced the notion of tracing allelic lineages back to common ancestors, Malécot is credited with fundamental idea behind the gene-genealogical or coalescent approach to population genetics (Kingman, 1982; Hudson, 1983; Tajima, 1983).

Coalescent theory is reviewed in Hein *et al.* (2005) and Wakeley (2008). In the context of (pairwise) IBD, it is enough to consider the number of generations, G, back to the common ancestor between two alleles. Under the Wright–Fisher model above, G is geometrically distributed:

$$P(G = g) = \frac{1}{2N}\left(1 - \frac{1}{2N}\right)^{g-1}, \quad g = 1, 2, \ldots \qquad [6]$$

If, as typical in coalescent theory, time is rescaled so that it is measured in units of $2N$ generations and N is taken to be very large, technically taking the limit $N \to \infty$ and using $T = G/2N$, then the pairwise coalescence time, T, is exponentially distributed:

$$f_T(t) = e^{-t}, \quad t > 0 \qquad [7]$$

Thus the expected value of the time back to the most recent common ancestor of a pair of alleles is equal to one on the coalescent time scale, or $2N$ when measured in generations.

Two notions of close relationship have been used in gene genealogical or coalescent approaches to population genetics. The first defines IBD relative to an arbitrarily chosen past population, as for example: "The probability of identity by descent is defined as the chance that two genes are descended from the same gene in some ancestral population" (Whitlock and Barton, 1997). Fixing a given generation g in the past and using [6], the probability of IBD would be

$$P(G \le g) = \sum_{i=1}^{g} P(G = g) = 1 - \left(1 - \frac{1}{2N}\right)^g \qquad [8]$$

and for the corresponding coalescence time $t = g/2N$, the probability of IBD would be

$$P(T < t) = \int_0^t f_T(x)dx = 1 - e^{-t} \qquad [9]$$

When g and t are small, these probabilities are both small because it is unlikely for two alleles to be descended from a very recent common ancestor, while both probabilities approach 1 as g and t approach infinity. The occurrence of IBD under this definition is illustrated in **Figure 2(a)**.

Under this time-based notion of IBD, as under the pedigree definition of IBD in Section 'Pedigree Identity by Descent,' IBD has been defined alternately to require or not require identity in state. For example, Crow (1954, p. 544) considered that two alleles are identical by descent if both are 'derived from a single gene in some common ancestor' but added that they 'may be unlike in state if there has been a mutation since their common origin.' Similarly, Cotterman (1940, p. 171) considered that all eight combinations of his three kinds of sameness/difference are possible. In contrast, studying

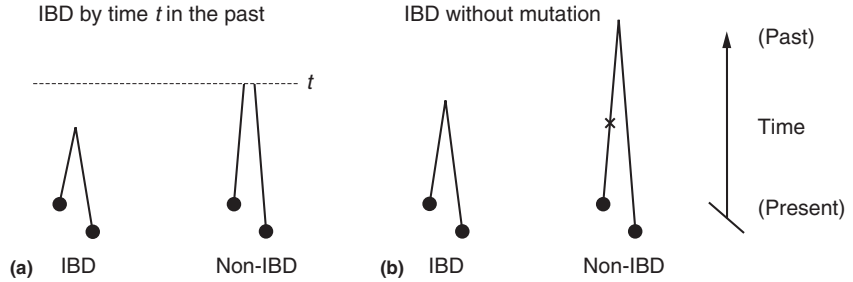

Figure 2 Two different definitions of IBD in gene-genealogical or coalescent models: (a) two alleles are identical by descent if they are descended from a common ancestral allele by time t (or $g = 2Nt$ generations) in the past, and (b) two alleles are identical by descent if they are descended from a common ancestral allele without mutation (x).

F in the face of mutation with probability u per allele per generation (so that $(1-u)^2$ gives the probability that neither allele underwent mutation in the previous generation) and writing

$$F_g = \frac{(1-u)^2}{2N} + (1-u)^2\left(1-\frac{1}{2N}\right)F_{g-1} \qquad [10]$$

Malécot (1946) implicitly assumed that IBD precludes mutation. Nagylaki (1989), interpreting Malécot, states: "Two homologous genes are identical by descent if and only if they are derived from the same gene or one is derived from the other (in both cases without mutation)."

The second coalescent definition of IBD follows Malécot's logic, and can be restated under the backward-time view of coalescent theory (Ewens, 1990) as follows. The probability of IBD is equal to the probability that coalescence, rather than mutation, is the first event encountered when the ancestry of two alleles is traced back into the past. Equations [6] and [7] offer two ways of computing the probability that no mutation occurs on either allele's lineage back to their common ancestor:

$$\sum_{g=1}^{\infty} P(G=g)(1-u)^{2g} = \frac{(1-u)^2}{(2N-1)(2-u)u+1} \qquad [11]$$

$$\int_0^{\infty} f_T(t)e^{-\theta t}dt = \frac{1}{\theta+1} \qquad [12]$$

in which $\theta=4Nu$ is the usual population mutation rate parameter in coalescent theory. A graphical illustration of this definition of IBD is given in **Figure 2(b)**. Equation [11] is also the solution to [10] for $F_{g-1}=F_g=F$. Malécot (1946) obtained [12] as an approximation to this solution, and noted its accuracy for most biologically reasonable values of N and u, which is to say when N is large and u is small.

The coalescent definitions of IBD described in this section apply to allelic variation at a single locus without recombination. Important extensions include considerations of subdivided populations (Wright, 1951; Slatkin, 1991; Rousset, 2004) and patterns of identity and difference in samples of more than two alleles (Ewens, 1972; Thompson, 2013).

Ancestral-Segment Identity by Descent

With the advent of DNA sequencing, and now whole-genome sequencing and genotyping, single-locus concepts of IBD have given way to a genomic perspective which has yielded new insights, particularly in the field of human population genetics (Thompson, 2013). In this context, it is the joint action of coalescence and recombination that determines close relationship, and IBD refers to segments of genomes which descend from recent common ancestors.

To illustrate, consider a focal site which is identical by descent under the pedigree definition of IBD. If there were no recombination, every site would have the same ancestry as the focal site and the entire chromosome would be identical by descent. Recombination decouples the ancestries of different sites and allows IBD to vary along the chromosome because each recombination event chops the chromosome into maternal and paternal pieces which then may have different ancestries. The result is that IBD will occur in segments, as depicted in **Figure 3**, such that some number of sites on either side of the focal site will share the same ancestry and be identical by descent.

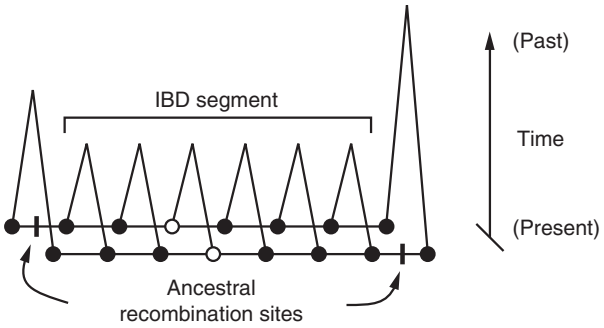

Figure 3 An IBD segment defined by two sites of recombination in the ancestry of two chromosomes. Every site in the IBD segment has the same most recent common ancestor and hence the same time until most recent common ancestry. The focal site is denoted by the open circles.

Under this ancestral-segment view of IBD, two homologous segments in an alignment of two chromosomes are identical by descent if they share a most recent common ancestor, and hence the same coalescence time, at every site. Here these are referred to as IBD segments, but they could also be called IBD tracts or IBD blocks. Note that this definition of IBD implies that every pair of chromosomes is IBD everywhere, because at every site there is a most recent common ancestor which is the same for some number of adjacent sites. As IBD is meant to indicate close relationship, interest is focused on recently co-inherited IBD segments. Younger IBD segments tend to be longer due to the limited opportunity for recombination in their ancestries. Thus, ancestral-segment IBD is typically defined in terms of a minimum length cutoff for segments.

Detection of IBD segments involves finding long haplotypes that are identical in state, although some mismatches are allowed due to the presence of genotyping or sequencing error, mutations that have occurred since the most recent common ancestor defining the IBD segment, and even short gene conversion events that effectively incorporate mutations from other haplotypes. Some IBD detection methods use dense genotype data (Purcell *et al.*, 2007; Gusev *et al.*, 2009; Browning and Browning, 2011), allowing reliable detection of IBD segments as short as two centiMorgans (cM). Recently, other methods have been developed to take advantage of sequence data, enabling reliable detection of IBD segments as short as 0.2 cM (Browning and Browning, 2013; Tataru *et al.*, 2014). Tataru *et al.* (2014) present comparisons of current IBD detection methods.

Fisher (1949, Chapter 3) made the first theoretical examination describing the lengths and counts of IBD segments (bordered by what he termed 'junctions') in regular mating systems, for example, full-sib or parent-offspring mating. Stam (1980) considered the same quantities in the context of random-mating populations, and Chapman and Thompson (2003) studied IBD tracts in subdivided

populations. These studies attempt to simultaneously model many IBD segments across the genome, which is a very difficult problem.

More recently, coalescent theory has been employed to model IBD-segment distributions. The foundation for this work is the coalescent with recombination, which describes the genetic ancestry of recombining chromosomes as an 'ancestral recombination graph' (Griffiths and Marjoram, 1997), allowing different ancestries at different loci. Wiuf and Hein (1999) provided a formulation of this model that proceeded along the chromosomes rather than backward in time, laying the groundwork for practical applications of coalescent theory to IBD segments. McVean and Cardin (2005) proposed a simplified Markov process to approximate this model, immensely improving its computational efficiency, with a subsequent improvement by Marjoram and Wall (2006). These latter models are referred to as sequentially Markov coalescent (SMC) models.

Palamara *et al.* (2012) derived the IBD-segment length distribution as follows. Consider a single focal site in two aligned chromosomes, and assume that the two copies at that site last shared a common ancestor g generations ago. If every ancestral recombination event defines a new IBD segment, which is equivalent to assuming the SMC model of McVean and Cardin (2005), the length of an IBD segment can be modeled by considering the nearest ancestral recombination events on either side of the focal site. This SMC assumption, that a recombination event invariably terminates an IBD segment, is reconsidered below.

There are $2g$ meioses separating the two chromosomes since the most recent shared ancestor at the focal site. If recombination occurs without interference and the distance between recombination sites is measured in Morgans, then in each meiosis recombination can be modeled as a Poisson process along the chromosome with mean equal to 1. Recombination across all $2g$ meioses is then a Poisson process with mean $2g$, and the length L of the IBD segment surrounding the focal site can be described as the sum of two independent exponential random variables with rate $2g$, each representing the distance along the chromosome in one direction away from the focal site.

Given g, the length L is gamma distributed (Palamara *et al.*, 2012):

$$f_{L|G}(l|g) = 4g^2 e^{-2gl} \qquad [13]$$

However, in most contexts the age g is not an observable quantity, so it is integrated out to give the overall IBD-segment length distribution. Using an exponential distribution with rate $1/2N$ to approximate the geometric distribution in [6],

$$\begin{aligned} f_L(l) &= \int_0^\infty f_G(g) f_{L|G}(l|g)\, dg \\ &= \int_0^\infty \frac{1}{2N} e^{-g/2N} (2g)^2 l e^{-2gl}\, dg = \frac{32N^2 l}{(1+4Nl)^3} \end{aligned} \qquad [14]$$

which is a power-law distribution, with infinite mean and variance.

Equation [14] gives the distribution of L when IBD segments are sampled by selecting a position (the focal site) uniformly at random over a long chromosome. Each IBD segment is effectively weighted by its length. It is perhaps more intuitive and more useful to consider the distribution of IBD segments that arises from sampling entire segments uniformly at random rather than weighting them by their lengths. The difference between the two distributions is often referred to as the inspection paradox. If the length of a uniformly sampled IBD segment is denoted S, the density function of S can be derived by weighting [14] by $1/l$ and normalizing, which gives

$$f_S(s) = \frac{\frac{1}{s}\frac{32N^2 s}{(1+4Ns)^3}}{\int_0^\infty \frac{1}{l}\frac{32N^2 l}{(1+4Nl)^3} dl} = \frac{8N}{(1+4Ns)^3} \qquad [15]$$

This is also a power-law distribution, with mean $1/4N$ and infinite variance. Note that deriving [15] without first deriving [14] would require knowing the distribution of coalescence times at IBD-segment edges. The derivation proceeds as above because the distribution of coalescent times at a fixed site arises more naturally from coalescent theory.

The density [15] suggests that in a diploid population of size $N = 10\,000$, the fraction of all IBD segments that are longer than 0.2 cM is $\sim 1.5 \times 10^{-4}$, while the fraction longer than 2 cM is $\sim 1.5 \times 10^{-6}$. Viewed from the other perspective, the density [14] indicates that the mean fraction of the genome contained in IBD segments larger than 0.2 cM is ~ 0.025, while the mean fraction contained in IBD segments larger than 2 cM is 0.0025.

The shapes of these distributions change under variable population sizes or when migration occurs between subpopulations, allowing data on IBD to be used for demographic inference about the recent past. Palamara *et al.* (2012) used this to infer recent population size changes in the Ashekenzi Jewish and Maasai populations, later generalizing to include migration between two subpopulations (Palamara and Pe'er, 2013). Independently, Ralph and Coop (2013) used IBD sharing to characterize the recent geographic and demographic structuring of Europe.

Common to all of these studies is the assumption that recombination events always terminate IBD segments. In fact one-third of all recombination events do not create a new IBD segment (see Theorem 2.4 in Griffiths and Marjoram, 1997) because they are 'healed' by coalescent events in which the two ancestral lineages separated by recombination coalesce back together. These events were ignored in the original formulation of the SMC, but were included in the subsequent SMC' model (Marjoram and Wall, 2006). IBD-segment length distributions can be derived under the SMC' in the manner above, and predictions for overall IBD length distributions under the SMC' match simulations under the full ancestral recombination graph very well, which is not true of the SMC (Carmi *et al.*, 2014). The resulting equations are cumbersome and involve special mathematical functions. Furthermore, for recent IBD segments there is little opportunity for back coalescence, and the differences between the SMC and SMC' predictions are negligible. However, for older, or shorter, IBD segments (smaller than ~ 0.5–1 cM) and for populations that have a small recent effective population size, it is important to base calculations on the SMC'.

With this minor caveat, it is clear that IBD segment-based techniques offer novel and practical ways of using genetic data to learn about the very recent past. IBD approaches to inference depend heavily on accurate detection of IBD segments, emphasizing the importance of recent improvements in IBD detection (Browning and Browning, 2013; Tataru *et al.*, 2014). Considering these developments, along with the recent theoretical advances outlined above, there is promise that IBD-segment methods will continue to reveal new insights into recent demographic processes in ways that complement other traditional approaches of population genetics.

See also: Effective Population Size. Genetic Drift, Models of Random

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Codon Usage and Translational Selection

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The Codon Bias Phenomenon

Messenger RNAs (mRNAs) are translated into proteins according to the genetic code, which determines which triplets of nucleotides, termed codons, correspond to what amino acid. The genetic code is redundant. This means that most amino acids are encoded by more than one codon. Because of this redundant structure of the genetic code, approximately a quarter of mutations within protein-coding sequences do not result in an amino acid change. Such mutations are often, sometimes incorrectly, assumed to have no effect on function and/or fitness. Due to this assumption they are referred to as 'synonymous' or 'silent' mutations, and the sites at which such mutations may occur are referred to as synonymous sites.

Although different synonymous codons encode the exact same amino acid, they are not used equally within a genome. Instead, a phenomenon of 'codon-bias' by which various synonymous codons are systematically more or less frequently used within the protein-coding sequences of an organism is ubiquitous among all living creatures. Codon bias is driven by both biases in the background substitution patterns of the genome and by natural selection favoring certain codons over others (Hershberg and Petrov, 2008; Plotkin and Kudla, 2011).

Background Substitution Biases and Their Effect on Codon Usage

The GC-content of organisms is a highly variable trait. For example, in bacteria GC-content can range from lower than 25% to higher than 75% (Lynch, 2007; Bentley and Parkhill, 2004; Sueoka, 1962). Variation in nucleotide content is observed in a correlated manner across all types of sites, including nonprotein-coding regions, synonymous sites and nonsynonymous sites (Hershberg and Petrov, 2009, 2010, 2012; **Figure 1**). For many years it was thought that the nucleotide composition of a genome is determined by mutational biases (e.g., Andersson and Sharp, 1996; Chen *et al.*, 2004; Hershberg and Petrov, 2008; Shields, 1990; Muto and Osawa, 1987; Sueoka, 1962). In other words, it was thought that GC-rich genomes arose form GC-biased mutation patterns, while AT-rich genomes arose from AT-biased mutation patterns. However, recently it has been shown that mutational biases in themselves cannot explain nucleotide content variation, as for both AT-rich and GC-rich genomes, mutations are universally more likely to occur from G/C to A/T (Hildebrand *et al.*, 2010; Hershberg and Petrov, 2010). The causes of variation in GC-content have therefore not been satisfactorily resolved. In the end, the GC-content of an organism is likely determined by a complex combination of neutral and selective processes that can cause the substitution patterns of the genome to be biased either toward GC or toward AT (Duret and Galtier, 2009; Foerstner *et al.*, 2005; Hershberg and Petrov, 2010; Hildebrand *et al.*, 2010; Reichenberger *et al.*, 2015).

It is clear that even in the absence of any selection on codon usage, a certain level of codon bias can be generated by these biases in substitution patterns. For example, nucleotide substitution biases toward GC will increase a genome's GC-content and also increase usage of G and C ending codons.

Codon Usage Can Affect Translation Accuracy and Efficiency

It is thought that the identity of the codons used to encode amino acids within a protein-coding sequence can affect the efficiency and accuracy with which that coding sequence will be translated. The major reason for this effect is thought to be that codons recognized by transfer RNAs (tRNAs) that are more abundant within the cell will be translated more accurately and more efficiently.

It is largely thought that codons encoded by more abundant tRNAs will be translated more efficiently because more abundant tRNAs will become available more frequently. There has recently been much debate as to whether this model is indeed correct. Older studies, using a variety of low-throughput techniques have demonstrated a link between tRNA

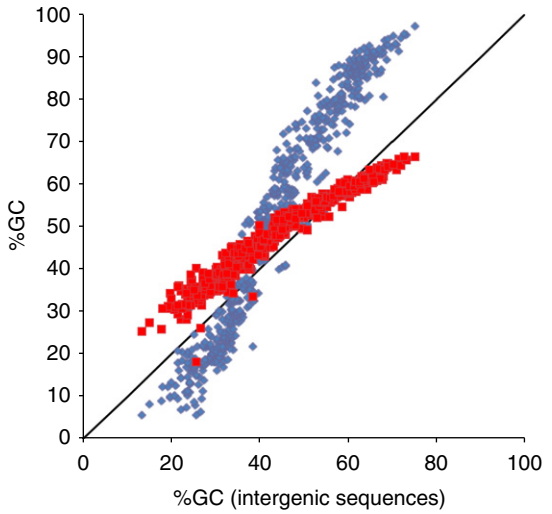

Figure 1 The GC-content of bacteria varies in a consistent manner across types of sites (reproduced from Hershberg, R., Petrov, D.A., 2010. Evidence that mutation is universally biased towards AT in bacteria. PLOS Genetics 6, e1001115). GC-content of nonsynonymous (red) and synonymous (blue) sites are plotted against the GC-contents of intergenic regions from the same bacteria. Each point represents a different fully sequenced bacterial genome. The plot demonstrates a very clear correlation between the GC-contents of all three categories of sites. For synonymous sites, GC-content follows that of intergenic regions, but values tend to be more extreme. In GC-rich genomes, there is higher GC-content of synonymous sites compared to intergenic sites. At the same time, in AT-rich genomes, synonymous sites are even more AT-rich than intergenic sites.

abundance and the speed with which codons are translated (Curran and Yarus, 1989; Sorensen *et al.*, 1989). However, these results were put into question by a number of more recent studies that utilized high-throughput ribosome profiling data and have failed to demonstrate such a relationship (Ingolia *et al.*, 2011; Li *et al.*, 2012; Qian *et al.*, 2012). The last word in this debate so far comes from recently published studies that used the same type of ribosome profiling data, but different analyses methodologies (Dana and Tuller, 2014; Gardin *et al.*, 2014). These studies contradicted previous ribosome profiling studies, observing highly significant correlations between tRNA concentrations and decoding times of the codons they recognize, both in prokaryotes and in eukaryotes (Dana and Tuller, 2014; Gardin *et al.*, 2014).

Codons that are recognized by more abundant tRNAs are expected to be translated more accurately because by arriving more frequently their tRNAs will provide less time for other tRNAs to arrive and incorporate an incorrect amino acid (Akashi, 1994; Precup and Parker, 1987; Stoletzki and Eyre-Walker, 2007).

Translational Selection Increases Codon Bias

Given that codon bias can affect both the efficiency and the accuracy of translation, it is possible that natural selection acting on the fidelity and efficiency of translation will increase usage of certain codons over others. Those codons that are favored by selection because they are translated more accurately and/or efficiently are referred to as favored or preferred codons. Selection to consistently increase usage of certain codons can of course increase codon bias. Whether or not selection at the level of translation will affect codon bias depends on whether the effects of codon usage on translation are strong and important enough to be noticed by selection. For this reason, across organisms, codon usage is more biased in highly compared to lowly expressed genes (Castillo-Davis and Hartl, 2002; Duret, 2002; Duret and Mouchiroud, 1999; Ghaemmaghami *et al.*, 2003; Goetz and Fuglsang, 2005; Gouy and Gautier, 1982). It is more important that a highly expressed gene be translated in an efficient manner. Otherwise, ribosomes will spend a disproportionate amount of time translating such a gene, which may hinder the cells' ability to properly express all the proteins needed for it to function. It is also more important for a highly expressed gene to be translated accurately. This is because mistranslated proteins may undergo misfolding. A highly expressed gene that is often mistranslated and misfolded could lead to misfolded protein aggregation and cell death (Drummond and Wilke, 2008). In contrast, mistranslation of a less abundant protein would not lead to such high levels of misfolded proteins and would therefore not lead to the same levels of aggregation.

It is likely that selection to use favored codons within highly expressed genes often acts at the level of the entire genome, rather than just at the level of individual genes. In other words, if a highly expressed gene is encoded by disfavored codons, the entire organism will be globally affected, rather than just the translation of that specific gene (Andersson and Kurland, 1990). While selection acting at the individual gene level may only rarely be strong enough to drive changes in codon bias, selection at the entire genome level might more often do so. This view is supported by a recent study in which the 'green florescent protein' (GFP) gene, under the regulation of a strong promoter, was introduced into *Escherichia coli* cells (Raghavan *et al.*, 2012). In *E. coli*, favored codons tend to be GC-rich (Hershberg and Petrov, 2009). The authors of this study introduced different copies of the gene with varying nucleotide contents (and thus varying codon usages). When they introduced the GFP gene encoded by disfavored AT-rich codons they found that cellular growth (a proxy for fitness) decreased (Raghavan *et al.*, 2012). Since the GFP gene in itself is not important for *E. coli* growth, this most likely demonstrates that the effect codon usage within this highly expressed gene has on growth is unrelated to the function of that specific gene. Rather, it is likely that the high expression of a gene with strongly disfavored codon usage led to a slowdown in protein translation and/or aggregation of misfolded proteins that affected the entire cell.

At the same time, selection at the level of individual genes has also been demonstrated. For example, it has been shown that even relatively moderate alterations to the codon usage of the *Drosophila* ADH gene can lead to substantial alteration in its expression, leading to reduced ability of the flies carrying the altered genes to tolerate ethanol (Carlini, 2004; Carlini and Stephan, 2003). Such changes in codon usage would likely be under selection due to their effect on the activity of the specific genes in which they reside. It is important to note, however, that it is not clear whether the changes made to the ADH gene affected its expression by affecting its translation accuracy or efficiency. Synonymous changes could, after all, also affect other stages of gene expression as well (see below).

Selection on Codon Usage Is Ubiquitous but May Vary between Organisms

Over the years there has been some debate as to whether selection on codon usage is ubiquitous to all creatures, or whether it is limited to only some. This debate seems to now be settled, as studies have shown that natural selection affects codon usage across all bacteria, and can also affect codon usage in eukaryotes, including mammals (Drummond and Wilke, 2008; Akashi, 1994; Stoletzki and Eyre-Walker, 2007; Hershberg and Petrov, 2009; Marais and Duret, 2001; Supek *et al.*, 2010). Whether or not selection acts for the same reason in all organisms is less clear. For example, it has been demonstrated that selection to increase translation accuracy acts in both prokaryotes and eukaryotes (including in humans) (Drummond and Wilke, 2008; Akashi, 1994; Stoletzki and Eyre-Walker, 2007). This has been demonstrated via the Akashi test (Akashi, 1994).

The Akashi test compares levels of codon bias between codons encoding highly conserved protein residues and codons encoding less conserved protein residues. The idea behind this approach is that amino acid residues that are more important for protein folding are expected to be more conserved than less important amino acid residues. Conservation is therefore used as a proxy for the importance of an amino acid residue to the folding of its protein. By showing higher levels of codon bias in codons encoding more conserved

amino acid residues the Akashi test shows that selection favors usage of specific 'accurate' codons precisely at residues for which accuracy would be more important. This in turn indicates that selection is acting on codon usage to increase translation accuracy.

Whether or not selection on codon usage to increase translation efficiency is also ubiquitous across organisms is, at this point, less clear (Plotkin and Kudla, 2011; Hershberg and Petrov, 2008).

Selection on Codon Usage – Weak and Constant, or Variable and Sometimes Strong?

As mentioned above, synonymous mutations are often assumed to have no or very low effects on function and fitness compared to nonsynonymous mutations. Therefore it is largely assumed that even if there is selection acting on codon usage, such selection will tend to be weak.

While codon bias greatly varies between organisms it is generally intermediate. This means that there are no cases in which only a single codon is used to encode a given amino acid within a genome. The generally accepted model of codon bias maintains that these, generally intermediate, levels of codon bias are maintained by a balance between background substitutions biases (often mis-referred to as mutation), natural selection and genetic drift. Selection increases codon bias in the face of nucleotide substitution biases that counter this increase. In most models, selection on codon usage is implied to be constant (Akashi and Schaeffer, 1997; Akashi, 1995; Mcvean and Vieira, 2001; Nielsen *et al.*, 2007). It is easy to demonstrate that if selection on codon usage is indeed constant, selection would have to be weak (fitness coefficient (s) $\sim 1/N_e$, where N_e is the organism's effective population size), across all organisms, in order to explain intermediate levels of codon bias (Figure 2). Given that different organisms can have very different effective population sizes, it is unclear why the fitness coefficient of synonymous substitutions would be so fine-tuned to each organisms N_e.

A possible solution to this conundrum could be that selection on synonymous sites is not in fact constant. Selection may act in a nonconstant manner on codon bias in two different non-mutually exclusive manners:

(1) It is possible that selection on codon usage varies as a function of levels of codon bias (Figure 3; Hershberg and Petrov, 2008). Suppose there is an optimal level of codon bias for a certain gene. This optimal level may be determined by such parameters as the level of expression of that specific gene. Let us imagine, for example, a highly expressed gene that is at its optimal codon usage. This would mean this gene is encoded by a sufficient proportion of favored codons as to allow for efficient and accurate enough translation as to not cause aggregation of misfolded proteins and/or reduction in the global efficiency of translation. Any increase in the proportion of favored codons used within this gene may either not change fitness at all, or under certain scenarios may even decrease fitness. It is possible that a single mutation at a single favored codon leading to a small downward shift from the optimum proportion of favored codons will be subject to only weak selection. After all, such a single change may not greatly affect the accuracy and efficiency with which a whole gene is translated. However, if more synonymous mutations were to occur reducing the proportion of favored codons further and further, such additional mutations may be subject to stronger selection. Indeed, they may even prove lethal (Figure 3). Similarly, if a new gene is introduced into a genome via horizontal gene transfer (HGT), and if that gene has very different codon usage than the optimum, there may initially be very strong positive selection for mutations that move codon usage of this gene toward the optimum. Once codon usage of this newly introduced gene becomes more similar to the optimum such selection may become weaker and weaker.

(2) It is possible that selection on synonymous sites varies greatly between sites. As I mentioned above, codon bias tends to be intermediate across all organisms studied. In other words, there are no cases in which only the most preferred codon is used to encode a certain amino acid

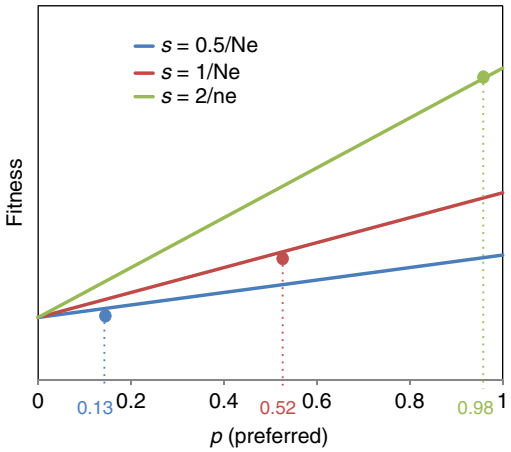

Figure 2 (Reproduced and adapted from Hershberg, R., Petrov, D.A., 2008. Selection on codon bias. Annual Review of Genetics 42, 287–299). Under a model of constant selection on codon usage, fitness is a linear function of the level of codon bias. Under such a model fitness $= s \times p$ (preferred) + constant, where s is the selection coefficient and p (preferred) is the proportion of codons that are fixed for the preferred state. The relationship between fitness and p(preferred) is drawn for three different selection coefficients ($s=0.5/N_e$, $s=1/N_e$, and $s=2/N_e$). For this figure $N_e=10^6$, constant = 0.2. The equilibrium proportion of codons that are fixed for the preferred state (small circles) was calculated for each value of s using the formula: $\hat{p}\,(\text{preferred}) = \frac{1}{\left(1+\frac{\text{bsub}_p}{\text{bsub}_u}\times e^{-4Nes}\right)}$ where bsub_p and bsub_u are the background substitution rates from preferred to unpreferred and from unpreferred to preferred, respectively. For this calculation the ratio between the two was arbitrarily set to 50. For the equilibrium proportion of codons that are fixed for the preferred state to be intermediate (as is true for most genes), the selection coefficient, s, must be in the range of $1/N_e$, as larger values of s lead to genes that are almost entirely encoded by preferred codons (exemplified here by the case of $s=2/N_e$), whereas lower values of s lead to genes with levels of codon bias determined solely by the background substitution patterns of the genome in question (exemplified here by the case of $s=0.5/N_e$).

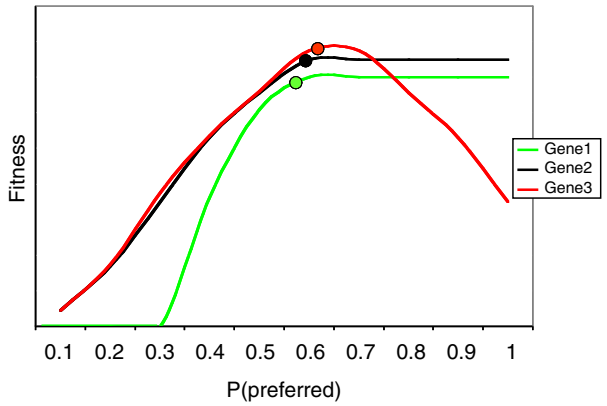

Figure 3 Schematic of the model under which the strength of selection on synonymous mutations changes as a function of codon bias (reproduced and adapted from Hershberg, R., Petrov, D.A., 2008. Selection on codon bias. Annual Review of Genetics 42, 287–299). When the proportion of preferred codons nears an optimum, a selection-background substitution-drift equilibrium is reached. Under this equilibrium, selection is weak. However, shifts from equilibrium can result in strong selection on additional synonymous mutations. The equilibrium proportion of codons that are fixed for the preferred state are marked by small circles. Three possible relationships between fitness and codon bias are depicted: For all three genes at low levels of codon bias, selection for preferred mutations is strong and the strength of selection decreases as codon bias nears equilibrium. For gene 1 very low levels of codon bias are lethal. For both gene 1 and gene 2 fitness reaches a plateau after which additional preferred mutations neither increase nor decrease fitness. For gene 3 there is an optimal level of codon bias that is close to the equilibrium level of codon bias for that gene, and once the gene reaches this level, additional preferred mutations are selected against.

(unless that amino acid is encoded by only one codon). A second way to explain this, without selection on synonymous mutations being constantly weak, is to invoke variation in the fitness effects of synonymous mutations as a function of the genes in which they reside and their positions within these genes. Under such a model, selection could be weak or even absent on synonymous mutations occurring in many genes and at many sites, but much stronger on such mutations occurring at other sites.

As we have already discussed above, selection on codon usage is expected to be stronger on highly expressed genes, and within these genes more selection may be applied on codons encoding amino acid residues important for protein folding. In addition to these well-established levels of variation, other sources of variation may also apply. For example, it is possible that certain synonymous sites may be important for creating mRNA secondary structures. Such secondary structures were suggested to play an important role in stalling translation at codons encoding amino acids important for protein folding and function. This in turn is thought to slow down translation at these important residues, allowing for higher accuracy of translation (Yang *et al.*, 2014). mRNA secondary structures may also play an important role in other levels of regulation, for instance, by affecting mRNA stability in the face of degradation. Additionally, synonymous mutations could also affect such regulatory functions as splicing and the binding of transcription factors and other regulatory proteins.

The best line of evidence currently available showing that selection on synonymous sites can be strong comes from a study carried out by Lawrie *et al.* (2013). In this study the frequency spectra of polymorphisms segregating within *Drosophila melanogaster* was utilized to examine the intensity of selection. Mutations under purifying selection will segregate at lower frequencies within populations. This is why the frequency spectra of polymorphisms can be used to estimate the efficiency with which natural selection acts on a group of mutations. Indeed, examination of the frequency spectra of synonymous polymorphisms in *Drosophila* was among the first ways in which selection on synonymous sites was demonstrated (Akashi and Schaeffer, 1997). A limitation of this approach is that, unless a very large number of individuals are sequenced from a population, only weak selection will allow mutations to persist at frequencies high enough to be observed. Any mutation subject to stronger purifying selection will simply be too rare to be observed. Because the polymorphism data Lawrie *et al.* (2013) utilized was extensive, they were able to also consider the class of 'missing polymorphisms.' This allowed Lawrie *et al.* to estimate what proportion of synonymous sites within *D. melanogaster* was subject to strong selection.

The results were very surprising. Lawrie *et al.* (2013) found that an estimated 22% of fourfold synonymous sites in *D. melanogaster* are subject to very strong purifying selection. Much fewer sites were found to be subject to weak selection. It did not seem that mutations from favored to unfavored codons were more likely to be strongly selected against than other synonymous mutations. Thus, it appears that the main reason for this strong selection is not directly linked to selection in favor of codons that optimize translation. At the same time a significant correlation was observed between levels of gene expression of a gene and the fraction of synonymous sites within that gene that were subject to strong constraint. Highly expressed genes are often more functionally important, and indeed genes with higher numbers of strongly constrained synonymous sites tended to be functionally important, and were found to often be involved in key developmental pathways (Lawrie *et al.*, 2013). Synonymous mutations, located in such important genes and subject to such strong constraint may sometimes be involved in genetic diseases on the one hand, and in adaptation on the other. Indeed, several recent studies have begun reveling examples of synonymous alleles that are involved in disease and adaptive phenotypes (Bailey *et al.*, 2014; Fung *et al.*, 2014; Supek *et al.*, 2014; Gartner *et al.*, 2013; Sauna and Kimchi-Sarfaty, 2011).

Favored Codon Identity and Shifts in Codon Usage

Different organisms have distinct codon usages. Such variation in codon usage between organisms is likely to be contributed by differences in background substitution biases. At the same time, they are also likely to be the result of differences in the identities of the codons that are favored by selection within different organisms.

Variation in the identity of favored codons between organisms begs two questions: First, one must ask which

codons are favored in each organism, and whether there are general rules determining the identities of favored codons. Second, the question arises of how changes are achieved in the identity of favored codons. After all, in order to obtain a change in codon usage one would have to change a very high number of sites within a genome. It is hard to imagine how such a change would be feasible in the face of selection to maintain codon usage. For a long time it was assumed that the identity of favored codons within each organism is a frozen accident. Meaning, the choice of tRNAs that are most abundant was more or less random and this drove a certain codon usage. Once this codon usage was established it was more or less frozen, since changing it would be very difficult in the face of selection. Changes in codon usage under this model would be made possible during long periods of relaxed selection (Bulmer, 1987).

Contrary to the idea that the choice of favored codon is random, we have demonstrated that in bacteria the identity of favored codons tracks the background substitution patterns of the genome (Hershberg and Petrov, 2009). In other words, in GC-rich bacteria favored codons will tend to be GC-rich, while they will be AT-rich in AT-rich bacteria. This leads to a trend, we have dubbed 'going with the flow.' According to this trend codon bias will always go in the direction of the background substitution biases of a bacterial genome, but to a more extreme extent. If the background substitution biases of a genome determine that this genome will be GC-rich, this will mean the synonymous sites of this genome will be even more GC-rich (Hershberg and Petrov, 2009; 2012). This can be seen by comparing the GC-content of synonymous sites to that of intergenic regions of the same genome (**Figure 1**).

It is important to note that there is no trivial a priori reason to expect that tRNA abundance and the background substitution biases of a genome would be linked in such a manner. However, it suggests another mechanism for changes in codon usage that does not require long periods of relaxed selection. Suppose an organism starts experiencing a shift in nucleotide substitution biases. (Note that our model does not claim to explain why such shifts would happen. However, clearly such shifts do occur, or all organisms would have similar nucleotide contents). At first, highly expressed genes will not change their nucleotide content alongside the rest of the genome, since they will be under selection to continue using the most favored codons, recognized by the most abundant tRNAs. Yet, the remainder of the genes which are not highly expressed will shift their nucleotide content and alongside it their codon usage. Once a sufficient amount of non-highly expressed genes are using codons that totally do not match the tRNA pool, it may become advantageous for the tRNAs matching the codons used most by this large group of genes to increase in abundance. After all, even though these genes are not highly expressed, there are many of them and if all of them are translated inefficiently it could reduce the global efficiency of translation and also lead to aggregation of misfolded proteins. Once these tRNAs increase in abundance it will allow the highly expressed genes to follow suit and also alter their codon usage. This may eventually result in a total shift in codon usage toward the direction determined by the genome's new background substitution biases (Hershberg and Petrov, 2009).

Concluding Remarks

Over the past few years we have made much progress in understanding the effects of selection at the level of translation on codon usage. For one, we have moved from thinking that synonymous mutations had very limited functional and fitness consequence, to an increasing realization that synonymous variation needs to be taken into account as a major source of functional variation. At the same time much remains to be understood. Open questions include: (1) What is the relative contribution of translation optimization considerations in the shaping of codon usage? (2) What are the selective pressures beyond translation optimization that affect codon usage? (3) How ubiquitous is selection on codon usage at the level of translation efficiency, and what determines whether a genome will be subject to such selection? (4) What are the determinants of strong selection being applied on a large proportion of synonymous sites? (5) How do shifts in codon usage occur? The next few years should bring forward many new answers to these and other open questions regarding this most intriguing 'codon-bias' phenomenon.

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See also: Neutral Models of Genetic Drift and Mutation. Protein Biophysics and Evolution

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Coevolutionary Fitness Landscapes

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Glossary

Coevolution Evolutionary change that is reciprocated between two interacting groups (usually species). For example, evolutionary change in group A leads to evolutionary change in group B which in turn causes evolutionary change in group A and so forth.

Covariance A statistical measure that indicates to what degree two variables change in concert. The covariance is formally defined as the difference between the mean of the product and product of the means (i.e., $\mathrm{Cov}(x,y) = \overline{x \cdot y} - \bar{x} \cdot \bar{y}$ where the bar indicates the mean). A negative covariance indicates that as one variable increases, the other decreases; a positive covariance indicates that as one variable increases, the other increases as well.

Gene flow The movement of genes from one population to another. Note that gene flow is considered different from migration of individuals because of the added requirement that the genes of transitory individuals get passed on in the populations into which they travel. Thus, for example, gene flow could happen without migration if an individual traveled to another population, successfully mated, and then returned to their home population.

Genetic drift Sampling error on gene frequencies that occurs in populations. For example, assume a population has two individuals, one with genotype A and one with genotype B. Thus the frequency of both A and B is 50%. If one of the two individuals, say individual B, is randomly lost from the population, then the frequency of genotype A becomes 100%. The effect of genetic drift on evolution scales with population size, where small population sizes have the largest sampling error (such as the previous example).

Phenotypic interface The suite of traits in two species that determine the outcome of an interaction. An example of this would be antigens of pathogens and antibodies in hosts. The ability of antibodies to detect antigens determines whether a disease infects a host and thus these two traits form a phenotypic interface.

Phenotype matching A system where the traits of each species have distinct fitness optima or minima based on the phenotypic value of the individual they interact with. For example, in Darwin's orchids and moths, the orchids (*Angraecum sesquipedale*) have a very long nectar-bearing spur and the moths (*Xanthopan morganii*) have a matching proboscis length to guarantee that pollination occurs.

Population and metapopulation The traditional definition of a population is a group of individuals who are breeding with each other at random. A metapopulation is a group of populations that are connected via migration. Within a metapopulation other processes besides migration, such as extinction and recolonization, may occur.

Population and quantitative genetics Population genetics studies the frequencies of different, explicit genotypes or alleles within populations of organisms (e.g., tracking the frequency of the *AA*, *Aa*, and *aa* genotypes for a diploid organism at a gene locus having alleles *A* and *a*). Quantitative genetics focuses on phenotypic values – such as body weight – and their means and variances, and assumes that these traits are controlled by many genes that each have small effects on the expressed trait.

Shifting balance theory Proposed by Sewall Wright in 1932, this theory examines how local adaptation occurs in subdivided populations. Essentially, populations in a metapopulation may transiently become maladapted by genetic drift but then attain a new higher fitness peak by natural selection after crossing through a maladaptive phenotypic space. Once a new fitness peak is achieved, gene flow can then export more fit genotypes to other populations.

Introduction

The process of species coevolving with each other has been central to the formation of the broad diversity of life that currently exists on Earth. Of the three domains of life – Archaea, Bacteria, and Eukarya – eukaryotes are thought to have arisen due to a coevolutionary, mutualistic relationship between Archaea and Bacteria. Strong evidence indicates that mitochondria were once free-living Alphaproteobacteria that formed an endosymbiotic relationship with Archaea (Margulis, 1993). Further evidence suggests that this capture of a free-living bacterium has been repeated again in eukaryotes capable of photosynthesis; phylogenetic and other analyses indicate that chloroplasts are descendent from Cyanobacteria (Cavalier-Smith, 2000). The mutualistic relationships between mitochondria, chloroplasts, and their hosts led to the formation of complex organisms with sophisticated nutrient uptake mechanisms, which now make up all macroscopic biota. While this example is a notable event, coevolution is continually shaping biological diversity at both the macroscopic and microscopic levels.

This article focuses on how interacting with another species affects the fitness of a focal species. These fitness 'outcomes' then feedback into and define how species coevolve. Evolutionary (or coevolutionary) biologists are particularly interested in fitness because the strength of natural selection depends on how fitness varies among individuals. Thus, fitness drives the process of adaptation to local conditions and ultimately determines whether a species persists or goes extinct. It has long been realized that coevolution has shaped the

adaptation of most (if not all) species on Earth (Mode, 1958). In fact, within the coevolutionary literature it is not uncommon to see the "Tangled Bank Hypothesis" referenced. This hypothesis comes near the end of *On the Origin of Species* by Darwin (1859), where he states: "It is interesting to contemplate an *entangled bank*, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and *dependent on each other in so complex a manner*, have all been produced by laws acting around us" [emphasis added]. Clearly, even at this early time in the study of evolution, the importance of interactions between species in shaping biological diversity was already being noted.

Natural Selection

To understand how fitness plays a role in natural selection and adaptation, one needs to understand how selection is defined. In general, evolutionary biologists work with 'soft selection'; soft selection means that a population is neither increasing nor decreasing in size. The opposite of this is 'hard selection' where population size fluctuates (Wallace, 1975; Débarre and Gandon, 2011). The fitness measure used for soft selection is called 'relative fitness' and it is typically denoted with the symbol w; a fitness measure is considered relative when its average value (symbolized as $\overline{w}$, where the bar denotes the average value of w) is 1. Selection (s) is formally defined as the change in the average of some trait z (e.g., body weight) between two time points (call them $\overline{z}^*$ and $\overline{z}$ with the bar notation again indicating an average and the asterisk noting the mean after selection), but prior to inheritance (i.e., the organism has not reproduced); this gives the simple equation $s = \overline{z}^* - \overline{z}$ (Lande, 1979). By definition, mean relative fitness is 1, so we can rewrite this as $s = \overline{z}^* - \overline{z} \cdot \overline{w}$. To find the mean at the later time point ($\overline{z}^*$), we need to know that relative fitness tells us what the change in frequency of the trait will be after a selective event. More intuitively, if $w > 1$ then a particular trait value will be over-represented after selection; conversely, if $w < 1$ then a particular trait value will be under-represented after selection. Therefore, the mean of the product of z and w gives the mean post-selection (see Lande and Arnold, 1983 for more detailed explanation of this), and our equation becomes $s = \overline{w \cdot z} - \overline{z} \cdot \overline{w}$. The right-hand side of this last equation is the statistical definition of a covariance between two variables, thus producing $s = \mathrm{Cov}(w, z)$ (Lande and Arnold, 1983). This simple derivation leads to often-used phrase, "selection is the covariance of (relative) fitness and phenotype." While the derivation shown comes from the field of quantitative genetics, the same – or at least nearly so – result has been found starting from first principles in population genetics (Price, 1970). This equation highlights how fitness ties directly into natural selection and adaptation.

Building upon this simple definition, it is then helpful to investigate two questions in order to understand how selection driven by coevolution affects biodiversity. First, we wish to know how interactions between species affect fitness. At a more basic level we simply need to understand what causes something to be more or less fit and why it varies. Second, we need to understand the coevolutionary process and the factors outside of natural selection (e.g., genetic drift) that are also important to that process. The following sections address these two questions separately.

A Multi-Scale View of Fitness

What is fitness? This seems like a very basic question for evolutionary biology, yet the answers that are given are often varied and complex. Furthermore, fitness is often difficult to measure experimentally and proxies are often used. For example, for many plant studies, seed set is used as measure of fitness, but it is usually unknown whether the seeds in question are viable and contribute to the subsequent generation. For microorganisms like bacteria, doubling-time estimates are used as measure of fitness, but this is more of a population-level estimate of fitness rather an estimate of fitness for an individual bacterium. A safe definition of fitness is simply the number of progeny that survive to reproductive age. The issue here is that 'progeny' can be defined in multiple ways.

Traits of individuals are shaped by genetic and environmental factors. If we assume for simplicity that one gene controls one trait (e.g., flower color in Mendel's pea plant experiments), then we can think of fitness at the gene level. Fitness would then be the number of copies of a gene that are found in the next-generation. This definition of fitness is overly simplistic because genes do not exist in a vacuum; rather, many genes make up an individual and selection (potentially) acts on the individual. Despite this, many evolutionary (or coevolutionary) models – including some of those discussed later in this article – use a gene-centric definition of fitness. However, this difference between thinking at the gene level and at the individual level gives rise to ideas such as 'coadapted gene complexes' (Dobzhansky, 1948, 1950; Wallace, 1968); in other words, the genes within an individual must interact in such a way as to produce a fit individual to ensure their survival. But conflict does arise between the gene and individual level; a gene should favor any mechanism that favors its continuation in the next-generation, whether or not the organism survives (Williams, 1966). This thinking has led to the concept of inclusive fitness (Hamilton, 1964a, 1964b), wherein a gene aids the success of copies of itself passed on globally via related individuals; this argument has been popularized in Dawkin's (1976) *The Selfish Gene*. Inclusive fitness has been used to explain the evolution of altruism and caste systems in eusocial insects (such as honey bees where most individuals forego reproduction).

Regardless, fitness at the individual level is clearly the result of the expression of many genes that underlie many traits. This leads to a 'multivariate' view of natural selection and evolution (Lande, 1979). The theory underlying multivariate evolution makes it possible to study selection on specific traits while taking into account genetic correlations that may exist between the genes responsible for each trait. From the coevolutionary perspective, being able to study traits separately using multivariate approaches is very important. Within a coevolutionary system, a trait (or perhaps a suite of traits) is often thought to form a 'phenotypic interface' between coevolving species; how

these traits interact at the phenotypic interface determines the fitness derived from interactions with members of other species (Brodie and Ridenhour, 2003).

Combining the knowledge that fitness may be derived from various sources and that multivariate analysis is needed to study specific traits, the selection coefficient (s) can be broken down in the following manner: If we consider fitness from two different sources, one coevolutionary (w_c) and one not (w_0), we can simply rewrite our selection coefficient as $s = \mathrm{Cov}(w_c + w_0, z)$. Using some statistical properties of covariances, this equation can be simplified to $s = \mathrm{Cov}(w_c, z) + \mathrm{Cov}(w_0, z) = s_c + s_0$, where s_c and s_0 respectively represent the selection pressure on a trait due to coevolutionary and noncoevolutionary sources (Ridenhour, 2005). For the sake of simplicity, this example of breaking down selection into different sources does not describe the full multivariate approach but the results are easily extended to such approaches.

So far, selection at the gene and individual level has been described, but both population-level and metapopulation-level perspectives of selection are needed to understand how coevolution affects adaptation. Natural selection causes the mean fitness of the population to increase; therefore at the population level it is useful to look at the 'adaptive surface' (Wright, 1967; Cresswell and Galen, 1991; Fear and Price, 1998). An adaptive surface is obtained when individual fitnesses are averaged across a distribution of traits whose mean is allowed to vary (Figure 1). Conceptually one can think of evaluating the individual fitness function at the population mean phenotype, however the variance in trait values affects the adaptive surface. Metapopulation processes (e.g., migration) and population processes (e.g., genetic drift) will also affect the means and variances of traits in populations, and thus also play a role in adaptation. Sewall Wright's 'shifting balance theory' (1932) was perhaps the first significant scientific argument regarding how processes that occur with metapopulations drive the adaptive process. These ideas still play a critical role in modern coevolutionary thinking.

Coevolution and Fitness

Janzen (1980) defined coevolution as reciprocal evolutionary change that occurs between interacting groups (typically species). This is the most commonly used definition of coevolution, however, other broader definitions of coevolution are sometimes used (e.g., diffuse coevolution; Iwao and Rausher, 1997; Fox, 1988). Regardless of the definition, interactions between species are the building blocks of the coevolutionary process and can be broken down into several categories: competitions, mutualisms, antagonisms, commensalisms, and

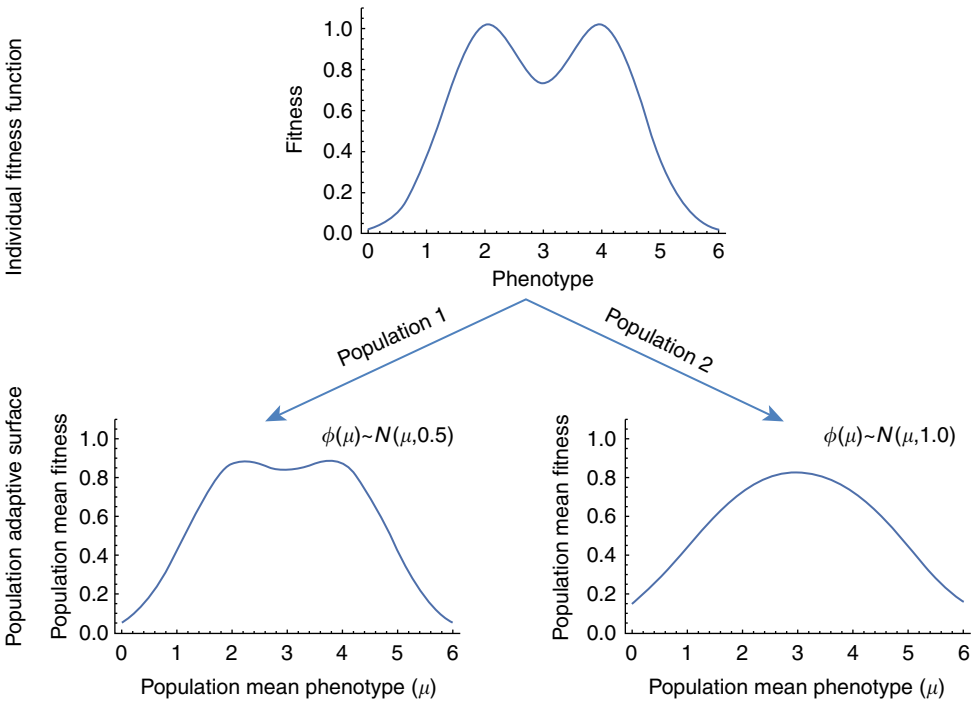

Figure 1 Individual fitness versus the population adaptive surface. The top plot shows an individual fitness function where the optimal phenotype is either $z=2$ or $z=4$. The lower row of plots shows the adaptive surface for two populations. Population 1 has phenotypic distribution that is normal with a standard error of 0.5 ($\varphi(\mu) \sim \mathrm{N}(\mu, 0.5)$); in contrast, population 2 has a distribution with a larger standard error of 1.0 ($\varphi(\mu) \sim \mathrm{N}(\mu, 1.0)$). Note that population 1 and population 2 have different optima favored by natural selection. In population 1, two potential evolutionary optima exist (i.e., two peaks) while only one exists for the population with greater variance in phenotypes. Furthermore, the maximum population mean fitness in population 1 is greater than the maximum for population 2. Also, the population mean fitness is less than the maximum individual fitness. The only way the population could have the maximum individual fitness would be for the phenotype to become fixed at a value of either 2 or 4. In situations like this, there should be stabilizing selection that reduces the phenotypic variance in the population so that the population mean fitness can increase. Coevolution occurs within metapopulations which makes the fitness landscapes even more complicated because then metapopulation processes (e.g., migration) affect the adaptive surface.

amensalisms. These categories come from the fitness consequences of two species interacting together. If we let (+) indicate a fitness benefit, (−) indicate a fitness loss, and (0) indicate neutral fitness consequences, then a (−,−) species pair is a competition, a (+,+) species pair is a mutualism, a (+,−) species pair is an antagonism, a (+,0) species pair is a commensalism, and a (0,−) species pair is an amensalism. For completeness, (0,0) is sometimes referred to as a neutralism, but is typically considered a lack of interaction. Understanding these types of interactions and their fitness consequences is central to understanding coevolutionary outcomes (Thompson, 1982).

Because host–pathogen (e.g., Burdon and Thrall, 2009) and host–parasite systems (Morran *et al.*, 2011) have strong public health, agricultural, and conservation implications, antagonisms are probably the most commonly studied form of coevolutionary interactions. However, many classic examples of antagonisms exist that are not host–pathogen/parasite interactions. For example, Brodie *et al.* (2002) demonstrates how garter snakes have evolved high levels of resistance to deadly tetrodotoxin that is found in elevated levels in their prey, the rough-skinned newt. Toju and Sota (2006) explores similar 'arms race' dynamics in the coevolution of Japanese camellias and camellia weevils. Other classic studies of antagonisms display 'phenotype matching' dynamics, such as seen in parsnip and parsnip webworms (Zangerl and Berenbaum, 2003) or nest-parasitic cuckoos (Krüger, 2007). Cyclical dynamics are also possible in antagonisms as observed in snails and their parasites (Morran *et al.*, 2011).

While mutualisms have possibly been less studied than antagonisms, mutualism has been of enormous interest to evolutionary biologists throughout history. Early research on mutualisms includes Darwin's (1862) treatise on orchids and their pollinators. Mutualisms between plants and their subterranean mycorrhizal fungi may be one of the most important interactions between all biotic organisms, and display a range of forms and evolutionary strategies (Powell *et al.*, 2009); in fact, mutualisms with mycorrhizal fungi may exist for 80% of flowering plants (Bronstein *et al.*, 2006). Some other classic examples of mutualisms are: the relationship between fig and their pollinating fig wasps (Kerdelhue *et al.*, 2000); ants that practice 'agriculture' by farming fungi that provide antibiotics (Mueller *et al.*, 2005); and the obligate mutualism has evolved between yuccas and yucca moths (Althoff *et al.*, 2006).

Regardless of the form of the coevolutionary interaction that is taking place, the 'geographic mosaic theory' (Thompson, 1994, 2005) has been one of the central frameworks for coevolutionary thinking since its introduction. This theory is made up of a set of three 'ecological predictions' and three 'evolutionary hypotheses.' The general ecological predictions of the geographic mosaic theory are as follows:

- trait 'matching' between species will happen only within communities,
- there will be variation in the mean phenotypes among populations, and
- there will be few species-level coevolved traits.

These general ecological predictions are typically supported by empirical studies but are not necessarily outcomes unique to the geographic mosaic theory (Gomulkiewicz *et al.*, 2007). However, with regard to coevolutionary fitness landscapes, the 'evolutionary hypotheses' are more interesting and are:

- different populations will become hotspots and coldspots of coevolution,
- selection mosaics will lead to variable evolutionary trajectories across populations, and
- trait remixing will alter the spatial distribution of coevolving traits.

Theoretical work has shown that selection mosaics and trait remixing have important – and sometimes nonintuitive – effects with regard to local adaptation (Gandon and Michalakis, 2002; Ridenhour and Nuismer, 2007). Empirical studies have also borne out some of the novel predictions related to the geographic mosaic theory. For example, Jokela *et al.* (2009) demonstrate that sexual reproduction of organisms can be favored by host–parasite coevolution, despite being costly. Thompson (2009) highlights some of the most important advances that have resulted from the geographic mosaic theory.

The three evolutionary hypotheses are central to understanding coevolutionary fitness landscapes. First, hotspots and coldspots represent sources and sinks of coevolution. Hotspots (sources) are populations in which reciprocal evolution (per Janzen, 1980) occurs; coldspots (sinks) are populations where either coevolutionary selection pressures are absent or no genetic variation exists for the traits involved in the interaction. Vogwill *et al.* (2009) and Thompson *et al.* (2002) both demonstrate the important role of hotspots and coldspots in the rate of evolution and local adaptation within coevolving species. There are various causes of hotspots and coldspots. For example, crossbills (*Loxia curvirostra*) are seed predators of lodgepole pine (*Pinus contora*). Lodgepole pine have evolved different pinecone morphologies to protect their seeds from crossbills, and, in response, crossbills have evolved different bill morphologies to continue their consumption of lodgepole pine seeds. However, populations that have both crossbills and lodgepile pine present are not always coevolutionary hotspots. Benkman *et al.* (2001) find that crossbills are not significant sources of selection on lodgepole pine when a third species, squirrels, is present to act as the dominant pinecone predator (thus creating a coldspot in the crossbill–lodgepole coevolutionary landscape). Differences in productivity among populations can also lead to the formation of hotspots and coldspots (Hochberg and van Baalen, 1998). While hotspots and coldspots are observed in the crossbill–lodgepole system at a broad geographic scale, evidence that hotspots and coldspots can occur at very localized scales has been found in plants and their pathogens (Laine, 2006).

Selection mosaics are the most novel – and perhaps difficult to understand – concept within the framework of the geographic mosaic theory. A selection mosaic occurs when fitness outcomes of a specific interaction vary due to some third factor (the first two factors being the genotypes/phenotypes of the two interacting species). Thus, selection mosaics have been described as being a genotype × genotype × environment (G × G × E) interaction that affects fitness (Thompson, 2005). To be more specific, the difference in fitness outcomes between two populations is a result of the

environment (E) if a selection mosaic is present. The environment that may cause this difference is often the biotic environment (i.e., the community of species in which an interaction takes place). Thus the presence or absence of tertiary species can lead to the formation of selection mosaics, such as described in crossbill–lodgepole–squirrel example given previously (Benkman, 1999). Actually, measuring G × G × E interactions that alter the resulting fitness of an interaction between species is not a simple task. (Some methods, such as those developed in Kirkpatrick and Heckman (1989) or Kingsolver *et al.* (2001) have potential for doing so.) Most studies that hint at selection mosaics have involved some form of experimental evolution. One example of this is a study by Rudgers and Strauss (2004) that demonstrates geographically variable selection by manipulating ant interactions with cotton across population. Fewer studies have attempted to look at selection mosaics *in situ* due to logistical difficulties. However, one example of this is a study of garter snakes (*Thamnophis sirtalis*) and newts (*Taricha granulosa*) by Hanifin *et al.* (2008) that suggests the nature of coevolutionary selection changes in populations in which predator or prey phenotypes are extremely exaggerated.

The third evolutionary process of the geographic mosaic theory, trait remixing, is probably the most easily demonstrable and least controversial aspect of the geographic mosaic theory. Trait remixing is broadly defined as any nonselective force that causes evolution in a metapopulation. Microevolutionary forces such as gene flow, mutation, and genetic drift can therefore all be sources of trait remixing. Many studies of gene flow – such as Dybdahl and Lively (1996) regarding snails and their castrating trematode parasite, or Ridenhour *et al.* (2007) concerning coevolving garter snakes and newts – have demonstrated that patterns of gene flow are important to the coevolutionary process. Gene flow's influence on coevolution has been extensively studied from a theoretical standpoint as well (e.g., Nuismer *et al.*, 1999). Particularly interesting are results demonstrating that gene flow, traditionally considered to be a maladaptive force, can improve the opportunity for local adaptation by introducing novel genetic variants (Gandon and Michalakis, 2002). Empirical demonstrations of these effects exist in plant–pathogen systems (Thrall *et al.*, 2002). Schulte *et al.* (2010) show that coevolution may speed up the process of reciprocal adaptation via increases in mutation rates. While these forces are, by definition, not part of coevolutionary selection they do influence the fitness landscape. Trait remixing is important to coevolutionary fitness landscapes for three reasons: First, gene flow imports/exports individuals (genotypes) from other populations thus either increasing or decreasing the population mean fitness and variance that changes the adaptive surface for a population (**Figure 1**). Second, any mutations that lead to the creation of novel phenotypes increase the variance of a trait in a population and furthermore may create a phenotype with higher fitness than any other extant phenotype. Third, genetic drift reduces the phenotypic variance within small populations. Reductions in phenotypic variance can potentially lead to achieving higher population mean fitness (**Figure 1**), but have the drawback of limiting strength of selection (the covariance of fitness and phenotype decreases) and the ability of the population to respond to that selective force (there is less genetic variation available). In summary, all of these sources of trait remixing affect the fitness landscape and more importantly adaptation via mechanisms described by the shifting balance theory (Wright, 1932).

Conclusions

Coevolutionary fitness landscapes are complex and require an understanding of the source of fitness components, the multivariate nature of selection at the organismal level, how populations adapt to their local environment, and finally how interactions between species change across both time and space. The process of adaptation on fitness landscapes – without regard to coevolution – has been thoroughly studied; much of this research stems from ideas put forth in the shifting balance theory (Wright, 1932). The geographic mosaic theory (Thompson, 1982, 1994, 2005) is, to some degree, an extension of the shifting balance theory that incorporates concepts specific to how interactions between (coevolving) species shape fitness landscapes. In particular, two of the evolutionary processes of the geographic mosaic theory, the formation of hotspots and coldspots and of selection mosaics, offer novel insights into how adaptation occurs for coevolving species. These contributions have illuminated how coevolutionary interactions may have nonintuitive outcomes. Further research is needed to understand and document the G × G × E interactions that form selection mosaics. The need to understand how coevolution shapes diversity is continually growing; the evolutionary and ecological principles of adaptive coevolution are being brought to bear on more modern data regarding bacterial biofilm formation, microbiomes (human, environmental, or otherwise), and the complex interactions (such as protein–protein interactions) that occur at the molecular level. These data potentially offer a wealth of knowledge regarding the coevolutionary process, and reciprocally, coevolution offers explanations for the diversity observed in these complex modern data.

See also: Adaptive Landscapes. Coevolution, Introduction to. Shifting Balance Theory, Sewall Wright and

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Coevolution, Bacterial-Phage

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Glossary

Adsorption Generally describes the process of phage recognition of and attachment to receptors on the host cell surface, often directed by the phage tail fibers.

Apparent competition Indirect interactions among competitors through a shared natural enemy, such as a predator or parasites.

Burst size The average number of phage particles produced per bacterial cell that is infected.

CRISPR/Cas system Array of clustered, regularly interspaced short palindromic repeats (CRISPRs) within the bacterial genome in which small segments of phage DNA can be integrated as 'spacers.' These spacers are used to generate RNA that is carried by the Cas complex to cleave the phage DNA at the matching 'protospacer.'

Host range A given phage type is only capable of infecting a subset of bacteria in the environment, and this subset is termed the phage's 'host range.' A phage's host range is a function of its ability to recognize and bind to receptors on the surface of each bacterial cell (adsorption) and on its ability to escape detection and silencing or degradation once inside the cell.

Lateral gene transfer (also called horizontal gene transfer) Movement of genes between genomes of unrelated individuals or species.

Lytic phage Phages in the lytic cycle reproduce and assemble within host cells and must lyse the cell in order to transmit to the next host.

Metagenomics The genomic analysis/characterization of microorganisms through direct sequencing of DNA from samples thought to contain a community of microorganisms.

Phage display Method for testing interactions among proteins, or proteins and peptides, using phage expression of genes encoding a protein of interest.

Phage therapy Describes the therapeutic use of bacteriophages to treat populations of pathogenic bacteria during infection.

Phase variation The variable expression of proteins, often in an on–off fashion, among genetically identical bacterial cells in a population.

Proteomic tree A new approach for assigning phage taxonomy in which whole phage genomes are compared to those of all other sequenced phages in order to predict many aspects of the phage's biology and phylogeny.

Temperate phage Phages that undergo lysogenic infection, whereby the phage genome is integrated into that of its host and phage reproduction happens concurrently with bacterial reproduction. Once within the genome, these phages are referred to as 'prophages,' which can retain their ability to excise from the host genome and enter the lytic cycle.

Time-shift experiment Approach used to explore the dynamics of coevolution between bacteria and phages in which bacteria (or phage) are challenged against phages (or bacteria) from either earlier points in time, contemporary time points, or future time points. This approach is possible because of cryogenic storage and subsequent resurrection of both phages and bacteria from either experimental or natural settings.

Introduction

Bacteriophages (or phages) are viruses that infect bacterial cells. Phage particles are made up of proteins encapsulating either a DNA or RNA genome that is either single or double stranded, and either circular or linear depending on the family of phage. Despite the codiscovery of phages by Twort in 1915 and d'Herelle in 1917 (Duckworth, 1976) and the ubiquity of phages across all examined ecosystems on earth (Clokie *et al.*, 2011), our understanding of the role that phages might play in shaping the genomes, populations, and communities of their bacterial hosts is still expanding rapidly. Early research into phages focused on their possible use as therapeutic agents against pathogenic bacteria, but this interest was mainly eclipsed by the discovery of antibiotics. In part, phages were considered as inferior treatment options to antibiotics because of their relative specificity to particular host species and even strains. However, even as interest in phage therapy waned, the relative specificity of phages was a key feature of the successful influence of phage biology in shaping contemporary biotechnology (Petty *et al.*, 2007) and building the central dogma of molecular biology (Cairns *et al.*, 1966). Two of the major advances offered by phage research have been the use of 'phage display' technology to express exogenous proteins or peptides on the surface of phages (Azzazy and Highsmith, 2002) and the use of phage vectors in DNA cloning (Amemiya *et al.*, 1994). This applied interest has led to an in-depth and impressive understanding of a few bacteria–phage model systems, but has only rarely led to exploration of novel phages in the environment or the impact of phages in natural systems.

Recent resurgence of interest in phage ecology and evolution has been driven by both the imminent need for alternatives to antibiotics (Lu and Koeris, 2011) and the technology-driven discovery of high phage abundance even in the most extreme habitats (Engelhardt *et al.*, 2014). Many of the most common phages have been hiding in plain sight, and it is only through the use of next generation sequencing and sophisticated microscopy that we have uncovered their true ubiquity. For example, genome sequencing of a bacteriophage infecting one of the most abundant marine bacteria, SAR11,

allowed for the assignment of up to 25% of previously unknown sequencing reads from viral metagenomes of the Pacific and Indian Oceans (Kang *et al.*, 2013). Similarly, the discovery of the so-called 'crAssphage,' which most likely infects the common human gut-associated *Bacteroides* genus of bacteria, allowed assignment of up to 90% of previously unknown sequence reads from the human fecal metagenome (Dutilh *et al.*, 2014). As our understanding of phage prevalence and host range in nature expands, so too does the possibility that phages play a central role in many key ecological and biogeochemical processes shaping life on earth.

Phage Life History and Adaptation

As would be expected given their long evolutionary history, contemporary phages display great diversity in terms of genome content and structure (Breitbart and Rohwer, 2005; Hendrix *et al.*, 1999), host range (Hyman and Abedon, 2010; Koskella and Meaden, 2013), and life cycle (**Figure 1**; Abedon, 2008; Clokie *et al.*, 2011). Indeed, due to the lack of conserved sequences across phage genomes, there are currently no individual molecular markers developed with which phage communities can be easily examined (i.e., no equivalent of the 16S rRNA gene most commonly used for characterizing bacterial communities (Adriaenssens and Cowan, 2014)). As such, taxonomic assignment of phages often requires a more complex approach such as whole genome sequencing, microscopy, or the use of 'proteomic trees' (Rohwer and Edwards, 2002). To date, there is relatively little known about the diversity or biogeography of phages in nature, and most of this understanding comes from either viral metagenomic studies or microscopy. For example, phylogenetic profiling of phage metagenomes from either free-living or host-associated environments suggests these two communities are remarkably distinct from one another; a result that mirrors the findings for the bacterial communities with which they interact (Caporaso *et al.*, 2011). Such genomic data can also be used to predict the phage life cycle with surprisingly high accuracy (McNair *et al.*, 2012).

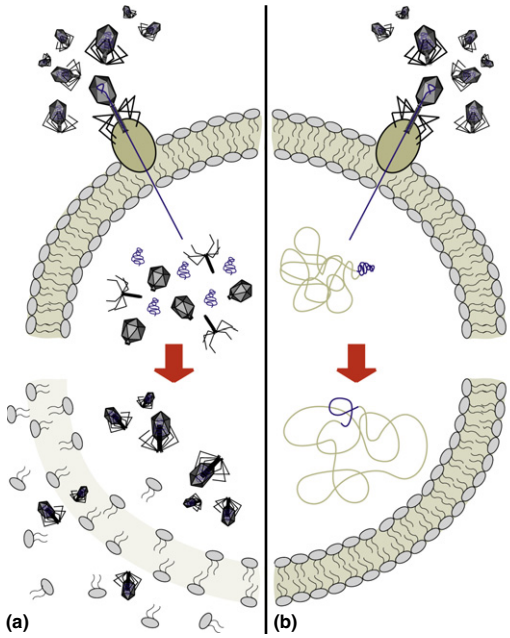

Figure 1 Illustration of the two most common phage life history strategies: lytic (a) and lysogenic (b) infection. In both cases, phages must first recognize and attach to bacterial host cells. This rate of 'adsorption' will be a function of both the likelihood that a given phage encounters a bacterium which is directly related to the ratio of bacteria to phage in the environment, and the probability that the phage successfully attaches to the cell. Once attached, the phage genetic material is injected into the cytoplasm and is either translated and replicated by the host cell machinery, thereby creating new phage virions which are released into the environment after lysis of the cell (a), or else integrated into the host genome and replicated along with the host genome during cell division (b).

Phages are unable to reproduce outside of host cells, but are able to survive for long periods outside of the cell. This means that phage presence is not necessarily indicative of host presence, especially if phages are able to better disperse to, or survive for longer in particular habitats than their hosts (Díaz-Muñoz and Koskella, 2014; Muniesa *et al.*, 1999). For free phage particles to successfully reproduce, they must first encounter and recognize a suitable bacterial cell, at which point phage 'adsorption' can occur (**Figure 1**). Phages recognize bacterial receptors, such as lipopolysaccharides or proteins in the outer membrane, where they attach to cells and attempt to inject their own genome (Labrie *et al.*, 2010) via ejection of genetic material mediated by the phage tail fiber (Molineux and Panja, 2013) or via endocytosis-like mechanisms (Romantschuk *et al.*, 1988). Upon successful infection, the phage DNA/RNA is either translated using the host cell's replication machinery, or integrated into the host genome (**Figure 1**).

Lytic Phages

For phages in the lytic cycle replication requires translation of viral proteins by the host cell machinery, where self-assembly of new virions occurs and the phage genomes are repacked into capsids (Aksyuk and Rossmann, 2011). Phage adaptations that increase replication efficiency include altered codon bias to better match that of its host (Chithambaram *et al.*, 2014; Lucks *et al.*, 2008) and selection on phage genome size (Bull *et al.*, 2004). During the process of assembly and repackaging, bacterial DNA as well as DNA from other coinfecting phages, can be incorporated into the capsid. This process is responsible for the highly dynamic nature of many phage genomes (Rokyta *et al.*, 2006) and allows for the possibility of phage-mediated lateral gene transfer from one bacterial genome to another (Canchaya *et al.*, 2003a). At the completion of phage replication, bacterial cell lysis is induced and phage progeny are released into the environment (Young, 2013). Infection of new hosts once again requires a chance encounter with susceptible cells, as phages are capable of only passive dispersal in the environment.

Lytic phages can vary in host range, adsorption efficiency, time to lysis, burst size, and survival in the environment. Phage host range is determined by many factors including the coevolutionary history, tail fiber length, and expression of particular receptors on host cells (Hyman and Abedon, 2010).

Furthermore, while some phages are only capable of infecting a small subset of bacterial hosts in the environment, others are capable of infecting bacteria from different genera (Holmfeldt *et al.*, 2007; Koskella and Meaden, 2013). Experimental coevolution of bacteria and phages can result in increased host range over time as a result of stepwise mutations (Hall *et al.*, 2013), but shifts from one bacterial host type/species to another is typically more constrained. As with many phage life history traits (Keen, 2014), host range is likely shaped by a trade-off whereby increased breadth of infectivity comes at a cost to other measures of phage fitness, such as adsorption rate or burst size (Duffy *et al.*, 2006), but such costs are not universal (Bono *et al.*, 2013; Poullain *et al.*, 2008). Adsorption rate, time to lysis, and burst size are characteristics shaped by both the phage and host genotype, and can also be influenced by interactions between phages within the cell (Leggett *et al.*, 2013). The optimal time to lysis will also depend on the environment, in particular the density of host cells, as shorter time to lysis typically comes at the benefit of shorter generation times but the cost of fewer phage progeny being produced per generation (Bull *et al.*, 2004; Wang, 2006). Finally, variation in phage survival in the environment is a trait that is much less well-studied, but there exists some evidence that survival might be negatively correlated with phage multiplication within the host cell (De Paepe and Taddei, 2006).

Temperate Phages

Unlike lytic phages, temperate phages can integrate into the host genome as 'prophages' and are replicated along with the host cell during division. These phages can retain the ability to enter the lytic cycle in order to transmit horizontally to new hosts, and this process is often triggered by stressful conditions (Oppenheim *et al.*, 2005). However, over evolutionary time, prophages can lose the ability to excise from host cells, at which point they can be considered 'cryptic prophages.' Bacteria hosting prophages are referred to as 'lysogens' and can attain many important and often beneficial traits from their phages. Indeed, there is now good evidence that prophage acquisition plays a key role in bacterial evolution (Bobay *et al.*, 2014). Among the bacterial traits known to be phage-encoded are the production of toxins (Waldor and Mekalanos, 1996), resistance to antibiotics (Modi *et al.*, 2013), pathogen virulence to eukaryotic hosts (Fortier and Sekulovic, 2013), and increased resilience under stressful conditions (Wang *et al.*, 2010).

Phage integration into the host genome is also a process that can be shaped by selection. The enzymes involved in mediating recombination between the phage and bacterial genomes, the phage integrases, are known to preferentially target particular sites such as intergenic regions or conserved genes (Bobay *et al.*, 2013). Once associated with the host genome, prophages will be under the same selection pressures as those faced by their bacterial hosts. For instance, there is good evidence that selection for bacterial genome reduction often results in the loss of prophages from the genome (Canchaya *et al.*, 2003b). Prophages can also undergo rapid decay and inactivation after integration, including point mutations, genome restructuring, and integration of further foreign DNA. However, recent evidence suggests that many so-called 'domesticated' prophages (i.e., those that have been stably integrated into the host genome for long periods of time) have undergone purifying selection on genes involved in host fitness as well as those encoding key phage traits such as lysis and structure (Bobay *et al.*, 2014). This is particularly relevant to bacteria–phage coevolutionary dynamics, as temperate phages can acquire DNA from defective prophages via recombination, thus increasing the potential for rapid host-specific adaptation (De Paepe *et al.*, 2014a).

Alternative Phage Life Cycles

Although lytic and temperate strategies represent the majority of phage life histories, there are cases in which phages neither integrate into the genome nor enter the lytic cycle, and this state has generally been referred to as 'pseudolysogeny' (Abedon, 2009). For example, 'filamentous' phages are able to replicate within host cells in a similar way to lytic phages but without requiring cell lysis to transmit to a new host (Rakonjac *et al.*, 2011). As with prophages, filamentous phages can influence the phenotype of their hosts. In the case of the human pathogen *Vibrio cholera*, for example, toxin production and increased pathogenicity have been associated with infection by a filamentous phage (Waldor and Mekalanos, 1996). More generally, the pseudolysogeny strategy has been shown to have some unique benefits both in terms of dispersing phages into the environment, and therefore potentially lysing unrelated host cells (Gama *et al.*, 2013), and increasing tolerance to certain environmental conditions (Siringan *et al.*, 2014). Although very little is known about how phages in this state may be adapting to their hosts, there is some evidence that filamentous phages can increase the damage they cause to host cells under conditions in which transmission horizontally from cell to cell is more common that transmission vertically from mother to daughter cell (Messenger *et al.*, 1999).

Phage Adaptation to Host Populations

In addition to general adaptations to the host environment, there is growing evidence that phages evolve quite specific adaptations to particular bacterial host genotypes and species. Natural phage populations can be particularly well adapted to local populations of their hosts, but the scale of such adaptation differs among systems. For example, phages isolated from soil in a flood plain were more likely to infect bacterial hosts from the same sample than from samples collected only centimetres away (Vos *et al.*, 2009). In contrast, phages collected from leaves of horse chestnut trees were just as likely to infect bacterial hosts from other leaves of the same tree, but were much less likely to infect bacteria from neighboring trees (Koskella *et al.*, 2011). Similarly, reanalysis of the infection network of 215 phage types and 286 host types from multiple sites in the Atlantic ocean found that phages were 10 times more likely to infect bacteria from the same sample than those isolated from a different sample (Flores *et al.*, 2013). Evidence for rapid and specific phage adaptation has also come from numerous experimental microcosm studies (Gómez and Buckling, 2011), although in many of these studies phage adaptation is eclipsed by bacterial resistance, and phages are found to be less infective to local relative to foreign bacteria

(Buckling and Rainey, 2002a; Morgan *et al.*, 2005; Vogwill *et al.*, 2010). Importantly, both the direction and magnitude of local adaptation for *in vitro* bacteria–phage systems can be influenced by the rate of migration (Forde *et al.*, 2004; Morgan *et al.*, 2005) and the heterogeneity of resources among populations (Lopez-Pascua *et al.*, 2012).

Phage-Mediated Selection

Given the evidence that phages can readily adapt to infect local host populations and the incredible abundance of phage particles in both aquatic (Fuhrman and Schwalbach, 2003; Waterbury and Valois, 1993) and terrestrial environments (Ashelford *et al.*, 2003; Fancello *et al.*, 2012; Iriarte *et al.*, 2007), it is no surprise that phages have been shown capable of influencing everything from bacterial evolution and diversity (Benmayor *et al.*, 2008; Buckling and Rainey, 2002b; Middelboe *et al.*, 2009) to global biogeochemical cycles (Fuhrman, 1999). Among the direct impacts phages may have on their host populations are mortality-driven changes in density (Fuhrman and Noble, 1995), altered bacterial phenotype and fitness (Bohannan and Lenski, 2000a; Koskella *et al.*, 2012), including changes in bacterial virulence to eukaryotic hosts (Boyd and Brüssow, 2002; De Paepe *et al.*, 2014b), and horizontal transfer of genes among host cells (Canchaya *et al.*, 2003a; Kidambi *et al.*, 1994). Phage-induced mortality can have important consequences for nutrient cycles, and phages have been shown to alter the availability of carbon, nitrogen, and phosphorus in marine food webs (Lennon and Martiny, 2008). Furthermore, phage-mediated selection can influence the composition and stability of whole microbial communities both directly, for example, through apparent competition when phages are shared among species, and indirectly. A study across bioreactor populations found that phage abundance was both dynamic and correlated with the abundance of corresponding bacterial host species (Shapiro *et al.*, 2010), and phage genotypes were observed to fluctuate in accordance with the abundance of matching bacterial strains in an aquaculture environment (Rodriguez-Brito *et al.*, 2010).

The 'Kill the Winner' hypothesis puts forward the idea that population growth of otherwise dominant bacterial species should be hampered by increased prevalence of phages capable of infecting them (Thingstad, 2000; Winter *et al.*, 2010). Indeed, the introduction of phages in experimental microbial communities can alter the biomass of each species depending on the host range of the phage introduced (Middelboe *et al.*, 2003). Similarly, in a community of 15 human gut symbionts transplanted into germ-free mice, phages were found to affect both the density and diversity of bacteria (Reyes *et al.*, 2013). Given the abundance of phages found in human fecal samples (Dutilh *et al.*, 2014), there is therefore good reason to think phages may play a significant role in shaping human-associated microbiota (Abeles and Pride, 2014), as well as those of other eukaryotes (De Paepe *et al.*, 2014b; Koskella, 2013).

Mechanisms of Bacterial Resistance

The pervasive nature of phage-mediated selection, combined with the ubiquity of phages in the environment, has resulted in a wide array of bacterial resistance mechanisms and phage counter-adaptations. Indeed, despite an estimated marine cyanophage abundance of 10^3–10^4 ml^{-1} of water, coexisting cyanobacteria populations were found to be mostly resistant to local phages (Waterbury and Valois, 1993). Bacterial cells can resist phage infection by blocking phage attachment, reducing phage reproduction within cells, and/or interfering with phage transmission (**Figure 2**; Hyman and Abedon, 2010; Labrie *et al.*, 2010). As a first line of defense, bacteria can evade or block phage attachment, for example, by altering expression of the recognized receptor or by producing an extracellular matrix to prevent phages from coming into contact with receptors. If phages are able to successfully adsorb, hosts are still able to respond by either blocking phage replication or degrading phage DNA prior to translation. The currently best-studied mechanism for such recognition is the CRISPR-Cas system, which has been discovered in over half of bacterial species examined (Barrangou *et al.*, 2007; Jore *et al.*, 2012). This system allows for the integration of small segments of phage DNA into the host genome which, when expressed, leads to cleavage of invading phage DNA at matching sites (reviewed in Westra *et al.*, 2012). Even in cases where phages are able to replicate within host cells, bacteria are still able to prevent phage transmission to new hosts through abortive infection systems, which trigger cell lysis prior to phage assembly (Fineran *et al.*, 2009).

These diverse resistance systems differ from one another not only in mechanism, but also with regard to how readily phages can counter-adapt to each (Samson *et al.*, 2013), how costly each is to express (Bohannan and Lenski, 2000b), and how general/specific conferred resistance might be against different phages (Hyman and Abedon, 2010). Moreover, the mechanism of bacterial resistance and phage counter-adaptation will shape the coevolutionary dynamics observed (Betts *et al.*, 2014; Hall *et al.*, 2011; Jessup and Bohannan, 2008). This is in part due to the differences in costs associated with each, which have been found to range from an increased cost of carrying deleterious mutations (Buckling *et al.*, 2006), to altered metabolism of carbon (Middelboe *et al.*, 2009), to increased susceptibility to other phage types (Avrani *et al.*, 2011; Marston *et al.*, 2012).

Bacterial Adaptation to Local Phage Populations

Insight to the scale of bacterial adaptation to phage populations can be obtained from studies that match the CRISPR spacers found in the bacterial genome to phage types circulating in the environment. For example, CRISPR sequences of bacteria from bioreactors were found to diverge in response to local phage-mediated selection despite little divergence in bacterial strain diversity overall (Kunin *et al.*, 2008). Similarly, CRISPR sequences from the human microbiome project were found to differ substantially according to body sites and individual sampled (Rho *et al.*, 2012).

Phage Counter-Adaptation

In response to the diversity of bacterial resistance mechanisms that have evolved, phages have counter-adapted with an

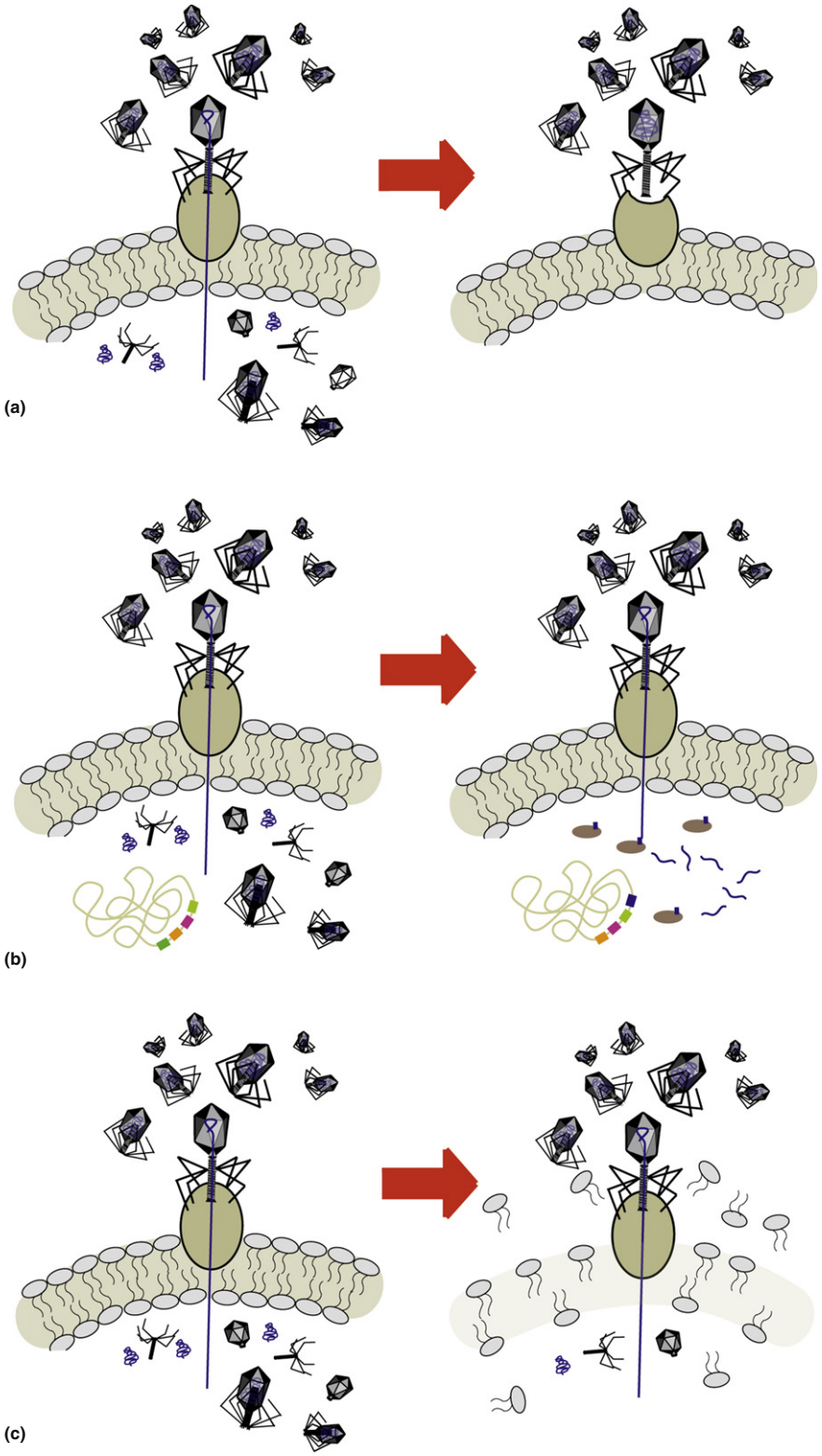

Figure 2 Illustration of common bacterial resistance mechanisms to phages. First, bacteria can alter or lose the receptor recognized by the phage, thus blocking phage adsorption (a). Next, bacteria can either degrade phage DNA or block the translation and replication of the phage genome, for example, using the CRISPR-Cas system (b). Finally, upon successful phage replication within the cell, bacteria can still interfere with phage transmission to neighboring cells by triggering cell lysis before the phage particles are fully assembled (c).

equally diverse array of mechanisms to overcome such resistance (reviewed in Samson *et al.*, 2013). In response to adsorption-blocking mechanisms, for example, mutations in phage tail fiber genes can lead to recognition of altered or entirely new receptors on the bacterial cell (Hyman and Abedon, 2010; Meyer *et al.*, 2012; Scanlan *et al.*, 2011). In addition, some phages are capable of degrading the extracellular matrix produced by bacterial cells, thereby gaining access to otherwise inaccessible receptors (Chibeu *et al.*, 2012; Yan *et al.*, 2014). In response to bacterial-mediated degradation of phage DNA, phage evolution can lead to loss of, or mutations at, sites recognized by bacterial endonucleases or even the acquisition of methylase genes within the phage genome (Samson *et al.*, 2013). Furthermore, counter-adaptations to the CRISPR-Cas system include mutations in the protospacer regions of the phage genome (Sun *et al.*, 2013), phage-encoded 'anti-CRISPR' genes (Bondy-Denomy *et al.*, 2012), and the recently discovered phage-encoded CRISPR-Cas system targeting bacterial chromosomes (Seed *et al.*, 2013).

Evidence for Bacterial-Phage Coevolution

As evidenced by the numerous adaptations and counter-adaptation observed in natural bacteria and phage populations, coevolution is likely to be a common process shaping microbial populations and communities. Direct evidence for bacteria–phage coevolution has come primarily from controlled laboratory studies, where bacteria and phage populations can be frozen and later resurrected to examine (co)evolutionary change over time (**Figure 3**; Koskella and Brockhurst, 2014). Early studies focused on the tailed-phages of *Escherichia coli* B or cyanophages often resulted in only a few coevolutionary cycles before the emergence of a resistant bacterial type that phages were seemingly unable to overcome (Barnet *et al.*, 1981; Chao *et al.*, 1977; Cowlishaw and Mrsa, 1975; Lenski and Levin, 1985). However, evidence for sustained and persistent coevolution has subsequently been uncovered in a number of systems, including *Pseudomonas fluorescens* (Buckling and Rainey, 2002a; Hall *et al.*, 2011), *Cellulophaga baltica* (Middelboe *et al.*, 2009), and *E. coli* O157:H7 (Mizoguchi *et al.*, 2003). Experimental coevolution studies have also highlighted the potential role of coevolution in shaping diversity (Benmayor *et al.*, 2008; Buckling and Rainey, 2002b; Poullain *et al.*, 2008), molecular evolution (Pal *et al.*, 2007; Paterson *et al.*, 2010; Scanlan *et al.*, 2011), and apparent competition among bacteria (Bohannan and Lenski, 2000b; Joo *et al.*, 2006; Koskella *et al.*, 2012). The rate of dispersal is a critical determinant of the speed and scale of coevolution, with low to intermediate rates acting to accelerate the coevolutionary process (Brockhurst *et al.*, 2007a; Vogwill *et al.*, 2008) while high rates act to synchronize dynamics across populations (Vogwill *et al.*, 2009, 2011).

Insight to the mode of bacteria–phage coevolution has been gained through the use of so-called 'time-shift' experiments, whereby bacteria/phage from one point in time are crossed against phages/bacterial hosts from either earlier,

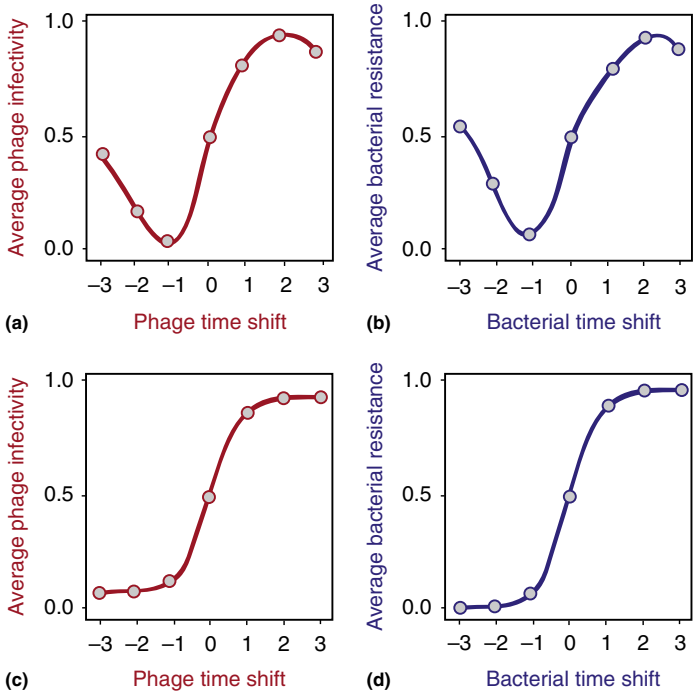

Figure 3 Simplified illustration of the predicted results of a time-shift experiment where either time-shifted phages are tested against fixed hosts (panels (a) and (c)) or the time-shifted hosts are tested against fixed phages (panels (b) and (d)). In the case of fluctuating coevolutionary dynamics (a and b), bacteria/phages from the future are predicted to be most resistant/infective. However, moving further forward in time, we expect resistance/infectivity to drop off as a result of loss of specific adaptations to those earlier populations. In contrast, under arms race coevolution (c and d), we expect phage infectivity and bacterial resistance to increase over time.

contemporary, or later time points (**Figure 3**; Gaba and Ebert, 2009). In this way, researchers can examine whether phage infectivity/bacterial resistance increases in a linear fashion through time, such that phages/bacteria from the future always perform best, or whether they fluctuate through time, as might be expected if infection genetics are highly specific or there are costs associated with increased range of infectivity/resistance (Koskella and Brockhurst, 2014). Experimental coevolution results suggest that the speed and/or mode of coevolution can be influenced by resource availability (Lopez-Pascua and Buckling, 2008) and the presence of other bacterial species (Gómez and Buckling, 2011). Furthermore, the mode of coevolution has been shown to change over time (Hall *et al.*, 2011) and can differ across phage types, even when interacting with the same bacterial host (Betts *et al.*, 2014).

Coevolution in Nature

Evidence of bacteria–phage coevolution in natural populations, although scarce, has confirmed many of the results observed *in vitro*. First, seminatural mesocosm experiments, in which ecological complexity is introduced into otherwise controlled experimental settings, have been used to explore how coevolution in nature might differ from that in the laboratory. Bacteria–phage coevolution in soil mesocosms with the natural microbial community present has demonstrated that coevolution in this environment is more in line with fluctuating selection than arms race dynamics (Gómez and Buckling, 2011), and that coevolution does not necessarily lead to increased mutation rates (Gómez and Buckling, 2013). These results are both in contrast to those observed in the same system when coevolved *in vitro* (Brockhurst *et al.*, 2007b; Pal *et al.*, 2007). Fully natural systems are also amenable to time-shift experiments, as bacteria and phages can be sampled and stored through time. In this way, microbial communities from horse chestnut leaves were collected over the course of a growing season to test for signatures of bacteria–phage coevolution within trees over time (Koskella, 2013). Bacterial isolates were found to be more resistant to phages from 1 month earlier and less resistant to phages from 1 month in the future, suggesting a rapid response to phage-mediated selection. A follow on study examining infectivity of individual phage clones demonstrated that phages were, on average, most infective to bacterial hosts from the recent past, but lost infectivity on bacterial hosts from earlier in the season (Koskella, 2014). This is again in line with fluctuating, rather than arms race dynamics, suggesting that this mode of coevolution might be less common in natural populations than it is in laboratory experiments. Overall, it is increasingly clear that phage-mediated selection can and does play an important role in shaping the ecology and evolution of host populations and that bacteria–phage coevolution can have impressive and often unexpected impacts on the health of individual eukaryotes (Abeles and Pride, 2014; De Paepe *et al.*, 2014b) and ecosystems (Peduzzi *et al.*, 2014).

See also: Antagonistic Interspecific Coevolution. Endogenous Retroviruses and Coevolution

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Coevolution, Introduction to

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Glossary

Antagonism An interaction in which one species gains a benefit at the expense of another.

Arms race A coevolutionary dynamic in which two interacting species gain advantage if they have greater values of the traits that mediate the interaction – such that over time these traits evolve to greater and greater values.

Competition An interaction in which two species make use of the same resource, in which the use of the resource by one species precludes the other from using it, and vice versa.

Mutualism An interaction in which one species provides a benefit to another, and receives a reciprocal benefit.

Parasitism An antagonistic interaction, usually symbiotic, in which individuals of one species live inside another and derives sustenance from this host. This is usually damaging to the host. Typically, hosts are much longer-lived than their parasites, and many parasite individuals infest a single host.

Predation An antagonistic interaction in which individuals of one species capture and consume individuals of another species. Typically, predators and prey have similar lifespans, and predators consume many prey individuals over the course of their lives.

Symbiosis Any interaction, antagonistic, mutualistic, or even non-coevolutionary, in which one species lives most of its life on or within individuals of another species.

Virulence The damage done to a host individual by parasites.

Introduction

No organism is an island. Every living thing contends with predators, parasites, and competitors, and most also receive benefits from mutualists (Table 1). These interactions with other species exert natural selection – and predators, parasites, competitors, and mutualists may also experience selection in return. The mutual evolutionary change that results from this reciprocal selection is 'coevolution' (Janzen, 1980; Thompson, 2005).

The idea that other living things are a significant source of natural selection is found as early as Charles Darwin's (1859, pp. 94–95) description of reciprocal evolutionary changes occurring in populations of clover and honeybees:

> The tubes of the corollas of the common red and incarnate clovers (*Trifolium pratense* and *incarnatum*) do not on a hasty glance appear to differ in length; yet the hive-bee can easily suck the nectar out of the incarnate clover, but not out of the common red clover… Thus it might be a great advantage to the hive-bee to have a slightly longer or differently constructed proboscis. On the other hand, I have found by experiment that the fertility of clover greatly depends on bees visiting and moving parts of the corolla, so as to push the pollen on to the stigmatic surface… Thus I can understand how a flower and a bee might slowly become, either simultaneously or one after the other, modified and adapted in the most perfect manner to each other, by the continued preservation of individuals presenting mutual and slightly favourable deviations of structure.

Darwin applied this principle to predict that the Malagasy orchid, *Angraecum sesquipedale*, which produces nectar at the bottom of a dangling spur up to 30 cm (12 in) long, must be pollinated by a moth with a proboscis long enough to reach the nectar, picking up or depositing the orchid's pollen in the process (Darwin, 1877, pp. 162–163) – and, decades later, such a moth was found (Rothschild and Jordan, 1903).

As with any question about evolutionary processes, it is important to differentiate patterns consistent with coevolution from direct evidence of reciprocal selection. Complementary traits in interacting species may arise for reasons unrelated to coevolutionary selection (Nuismer *et al.*, 2010; Gomulkiewicz *et al.*, 2007). Moreover, an interaction that has a strong selective effect on one species may be much less important in the evolutionary history of the other. For example, congruent traits may occur because the two interacting species encounter each other in a common environment that selects directly for the traits observed in both species, or because one species interacts

Table 1 Major categories of coevolutionary species interactions defined by their selective effects on interacting species

Interaction type	*Selective effect for*		*Examples*
	Species 1	*Species 2*	
Mutualism	+	+	Plants and their pollinators; legumes and nitrogen-fixing rhizobia; plants and mycorrhizal fungi; corals and photosynthetic algae
Antagonism	+	–	Hosts and parasites; predators and prey; herbivores and plants
Competition	–	–	Competitive interactions are well documented between different species of *Anolis* lizards, between subspecies of three-spined sticklebacks, and between carnivorous mammals – among many other cases

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with many other species that all have similar requirements. Identifying which interactions have long-term evolutionary importance, and how they shape the evolution of the species involved, has proven to be a complex, yet fascinating, question.

Coevolutionary Dynamics

Coevolution emerged as a specialized field of research in the second half of the twentieth century. One of the earliest examples is H.H. Flor's discovery that genetically different strains of flax rust fungus were able to infect some lines of flax, but unable to infect others (Flor, 1955, 1942). Flor concluded that the parasite and its host had 'complementary genic systems' (Flor, 1956). Based on Flor's data, Charles J. Mode (1958) constructed a mathematical model of flax rust 'coevolution' – perhaps the first use of this term – which predicted that selection by infectious rust should lead to an increased frequency of host genotypes that can resist infection, and that these more resistant hosts would in turn select for more virulent strains of rust. Coevolutionary dynamics differ from simple responses to natural selection in that a species' response to coevolutionary selection leads to a change in the original source of selection: the other interacting species. This means that coevolution is an ongoing process even when every other element of the interacting species' environment is stable. Coevolutionary dynamics take several different forms.

Arms Races

Consider a mollusk with a shell that protects it against predators. Selection should favor predators with jaws strong enough to break the shell. If strong-jawed predators become common enough, they may select for mollusks with stronger shells. Exactly such 'arms races' have been identified in the fossil record of mollusks and their predators (Vermeij, 1994). Patterns of escalating defense suggestive of arms races have also been documented in many contemporary species interactions. Milkweeds (genus *Asclepias*) evolved greater production of toxic phenolic compounds over a long history of coevolution with insect herbivores (Agrawal *et al.*, 2009). Over just a few decades, North American populations of wild parsnip (*Pastinaca sativa*), a weed introduced from Europe, evolved greater production of toxic furanocoumarins after parsnip-eating webworms (*Depressaria pastinacella*) were brought over from Europe (Zangerl *et al.*, 2008).

There are limits to coevolutionary arms races. The rough-skinned newt, *Taricha granulosa*, secretes tetrodotoxin, which can incapacitate the garter snakes that prey on the newts. In many cases, populations of newts that produce more tetrodotoxin live alongside coevolved snakes that can tolerate greater doses of the poison; but in some populations the snakes are able to tolerate far more poison than the local newts produce, suggesting that the snakes have 'escaped' (or maybe simply won) the arms race (Hanifin *et al.*, 2008).

Cycles

When a coevolutionary arms race is constrained by the costs of producing a defense or a counter-defense, coevolutionary cycles are possible (**Figure 1**). As one species reaches the upper limits of defense production, individuals with reduced defenses may gain a selective advantage if the cost of increased predation or herbivory is offset by the savings of not producing a massive shell or lots of toxin. Greater frequency of undefended prey creates an advantage for predators or herbivores with reduced counter-defenses, and so this species evolves to a lower-cost state as well. When both defense and counter-defense have evolved to a lowered state, the arms race can begin anew (Berenbaum *et al.*, 1986; Agrawal and Lively, 2002).

Cycles may also occur when different genetic lines of an antagonist species specialize on specific genetic variants in its victim, as in Flor's (1942, 1955) wheat rust. Selection first favors the antagonist genotype that specializes on the most common victim genotype, but the increased frequency of this antagonist genotype selects against the victim genotype it targets – and eventually a different victim genotype will be the most common in the population, which in turn favors a different antagonist genotype. This negative frequency-dependent selection is found in populations of the New Zealand mud snail, *Potamopyrgus antipodarum*, which are attacked by a trematode worm in the genus *Microphallus*. Within individual lakes, the most common genetic line in the local mud snail

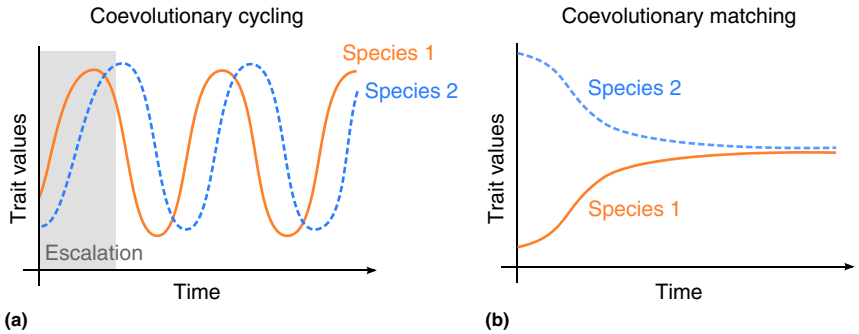

Figure 1 Coevolutionary dynamics. (a) Coevolutionary escalation (shaded area) occurs when prey or hosts evade their antagonists by evolving defenses superior to their predators' or parasites' counter-defenses; and if greater defenses and counter-defenses are costly, then coevolutionary cycles may occur. Cycles can also occur if antagonists are more successful when they match the most common host genotypes, creating negative frequency-dependent selection. (b) In many mutualisms, interacting species benefit from closer matching to each other, which creates selection for better-matched traits.

population changes in a cyclical fashion, and trematodes are more infective against snails from the previous year's population (Dybdahl and Lively, 1998). This dynamic is thought to maintain diversity in the genes that mediate immune responses in mammals, birds, and even plants – and in the genes of parasites and disease organisms that mediate resistance to immunity (Tiffin and Moeller, 2006; Westerdahl, 2007; Tellier and Brown, 2007).

Matching

Mutualism is often expected to favor individuals whose traits are better matched to their mutualistic partner, leading to a stable equilibrium when each species is optimally matched to the other (Kiester *et al.*, 1984). This may be the case for yucca plants and their pollinators, specialized moths in the genus *Tegeticula* that lay eggs in yucca flowers before depositing pollen. The moth larvae feed on developing yucca seeds, so the moths drill into the floral ovary with a needle-like ovipositor, laying their eggs adjacent to the ovules that will develop into seeds. However, if the moth does too much damage in the course of oviposition, it risks triggering the plant to abort the damaged flower. Consequently, the length of the pollinator's ovipositor is closely matched to the thickness of the yucca's ovary wall. In the case of one yucca species, Joshua tree (*Yucca brevifolia*), this selection for matching seems to have led to differentiation in floral shape among populations serviced by different pollinator species with different ovipositor lengths (Yoder *et al.*, 2013; Pellmyr and Huth, 1994).

However, it is not clear how well this simple model describes many other mutualistic interactions. Darwin's original prediction of coevolutionary dynamics between the orchid *A. sesquipedale* and its pollinator is more like an arms race, in which the orchids with longer spurs gain more effective pollination by forcing pollinators to reach deeper for a nectar reward, and pollinators with longer proboscises gain easier access to nectar (Darwin, 1877). Similarly, evidence for simple matching is lacking in the mutualistic interaction between plants in the legume family and rhizobial bacteria, which colonize their hosts' roots and fix atmospheric nitrogen in exchange for sugar resources. Patterns of compatibility between different strains of rhizobia and different host plant family lines suggest that there is no single 'best' combination of host and rhizobia, as would be expected from matching (Heath, 2009; Heath *et al.*, 2012).

The above-described dynamics explain coevolution over just a relatively small number of generations. Many lines of evidence suggest that coevolutionary interactions have also shaped species over millions of years, but how specific coevolutionary dynamics connect to these larger patterns of diversity remains an area of ongoing research.

Coevolution and Speciation

As Flor and Mode initiated the modern study of coevolutionary dynamics, their contemporary, Verne Grant began to document the importance of coevolutionary interactions in speciation. In a 1949 study, Grant compiled taxonomic descriptions of plant species and grouped them by the manner in which they exchange pollen. This data showed that in comparison to plants pollinated by wind and water, plant species pollinated by birds or by specialized insects were more easily distinguished based on features of their flowers (Grant, 1949; **Figure 2**). That is, interactions with animal pollinators meant that traits involved in pollination were more likely to define differences between animal-pollinated plant species.

Paul Ehrlich and Peter Raven (1964) further popularized the notion that species interactions contribute to macroevolutionary processes, in a study of butterfly species and the plants upon which those butterflies feed as larvae, and specifically called this process 'coevolution.' Ehrlich and Raven

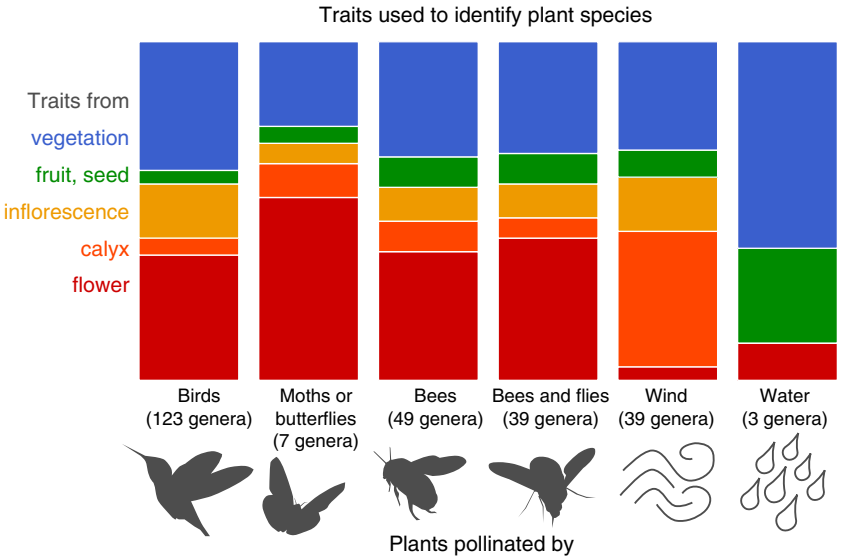

Figure 2 Plant–pollinator interactions affect traits important in plant species delimitation. Floral traits are more likely to be important in the taxonomic descriptions of plant species when those species are pollinated by animals, rather than wind or water. Data taken from Grant, V., 1949. Pollination systems as isolating mechanisms in angiosperms. Evolution 3, 82–97 (Table 1).

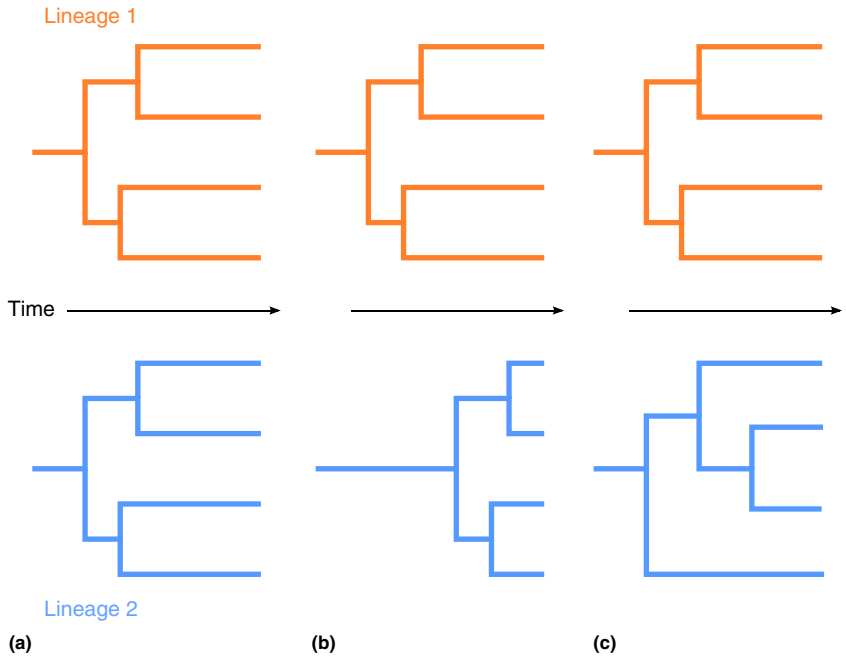

Figure 3 Stepwise and simultaneous diversification. Coevolution between two interacting lineages over evolutionary time may lead them to have congruent phylogenies (a), but this pattern may also occur if one lineage depends strongly on the other without exerting reciprocal selection. Coevolution over ecological timescales may also lead to phylogenies that are congruent in shape but without simultaneous speciation events (b) or largely incongruent (c) if pairwise associations between species shift frequently.

noted that butterfly species descended from a common ancestor frequently feed on plants from a single, closely related group of plants; and that such groups of plant species tend to have similar defenses against insect herbivores.

Ehrlich and Raven proposed that this pattern developed in a series of stepwise evolutionary transitions, starting when a plant species evolved a new anti-herbivore defense. If this defense was sufficiently effective to reduce or eliminate herbivore attacks, it would allow the plant species to expand its population, colonize new habitats, and, over time, diversify into an array of new species, all carrying the novel defense. As the group of plant species became more common in a landscape, it would become advantageous for any insect species that evolved a means to counter the new defense – and the lucky insect that did so would spawn its own array of new species as its descendants shifted and diversified into the already-diverse group of previously resistant plants. This stepwise diversification came to be known as 'escape-and-radiate' coevolution (Thompson, 2005).

In recent decades, our ability to reconstruct evolutionary relationships among species using DNA sequence data has allowed extensive verification of the general idea that coevolutionary interactions are associated with greater diversity. For example, lineages of beetles that feed on flowering plants contain orders of magnitude more species than their sister lineages that feed on gymnosperms – since the former plant group is itself much more diverse, this implies that insect specialization on individual host plant species contributed to this dramatic contrast (Farrell, 1998). A time-calibrated evolutionary tree for one of the largest groups of beetles, the weevils, shows that they did not begin to diversify until well after flowering plants had come to dominate terrestrial communities, which fits Ehrlich and Raven's model (McKenna *et al.*, 2009).

Similarly, lineages of plants with bilaterally symmetric flowers, which are often adapted to fit specific pollinators – like the orchid Darwin examined – are consistently more diverse than sister lineages whose flowers are radially symmetric, and which usually have less-specialized interactions with their pollinators (Sargent, 2004). Studies of the relative timing of diversification in such interactions suggest that in many cases clades of specialized plants may diversify by adapting to attract a preexisting diversity of pollinators, rather than speciating simultaneously and in parallel with their partner lineages (**Figure 3**; Ramírez *et al.*, 2011; Schiestl and Dötterl, 2012).

These complexities highlight the difficulty of connecting specific phylogenetic patterns to coevolutionary dynamics. When two interacting lineages have highly congruent evolutionary histories (**Figure 3(a)**), it is tempting to infer that their interaction is coevolutionary – but this pattern can also arise if one lineage depends strongly on the other without exerting reciprocal selection. Alternatively, the phylogenies of two lineages may be congruent in shape, but not in timing of their respective speciation events (**Figure 3(b)**); this is consistent with Ehrlich and Raven's model, but the same pattern may result when one lineage adapts to track the other, absent any reciprocal selection. Finally, two lineages that are currently coevolving may have entirely different evolutionary histories (**Figure 3(c)**) if species associations frequently change. Indeed, switching between pollinators may often contribute to the formation of new flowering plant species (Van der Niet and Johnson, 2012). In such cases, coevolutionary interactions spur speciation via a process that explicitly breaks down patterns of phylogenetic congruency.

It seems indisputable that the types of species interactions that often generate coevolutionary selection are associated with adaptive diversification. However, coevolutionary species interactions may often play a role in the formation of new species without causing both interacting species to diverge simultaneously – indeed, documented cases in which coevolutionary selection has led to speciation are quite rare (Althoff *et al.*, 2014; Hembry *et al.*, 2014). Increasingly, research on coevolutionary interactions and their long-term outcomes looks beyond simple, pairwise interactions to understand how groups of species influence each others' evolution, and how that influence changes in the context of different environments.

Coevolution in Mosaics and Networks

One reason coevolution may rarely result in tidy patterns of co-diversification is that few, if any, organisms interact with only one other species. Even a yucca moth living on and around the host plants on which she will mate and lay her eggs must also contend with disease, predators, and competition with many non-pollinating herbivores for plant resources. Sometimes the interaction of multiple species means that the effects of coevolution propagate from one level of the food chain to another. For example, when domesticated apples were introduced into North America, populations of the native fruit fly *Rhagoletis pomonella* evolved to specialize on apples – and parasitoid wasps that attack *R. pomonella* larvae have differentiated in parallel to specialize on the apple-feeding flies (Forbes *et al.*, 2009).

Variation in the environment and in the broader biological community can dramatically alter the outcome of a single, pairwise interaction. In western North America, the seeds of lodgepole pine trees, *Pinus contorta* subspecies *latifolia*, are eaten by pine squirrels, *Tamiasciurus hudsonicus*, and red crossbills (*Loxia curvirostra*). These two seed predators have rather different approaches. A squirrel bites cones off the tree's branches, and targets larger cones containing more seeds; but a crossbill uses the crossed tips of its parrot-like bill to pry open the scales at the tip of a pine cone and then extracts the seed under each scale using its tongue.

In most of lodgepole pine's range, more seeds are lost to squirrels than to crossbills, so the squirrels are the primary selective force acting on lodgepole pine cones. However, some isolated populations of lodgepole pine grow in habitats where no squirrels are present. In these sites, crossbills are the dominant seed predator; and the cones of lodgepole pines are larger and narrower than cones from populations attacked by squirrels, with thicker scales at their tips where crossbills prefer to attack (Benkman *et al.*, 2003; Edelaar and Benkman, 2006).

This complex interaction of pines, squirrels, and crossbills is considered one of the classic examples of a 'geographic mosaic' – in some populations, lodgepole pine cones are shaped by selection from squirrels; but in others, crossbills are the dominant selective force. In his geographic mosaic theory of coevolution, John Thompson (2005, 2013) predicted that interactions between species will rarely be straightforward, pairwise interactions – but rather that the form and consequences of coevolutionary selection will differ whenever species interact in different environmental contexts and ecological communities. Furthermore, if the interacting species move freely between contexts, it may often be the case that local populations are maladapted – badly suited to each other or the context in which they interact – because of immigration from populations experiencing different selective conditions (**Figure 4**; Gomulkiewicz *et al.*, 2000).

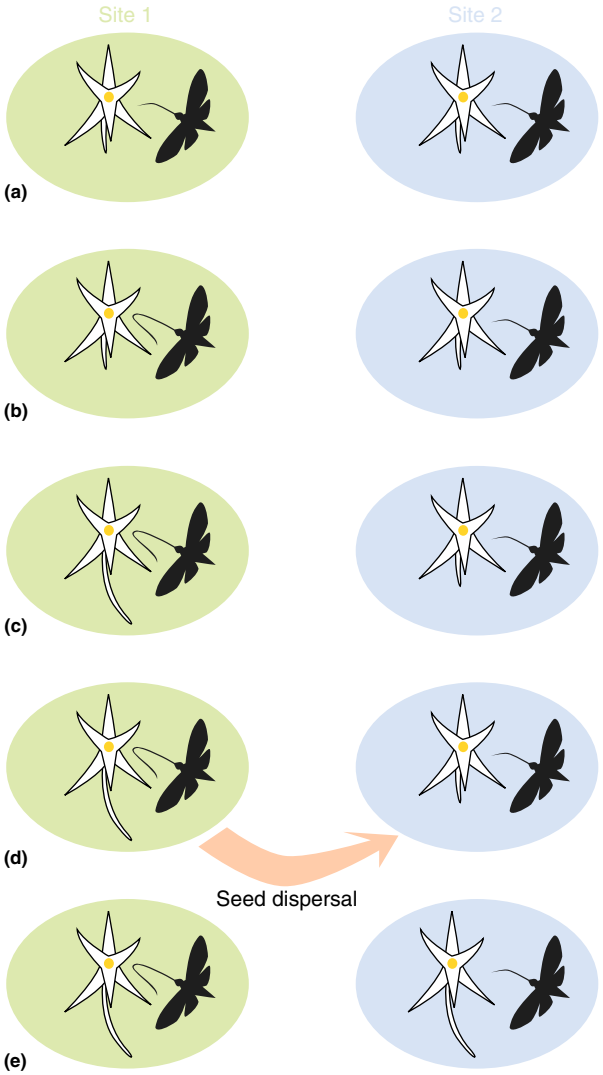

Figure 4 Migration across a geographic mosaic of coevolution can lead to local maladaptation. Consider a fictional pollination interaction similar to that between the orchid *Angraecum sesquipedale* and the hawk moths that pollinate them. In two separate sites, orchids attract moths with nectar secreted at the bottom of a dangling nectar spur. (a) Initially, the orchid's nectar tubes are just long enough that when a moth reaches its proboscis to the bottom of the spur, it comes into contact with the orchid's stigma and anthers, and picks up or deposits pollen. (b) Moths with longer proboscises can more efficiently reach the nectar, and at Site 1, this benefit causes the moth population to evolve longer proboscises. However, at Site 2, cooler temperatures make longer proboscises more physiologically expensive, enough to offset the benefit of better nectar removal. (c) The longer proboscises of moths at Site 1 select for orchids with longer nectar spurs. (d) Orchid seeds disperse from Site 1 to Site 2, introducing longer-spurred orchids into Site 2 – (e) where the local moths are poorly matched to them.

Because almost all species live in a variety of environments and encounter different communities of organisms across their native ranges, geographic mosaics of coevolution are probably common. They may also suggest a way in which coevolutionary interactions contribute to the formation of new species. If the nature and strength of coevolutionary interactions vary from population to population, different populations may follow very different evolutionary trajectories – over time, enough to become different species. For instance, the differences in lodgepole pine cone shape selected by the presence or absence of pine squirrels are sufficiently large that crossbills in squirrel-free pine populations have evolved deeper bills to open the thicker pine cone scales. This may have contributed to a change in the birds' mating calls that partially reproductively isolates them from other crossbill populations (Benkman *et al.*, 2003; Smith and Benkman, 2007).

The fact that species' evolution may be influenced by indirect interactions suggests that studying coevolution may often require an understanding that looks beyond simple, pairwise, and reciprocal adaptation. One line of research that offers such a view considers the structure of interaction networks, such as all the plant–pollinator relationships, within a given geographic area or biological community. In many such networks, the most specialized species that interact with only one or a few other species tend to interact with partner species that are more generalized and that interact with many other partners. This asymmetric specificity results in 'nested' networks (**Figure 5**).

Nestedness emerges in a wide variety of interaction types. These include networks of interaction between plants and pollinators, seed-dispersing animals, protective ants, and herbivores (Bascompte *et al.*, 2003; Guimarães, 2006; Cagnolo *et al.*, 2011) – and also networks of insect herbivores and parasitoid wasps, and cleaner shrimp species and the fish they service (Guimarães *et al.*, 2007; Cagnolo *et al.*, 2011). This phenomenon may be a simple consequence of species-abundance patterns; if more specialized species tend to be rarer, nestedness can arise because less-specialized species will be more likely to encounter each other, while the most specialized species will be most likely to encounter the most generalized species (Krishna *et al.*, 2008). However, mathematical modeling suggests that coevolution in large communities of interacting species can shape the structure of the interaction networks – and it can cause many species in similar ecological roles to converge on similar traits (Nuismer *et al.*, 2012; Guimarães *et al.*, 2011).

One example of such convergence may be the pollination syndromes of flowering plants, in which plant species pollinated by the same broad groups of animals tend to have flowers of similar shape and color (Rosas-Guerrero *et al.*, 2014). For example, species pollinated by hummingbirds often have red, tubular flowers that are open during daylight; whereas species pollinated by moths generally have white flowers that open in the evening, and produce strong fragrances. Pollination syndromes unite distantly related plant species via selection from a shared group of pollinators, and they suggest that even when individual pollinator species do not exert strong selection on individual plant species, the collective effects of selection to attract many similar species add up to a major evolutionary force. Such 'diffuse coevolution' would not create patterns of simultaneous speciation, or even reciprocal adaptation over the course of a few years, and yet it seems to be capable of shaping whole biological communities.

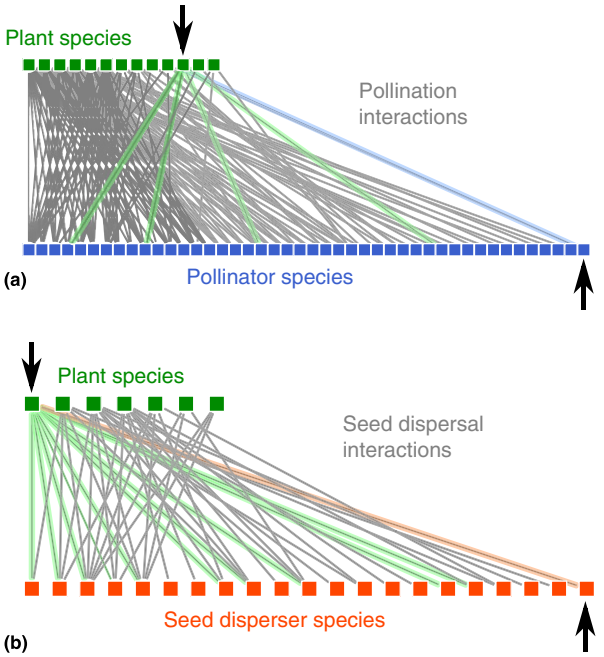

Figure 5 Nestedness in interaction networks. Nestedness, in which specialists interact mainly with generalists, and generalists interact mainly with each other, is common in species interaction networks. (a) In a network of flowering plants (green squares) and their animal pollinators (blue squares) in the forest of North Carolina, USA (Motten, 1986), a pollinator indicated with an arrow interacts with only one plant species (blue-shaded line), but that plant species is also visited by several other pollinators (green-shaded lines). (b) In a network of plants (green squares) and seed-dispersing birds (orange squares) in New Jersey, USA (Baird, 1980), a selected bird (arrow) disperses seeds from a single plant species (orange-shaded line), but that plant species also interacts with many other dispersers (green-shaded lines). Data downloaded from the Web of Life repository http://www.web-of-life.es (accessed 16.03.15).

Conclusions

Research on coevolution is only beginning to explore how pairwise species interactions are woven into the 'entangled bank' of a biological community, or a whole ecosystem. The big questions for future studies of coevolution are, in many ways, questions for all of evolutionary ecology – understanding how specific processes of reciprocal selection create patterns of speciation and extinction that manifest over millions of years:

How Often Does Coevolutionary Selection Directly Contribute to the Formation of New Species?

In Joshua trees, selection by pollinating yucca moths seems to be responsible for differentiation; but in many other

pollination interactions, pollinator switching seems to be important for species formation. In the case of crossbills, the presence or absence of squirrels changes the form of selection on exerted crossbills by lodgepole pine cones. If it truly does promote speciation, coevolutionary selection may often do so by reinforcing differences created by other evolutionary processes – but we lack data to differentiate between these possibilities, and to say which is more common.

How Do Different Kinds of Ecological Interactions Affect Diversification?

Mutualism, antagonism, and competition all produce different kinds of coevolutionary dynamics, so it makes sense to expect that they will have very different effects on long-term patterns of speciation. Establishment of a mutualistic relationship may have an effect similar to exploitation of a new resource – allowing lineages to diversify into new habitats that would otherwise be inaccessible. On the other hand, antagonistic interactions often directly create negative frequency-dependent selection that can promote divergence, particularly if played out across a geographic mosaic. Finally, competition has a widely documented role in creating and maintaining differentiation among species. However, the geographic mosaic theory of coevolution tells us that how we classify a particular species interaction may change dramatically with the context in which it occurs. Understanding the different effects of each interaction type, and how they relate to each other, will be a major topic for ongoing research.

How Do Coevolutionary Dynamics Extend to Highly Asymmetrical Interactions?

The body of research on species interaction networks suggests that tight pairwise interactions are relatively rare – often when a species is highly reliant on a single other species, that mutualist, prey, or host interacts with many other species. Similarly, new DNA sequencing methods have allowed us to track changes in the microbial diversity inside the bodies of multicellular organisms, including humans (Cho and Blaser, 2012). We can infer that hosts exert strong selection on their microbial communities, and we are beginning to understand how microbial community can affect their hosts' health. However, it is harder to say that individual species of bacteria are coevolving, in the classic sense, with the animals whose guts they inhabit. Can our existing theory of coevolution explain patterns arising in such asymmetric interactions?

The study of coevolution is literally the study of how all living things on Earth have evolved, together. In that sense, it promises to help us understand the origins and future of every biological community – and how we humans fit within them.

See also: Biogeography of Interactions. Cospeciation. Divergence and Diversification, Quantitative Genetics of. Ecological Speciation and Its Consequences. Endogenous Retroviruses and Coevolution. Pathogen Epidemiology. Pest Management, Evolution and. Plant–Pollinator Interactions and Flower Diversification. Population Structure and Gene Flow. Sequential Speciation. Symbiosis, History of. Symbiosis, Introduction to

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Commensalism, Amensalism, and Synnecrosis

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Glossary

Allolimy A biological interaction between two or more organisms in which one starves the other(s) without benefit or damage to the active party.

Allotrophy A biological interaction between two or more organisms in which one provides nutrition to the other(s) without benefit or damage to the donor.

Antibiosis A biological interaction between two or more organisms that is detrimental to at least one partner or an antagonistic association between an organism and the metabolic substances produced by another.

Endobiontic A reference to organisms that live within the substratum.

Ichnogenus Any genus known only from trace fossils, such as footprints, coprolites, or nests.

Metabiont A multi-celled organism.

Symbiotic relationships have been attractive study systems for evolutionary biologists because they frequently promote and/or result from coevolution between the interacting organisms. Long-term interactions between two or more organisms through evolutionary time can be powerful drivers of selection and adaptation. One result is that some symbioses – mutualisms, competition, and predator/prey interactions – are common among living organisms. However, three other kinds of symbioses – commensalism, amensalism, and synnecrosis – have received less attention from researchers. These include symbioses where the fitness of a species is unaffected by the interaction, or where all interacting species are strongly negatively affected. Some of these fitness relationships are not predicted to be favored by natural selection and are therefore not expected to commonly occur in nature. In this article we define these three types of symbioses, illustrate known examples of each, discuss some of the controversies regarding the definitions, and acknowledge the difficulties involved when classifying symbioses into established categories.

Commensalism

Commensalism is an ecological relationship between two organisms in which one species benefits from the association without impacting the fitness of the other. Originally this term described the use of waste food by scavengers (e.g., Wilson, 1975), but has been expanded to include any fitness benefit gained by the commensal.

Examples

Commensalism occurs in both the plant and animal kingdoms, and is also prevalent among bacterial species. We recognize three distinct types of commensal relationships, although more complex variants of these basic types can be established:

1. 'Phoresy' refers to a form of commensalism where one species (the phoretic) is mechanically transported by another species (the host) without exacting nutritional or developmental penalties on the host. Phoresy is a way of locating discrete or temporary microhabitats with a high degree of predictability, and provides a potential for emigration when a temporary habitat degrades or disappears (Houck and O'Connor, 1991).

The most commonly described phoretic associations are those occurring between different groups of arthropods. According to Houck and O'Connor (1991), the most spectacular radiation and diversification of adaptations for phoresy is found among the Acari (mites). Approximately 30% of hippoboscid flies exhibit phoretic associations with skin mites of the families Epidermoptidae and Cheyletidae as well as with chewing lice of the family Philopteridae. Skin mites attach themselves preferentially to the ventral surface of their host, between the metathorax and the first abdominal tergite and the ventral surface of wings. Chewing lice have been observed attached to the mesotibia of one hippoboscid fly (Amaral *et al.*, 2013). Another phoretic mite, *Trichouropoda shcherbakae* (Acari: Mesostigmata), is found on beetles of the genus *Tetropium* (Coleoptera: Cerambycidae) living in natural forest areas of Central Europe. Most mites were attached to the beetles' legs (Bloszyk *et al.*, 2013; **Figure 1**).

An overview of the phoretic mites of the Astigmata has led Houck and O'Connor (1991) to operationally define phoresy as a phenomenon in which one organism (the phoretic) receives an ecological or evolutionary advantage by migrating from the natal habitat while superficially attached to a selected interspecific host for some portion of the individual phoretic's lifetime. The phoretic species benefits from the host species' capacity to move more effectively among habitats than could the unaided phoretic. Phoresy compensates for the disadvantages of small size in long-distance migration, the lack of morphological adaptation (e.g., wings) for independent migration, and the vulnerability to predation during migration (Houck and O'Connor, 1991). Okabe (2013) has reviewed insect–mite symbiotic associations to develop hypotheses concerning the factors that favor different fitness relationships. He hypothesizes that many cases of species-specific symbiosis, including mutualisms and parasitic relationships, begin with phoresy.

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Figure 1 Phoresy is a common form of commensalism in which one species is transported by another species to locate discrete microhabitats or migrate. Phoresy is common among the mites (Acari) which frequently attach themselves to an arthropod host.

Phoresy among other arthropods (especially pseudoscorpions) is also common. Pseudoscorpions have the ability to attach themselves to a wide variety of arthropods more mobile than themselves, including at least 44 families of insects and three families of arachnids (Poinar *et al.*, 1998). For example, the pseudoscorpion *Cordylochernes scorpioides* is frequently found under the elytra of the giant harlequin beetle, *Acrocinus longimanus*. Evidence supports attachment being valuable in dispersal, and also indicates that male *C. scorpioides* defend beetles' abdomens as strategic sites for intercepting and inseminating dispersing females (Zeh and Zeh, 1992). Similarly, the pseudoscorpion *Semeiochernes armiger* (Pseudoscorpiones: Chernetidae) interacts phoretically with the giant tropical fly *Pantophthalmus tabaninus* in Amazonian rain forest habitats. As many as two hundred individual *P. tabaninus* mites have been found on the thorax of a single host fly (Santos *et al.*, 2005). Pseudoscorpions also frequently maintain phoretic associations with mammals and even birds (Villegas-Guzmán and Pérez, 2005; O'Connor, 2000).

2. 'Inquilinism' describes the use of a second species as a platform or cavity for the living circumstance of the beneficiary species. More broadly, an inquiline can also be any animal that lives commensally in the nest, burrow, or dwelling place of an animal of another species. This definition overlaps with the term metabiosis or tanatocresia, wherein one species modifies a habitat or creates a new habitat that can then be exploited by a second species. Oft-cited examples of the inquiline relationship include the gall wasps in the Cynipidae family. Most species in this family are gall formers on various plants (parasites), but the family also includes inquilines. The inquilines differ little in structure from the gall-inducing species but they do not induce galls themselves; instead, they deposit their eggs inside the galls induced by other cynipids. These cynipid 'cheaters' are not a clear-cut example of inquilinism: although it has been suggested that some inquilines do little harm to the original gall inducer, all inquilines studied in detail so far have been shown to affect the host gall-inducer negatively (Wiebes-Rijks and Shorthouse, 1992).

   A more straightforward example of inquilinism is found among barnacles that live on shells of mussels. Individuals found on living mussels were shown to grow significantly faster than those on empty mussel shells, and the presence of barnacles had no effects on growth of mussels. This relationship has been formally described as a commensalism (Lahionen and Furman, 1986).

   As an example of metabiosis, the holes excavated on trees by woodpeckers are used by a great variety of secondary hole nesters, either due to a scarcity of natural holes or because they provide better shelter than natural cavities (e.g., Santharam, 2004; Leonard, 2009; Cockle *et al.*, 2011; Wesolowski, 2012). It has also been proposed that a large proportion of soil organisms may be considered metabionts, as they modify soil habitats in a manner that allows other organisms to colonize (Waid, 1999).

3. 'Chemical commensalism' is most often, but not always, associated with two bacterial species, such that one bacterium metabolizes a chemical not useful to the second, producing a waste metabolite that is a useful energy source for the beneficiary second bacterium (Hogan, 2012). For example, a chemically defined medium can be established which support the growth of *Saccharomyces cerevisiae* but not *Proteus vulgaris*, when each is in pure culture. However, in a mixed culture alongside the yeast, *P. vulgaris* can grow. The bacterial population is dependent on the growth of the yeast because the latter produces a niacinlike factor required by the former. Since the numbers of *S. cerevisiae* are identical in pure or mixed culture, the interaction has been suggested as an example of a true commensalism (Shindala *et al.*, 1965).

   Another two-membered bacterial culture has been used to perform complete degradation of dodecyldimethylamine. *Burkholderia cepacia* can degrade the alkyl chain, whereas *Stenotrophomonas maltophilia* degrades dimethylamine, the product of the former. Batch culture experiments revealed that the two-membered culture consisting of *B. cepacia* and *S. maltophilia* is a commensalistic relationship under carbon-limited conditions but a mutualistic relationship under nitrogen-limited conditions (Kroon and Van Ginkel, 2001).

Ambiguity of the term 'Commensalism'

The term symbiosis has probably suffered much more misuse, changes in definition and controversy than any other definition concerning biological interactions (reviewed by Martin and Schwab, 2012, 2013). And among symbioses, confusion has surrounded commensalism more than other type of interactions, likely because mutualism, commensalism, parasitism, and predation are not categories with discrete boundaries; they occupy positions in a continuum of species interactions (Martin and Schwab, 2013; Douglas and Smith, 1989; Leung and Poulin, 2008). In a narrow sense, commensalism should be defined as an interaction between two organisms with strictly neutral fitness consequences for one

and positive consequences for the other. However, this narrow version of commensalism is difficult or even impossible to demonstrate due to its overly formal premises (see Zapalski, 2011 for details). In a broad understanding, commensalism can be viewed as an interaction that has positive effects for one interacting species, and a weak (either positive or negative) effect on the other. However, handling the term this way is very subjective, to the point where it is impossible to distinguish commensalism from other interactions (Zapalski, 2011).

Not only are objective measurements of commensalism difficult to apply, the costs and benefits that determine net effects of interactions can vary greatly in both space and time (e.g., Bronstein, 1994). For example, Lee *et al.* (2009) describe an annelid–crayfish association in which the interaction fluctuates between commensalism and mutualism depending on environmental conditions. Similarly, scaleworms of the genus *Arctonoe* have been found in symbiotic association with certain mollusk, echinoderm, and polychaete hosts. This relationship has generally been regarded as commensal. However, Wagner *et al.* (1979) suggest that the attraction of the sea star *Dermasterias imbricata* to its symbiotic scaleworm *Arctonoe vittata* implies that both host and symbiont derive benefit from the relationship, such that the association should better considered as mutualism. Another consideration is that organisms that usually commensal can become serious pathogens in certain contexts, as has been described for *Fusobacterium variu*, *Escherichia coli*, and other microorganisms (Leavis *et al.*, 2007; Drake, 2008; Ohkusa *et al.*, 2009). Similarly, some microorganisms that are benign for one individual can be pathogenic for another (Casadevall and Pirofski, 2000; Sachs and Wilcox, 2006).

It is also possible to describe many cases of species interactions as 'facultative commensalisms.' For example, white-fronted terns (*Sterna striata*) sometimes associate with Hector's dolphins (*Cephalorhynchus hectori*) while feeding near shore. Dolphins drive small fishes toward the surface where terns can more easily catch them, an activity that incurs no cost or benefit for the dolphins. This relationship may be particularly advantageous to terns during breeding seasons when energetic demands are high (Brager, 1998). Similarly, in shrimp-eating fish (Cichlidae) that forage in groups, an individual fish in a mixed-species group gets more food than when it forages singly. This varied searching and capturing behavior favors group foraging and utilization of prey that other fish may have overlooked (Yuma *et al.*, 1998). Various shrimps and crabs associate with the anemone *Bolocera tuediae* and the cerianthid *Pachycerianthus multiplicatus* by aggregating beneath their tentacles. The main benefit for these crustaceans to be associated with the anthozoans is protection against predators, but they also probably scavenge food (Jonsson *et al.*, 2001). Such associations are presumably facultative commensalism, as crustaceans live as non-symbionts on the sea floor as well, and the anthozoans do not seem to gain any benefits from the associations.

Coevolution and Selection in Commensal Organisms

In a strict commensal relationship we expect selection to operate on the commensal but not on the host, and as such commensalism is not a coevolutionary relationship. For example, remoras that hitch rides on the skin of sharks have deviated from a typical swimming fish design and possess derived sucker structures, but no parallel adaptations are seen in their shark hosts.

Long-lasting commensal associations that lead to highly specific adaptations may result in obligate commensalism. For example, phoresy in pseudoscorpions, which is obligatory in many cases, has a long history in the fossil record (Poinar *et al.*, 1998). Similarly, phoresy in mites is likewise the product of co-association through long evolutionary time. Commensal interactions lacking such a long history of interaction also occur in nature (Houck and O'Connor, 1991). A familiar example is the house sparrow (*Passer domesticus*), which became a sedentary human commensal 10 000 years ago, with wild populations having practically disappeared in present days (Saetre *et al.*, 2012).

Amensalism

Amensalism has received much less attention from researchers than commensalism. Amensalism is an interaction between organisms of two different species in which the individuals of one species adversely affect the fitness of a second species but receive no apparent fitness benefit themselves.

Examples

Amensalism is often used to describe strongly asymmetrical competitive interactions, such as those observed between ibex (*Capra pyrenaica*) and weevils of the genus *Timarcha* which feed upon the same type of shrub (Gómez and González-Mejías, 2002; **Figure 2**). In experimental manipulations, ibex exclusion increased abundance of weevils, whereas weevils had no effect on ibex. Ibex affected weevils both by exploitative competition and by incidental predation, with the total number of weevils emerging per shrub being more than fourfold higher in shrubs with ibex excluded than in shrubs open to ibex. In other words, while the presence of the weevil has almost no influence on food availability, the presence of ibex has an enormous detrimental effect on weevil numbers, as they consume significant quantities of plant matter and often incidentally ingest the weevils upon it. This study is a demonstration that small phytophagous insects may often be involved in amensal relationships with other herbivores highly dissimilar in size, ecology, and taxonomy, such as ungulates. Such asymmetric interactions are better classified as amensalism than competition.

Many cases described as competition for nesting space are also probably better ascribed to amensalism. For example, hole-nesting birds exhibit a size hierarchy in their access to tree holes, such that smaller species only can utilize holes that are inaccessible to bigger species. Some researchers have reported that several small birds recognize European starlings (*Sturnus vulgaris*) as aggressors and react aggressively toward them (Olsen *et al.*, 2008). Starlings in this study usurped and depredated nests of five other bird species. The difference between neutralism (a term that describes the relationship between two species that interact but do not affect each other) and amensalism may be a matter of resource availability. Starlings and nuthatches (*Sitta europea*) used the same tree

(a) (b)

Figure 2 Spanish ibex (*Capra pyrenaica*) (a) exhibits amensal relationship with weevils of the genus *Timarcha*. Ibex feed on the same plants as weevils so that ibex affect weevils both by exploitative competition and by incidental predation, as they consume significant quantities of plant matter and often incidentally ingest the weevils upon it. However, as weevils may cause significant damage to some plants they feed upon (b), no evidence was found that weevils affected the food available to ibex. This relationship may be better classified as amensalism than competition. © Marlin E. Rice.

holes for nesting in a study conducted in middle Europe (Mazgajski, 2000). The experimental increase of nest site availability providing nest boxes reduced considerably the negative effect of starlings on breeding success of nuthatches. When sufficient sites are available for all species, the relation between them can be considered neutralism. However, spotless starlings (*Sturnus unicolor*) studied in Spain occupied all 100 nest boxes erected by researchers. Only in a few occasions did smaller birds such as nuthatches and great tits (*Parus major*) attempted to use the boxes; spotless starlings invariably evicted the other species. There is no evidence that evicting other species from nest boxes causes any cost to starlings, so that this relationship seems amensal (Veiga and Polo, unpublished data).

Similarly, amensalism in the context of nesting space may describe the relationships observed between honeybees and wasps in tropical ecosystems of East Africa (Veiga *et al.*, 2013b). Nest boxes placed by researchers studying reproduction of birds were frequently occupied by either honeybees (*Apis mellifera*), wasps of the Vespidae family (paper wasps), or wasps of the Crabrionidae and Sphecidae families (mud daubers) (**Figure 3**). Wasps never used nest boxes occupied by bees during the previous breeding season while honeybees were observed to take over nest boxes irrespective of whether they were previously occupied or not by wasps. Although interactions between both groups of hymenopterans have not been described in detail, wasps were apparently reluctant to use any cavity where honeybees had bred even several months before. This is an apparent example of amensalism as there was not even a single case in which wasps were able to occupy a nest box previously used by bees. This implies that even eventual recolonizations of previously used cavities by bees are not hampered by the presence of wasps.

Ambiguity of the Term 'Amensalism'

Amensalism suffers, at least qualitatively, from similar problems of definition and classification as those discussed above for commensalism. Even some apparently clear examples of amensalism in which only one of two interacting species is obviously harmed by the relationship do not completely exclude the possibility that the other species is negatively affected in some way. For instance, the example of ibis and weevils has been alternatively described as an example of extremely asymmetric competition because the weevils may affect to some degree the quality of the ibex's food plants. Thus, although the negative effect of the weevil may be subtle and probably only perceptible under particular, infrequent ecological conditions, the relationships may not constitute a 'pure' case of amensalism. Similarly, the convergence of hole-nesting bees and wasps on tree cavities may entail costs for the dominant species under certain conditions. For example, dominant species may have to remove nesting material brought by other species to cavities before they have enough space to use the cavity, and this could entail energetic costs (Mazgajski, 2007). Also, cavities with multiple nesting species may become infested with nest parasites (e.g., Olsson and Allander, 1995), such that dominant species face non-negligible fitness costs in previously occupied cavities. Alternatively, some honeybee species are known to kill wasps (Ono *et al.*, 1995; Sugahara and Sakamoto, 2009), so it is conceivable that these bees also kill or actively displace nesting wasps. If attacks on wasps by bees elicit a cost for bees, this system may be an example of competition, not amensalism.

Coevolution and Selection in Amensals

As with commensalism, pure amensalism is not a coevolutionary relationship. Theoretical simulations using Lotka–Volterra equations have shown that although amensalism may be a stable solution, extinction of the negatively affected species may also occur (Ribeiro *et al.*, 2010; Cabella *et al.*, 2011). Amensalism is predicted to be a long-lasting relationship only if negative effects are not so high as to lead to total extinction of the handicapped species. In the example of honeybees and

Figure 3 Honeybees (*Apis mellifera*) frequently use nest boxes to build their combs (a), but also paper wasps of the Vespidae family (b) and mud daubers of the Cabrionidae and Sphecidae families (c) like these artificial cavities for nesting. Honeybees take over nest boxes irrespective of whether they were previously occupied or not by another hymenopteran, but wasps never used nest boxes occupied by bees during the previous breeding season. This is an example of amensalism in which the presence of bees hampers the use of cavities by wasps while bees use cavities irrespective of the previous occupation by wasps.

wasps presented above, the handicap suffered by wasps is probably small in most instances provided that there are other suitable cavities, as would seem to be the case in forest ecosystems (Veiga *et al.*, 2013a). However, in savanna habitats, where birds compete with wasps for the scarce cavities available (Veiga *et al.*, 2013b), additional restrictions imposed by honeybees to accede to cavities may seriously impact wasps populations.

Just as competition may promote selection for character displacement (Brown and Wilson, 1956), a similar mechanism may drive character displacement in amensal relationships. If the negatively impacted species cannot escape pressure from the harmful species (e.g., by migrating to new habitats), selection for characters that reduce the detrimental effect of amensalism may lead to changes in morphology or behavior.

Synnecrosis

Synnecrosis is probably the symbiotic interaction that has received the least amount of attention from researchers. The term is poorly defined in the literature. For the purpose of this entry, synnecrosis is a mutually detrimental interaction between two organisms that results in their mutual death. Although most authors agree with such definition (Christian *et al.*, 1974; Kasinger *et al.*, 2008; Martin and Schwab, 2013), synnecrosis has also been (incorrectly) applied to describe a coextinction event, wherein two obligately coevolved taxa die out in concert with one another (Kaiser and Boucot, 1996).

Synnecrosis is rarely studied. This is likely due to the idea that synnecrosis is a necessarily short-lived relationship, and is expected to be strongly selected against (Kasinger *et al.*, 2008). Theoretical modeling using Lotka–Volterra equations has confirmed on mathematical grounds that while amensalism or competition may lead to coexistence or to the extinction of one of the two interacting species, synnecrosis is not a stable phase in the model (Ribeiro *et al.*, 2010; Cabella *et al.*, 2011). For this reason, the term synnecrosis, as well as other similar terms including allotrophy, antibiosis, and allolimy have been avoided by most authors (Burkholder, 1952; Martin and Schwab, 2013). Note however that, unlike commensalism or amensalism, synnecrosis does describe a coevolutionary relationship.

See also: Coevolution, Introduction to. Evolution and Agriculture I. The Evolution of Domestication. Mutualism, the Evolutionary Ecology of. Predation and Parasitism. Symbiosis, Introduction to

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Compensatory Evolution

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Glossary

Compensatory mutations Mutations that ameliorate the deleterious fitness effects of other mutations.

Effective population size The number of individuals in an idealized population, which has the same genetic diversity as the population of interest. N_e is often used to represent effective population size.

Evolutionary capacitance The idea that cryptic genetic variation, which is hidden by the robustness in genetic systems, is released by environmental perturbation and help organisms to move to a new adaptive peak.

Fitness landscape A metaphor to represent the relationship between organismal fitness and other characters, such as phenotypes, allele frequencies, and genotypes. Height represents fitness.

$N_e s$ Product of effective population size and strength of natural selection, which indicate efficacy of natural selection in population.

Nearly neutral theory of molecular evolution The molecular evolution theory, which emphasizes the importance of mutations affected by both genetic drift and natural selection in molecular evolution.

Concept and Definition

History and Definitions of Compensatory Mutations

Compensatory evolution refers to the evolution through the fixation of compensatory mutations that ameliorate the deleterious fitness effects of other mutations. Although phenotypic masking effect of different mutations has been observed in classical genetic experiments, molecular mechanisms of compensatory mutations had been unknown until early molecular genetic experiments using bacteria started. In microbiological genetics, compensatory mutations are called suppressor mutations, which could mask the phenotype of preexisted (mostly deleterious) mutations. For example, insertions and deletions in open reading frames may cause frameshift and create a premature stop codon at the downstream of the mutation; the deleterious effect of such a frameshift could be restored if another insertion or deletion arises at the upstream of the stop codon. The study of suppressor mutations has greatly contributed to the understanding of translation mechanisms in early molecular biology research and the analysis of such revertants has also helped to estimate the rate of spontaneous mutations in a gene (Riddle and Roth, 1970) and the mutagenic effect of chemicals to DNA (Ames, 1979).

A wide range of molecular mechanisms contributes to compensatory evolution. In a broad sense, all back (reversal) mutations can be regarded as compensatory mutations. For example, if nucleotide A is mutated to G, the mutation from G to A at the same site is a compensatory mutation for the first mutation. In narrower sense, compensatory mutations occur between different site of molecules (intramolecular compensation), between different molecules (intermolecular compensation), or even between different genomes (inter-genomic compensation).

Population Genetics Model

In this subsection, compensatory evolution is defined in the context of population genetics. The theoretical population genetics model of compensatory evolution was studied by Haldane for deterministic case (Haldane, 1930) and by Kimura for stochastic case (Kimura, 1985). The model assumes simple biallelic two-locus model. Suppose there are two loci, A and B. Genotypes *AB* and *ab* represent wild type and mutant type, respectively. Under the compensatory neutral model of Kimura, individuals of genotypes *AB* and *ab* have the same fitness value but individuals of genotypes *aB* and *Ab* have smaller fitness value than wild type individuals. Kimura showed that under tight linkage between the locus A and B, the rate of evolution from genotype *AB* to *ab* is possible in a realistic order of timescale.

In the compensatory neutral model, there is little chance for intermediate deleterious states (*aB* and *Ab*) to fix in the population. However, the model could be extended to the compensatory weak selection model, where the fitness decline of *aB* and *Ab* is very small (i.e., slightly deleterious); such slightly deleterious alleles could fix in the population by genetic drift and the deleterious effect is later restored by slightly advantageous mutations (Ohta, 1992; Hartl and Taubes, 1996; Innan and Stephan, 2001; Carter and Wagner, 2002; Osada and Akashi, 2012). Although these two models have the same evolutionary outcome (fixation of *ab*), the trajectories of allele frequency are quite different. In the compensatory neutral model, fixation of mutations *a* and *b* simultaneously occurs and the genotype *ab* jumps over the deep fitness valley, whereas in the compensatory weak selection model, the genotype goes down through the shallow fitness valley. In **Figure 1**, the evolutionary paths of the compensatory evolution are shown. Distinguishing compensatory neutral and weak selection model is important for understanding how organisms evolve by crossing a fitness valley (see next subsection). The effect of population size on compensatory evolution may be complicated. In populations with small N_e, the compensatory weak selection would have higher contribution than the compensatory neutral because deleterious alleles are more easily fixed by stronger genetic drift. On the other hand, the elevated rate of occurrence of double mutants (*ab*) in large populations increases the

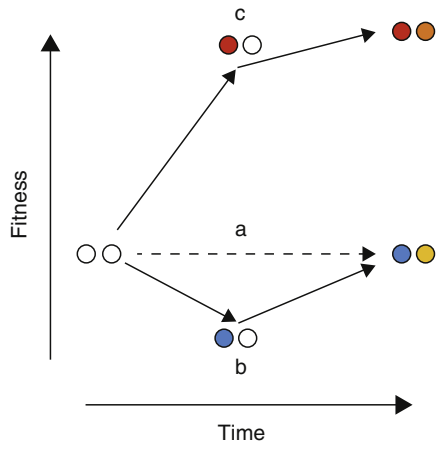

Figure 1 Schematic illustration of compensatory evolution in haploid system with two loci. Each circle represents a genotype at one locus. The blue and yellow circles denote compensatory mutations, which are deleterious without the other mutation. Three different paths are shown. (a) Compensatory neutral model, where two compensatory mutations (blue and yellow) reside in the same haplotype and become fixed together. (b) Compensatory weak selection model, where a slightly deleterious mutation (in this example, mutation in blue) fixes in the population and the fixation of slightly advantageous mutation (mutation in yellow) recovers the fitness decline. (c) Compensatory modifier model, where a strongly beneficial mutation (mutation in red) is initially fixed and the pleiotropic deleterious effect is restored by additional mutations (in this example by mutation in orange).

contribution of compensatory neutral evolution. Carter and Wagner (2002) showed that the rate of compensatory evolution can peak at intermediate population sizes for particular scenarios, when deleterious alleles (*a* and *b*) are recessive and the genotype *ab* has higher fitness than the genotype *AB*.

The term compensatory evolution is sometimes used in a very different context from the above model. If mutations are strongly advantageous in environments, those mutations become quickly fixed in the population. The initially beneficial mutations may have pleotropic deleterious effect. For example, some drug-resistant mutations in bacterial species reduce viability and the fitness cost is compensated by additional mutations (reviewed in Andersson and Hughes, 2010). In addition, fixation of strongly advantageous mutations could make other weakly deleterious mutations fixed together, which is referred to as genetic hitchhiking (Maynard Smith and Haigh, 1974). After the hitchhiking, the effect of deleterious mutations could be ameliorated by additional mutations or back mutations. Such processes are often called compensatory evolution but the evolutionary trajectory is quite different from the two models and the process can be termed 'compensatory modifier evolution,' to make a distinction (Akashi *et al.*, 2012).

Potential Evolutionary Role of Compensatory Mutations

The idea of compensatory evolution helps to understand how organisms could move to different adaptive peaks of fitness landscape, which is conceptualized by Wright (1932). **Figure 1** can be interpreted as fitness landscape in terms of genotypes. Compensatory neutral mutations would be one of the molecular mechanisms that create rugged fitness landscape. In contrast, Ohta incorporated the idea of slightly advantageous mutations in the nearly neutral theory of molecular evolution (Ohta, 1973). The fixation of slightly deleterious mutations in population must open up a space for slightly advantageous mutation that restore the fitness decline (Ohta, 1992), which is referred to as compensatory weak selection model here and shown in **Figure 1**.

Compensatory evolution is not restricted to the pair of loci. One example is the masking effect of deleterious mutations by molecular chaperons. One of the molecular chaperons, heat-shock protein Hsp90, is highly expressed under environmental stress and assist proper protein folding (Rutherford and Lindquist, 1998). It also helps folding of proteins with deleterious mutations (mostly destabilizing mutations) and could mask deleterious phenotypes. There is an idea of evolutionary capacitance, in which organisms could accumulate cryptic phenotypes and become the source of evolvability, which was developed by Waddington (Waddington, 1942). The compensatory process would promote the accumulation of many cryptic phenotypes and bring organisms to a totally different adaptive fitness peak when cryptic genetic variations are released by some reasons, such as environmental changes (reviewed in Paaby and Rockman, 2014). However, it remains unclear whether such genetic robustness through compensatory mutations plays a key role in organismal adaptation or simply as a by-product of robustness against environmental fluctuation (Lehner, 2010).

Molecular Mechanisms of Compensatory Evolution

Intramolecular Compensatory Evolution

Frameshift mutations are one of the examples of intramolecular compensation. However, there are many other mechanisms that promote intramolecular compensatory evolution. One of the clearest evidence of intramolecular compensatory evolution has been described in stem-loop structures in RNA molecules (Wheeler and Honeycutt, 1988). As shown in **Figure 2**, transcribed RNA molecules often form Watson–Crick pairs between A and U bases, and between C and G bases, in stem-loop structure. Mutations in one strand would dismiss the pairing and secondary structure of the RNA molecules may become unstable or may shift to a different state. However, if the base coding the opposite strand have another mutation that could form correct pairing, the two ribonucleotides could form proper pairing again. Many RNA structures such as tRNA and ribosomal RNA structure have the potential to promote compensatory evolution, and studies have shown that compensatory evolution is a prevalent mode of RNA sequence evolution (Wheeler and Honeycutt, 1988; Stephan and Kirby, 1993; Meer *et al.*, 2010).

Proteins are folded into complex three-dimensional structures and many amino acid residues interact with one another in the folding process, which provide huge opportunity for compensatory evolution between different amino acid sites (DePristo *et al.*, 2005). For example, positively charged amino

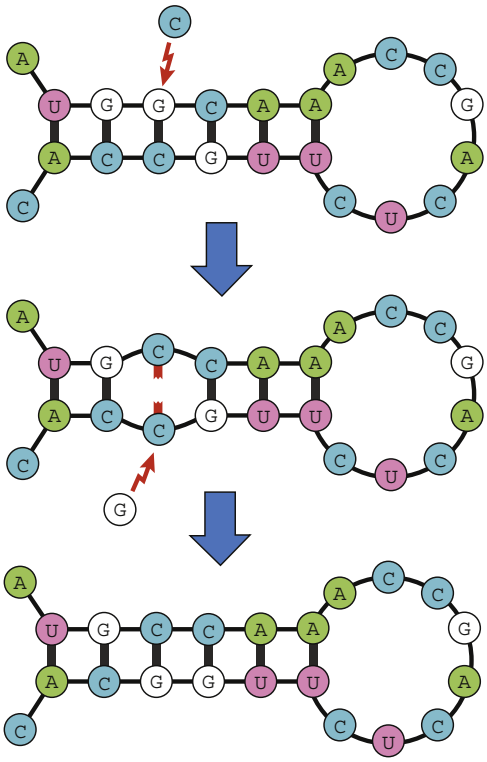

Figure 2 Example of compensatory evolution in RNA stem-loop structure. Watson and Crick pairs bound with each other with hydrogen bonds. Change of RNA sequence from G to C in the stem region (which is C to G mutation in the coding strand of genome) would break up the coupling, but the bond could be restored by additional mutation in the opposite strand.

acid site and negatively charged amino acid sites that are physically close to each other are bound with electrostatic interaction. Change of the one amino acid to lose proper charge may disrupt the interaction and may be detrimental for protein folding and stability, but the stability may be recovered by paired change at interacting sites. Complex protein structure offers large potential for other kinds of interaction between amino acid sites, such as hydrophobic interactions and covalent bonds between amino acid residues (reviewed in Ivankov *et al.*, 2014). In laboratory experiments, many compensatory mutations that could affect the stabilization of proteins have been identified (Lunzer *et al.*, 2010). Note that compensatory evolution here is not restricted to two-locus interaction as presented in the simple population genetics model. Indeed, experimental evidence showed that the effect of deleterious mutations are often compensated by many different mutations around deleterious mutations (e.g., Poon and Chao, 2005). In addition, many molecular evolution studies have identified coupled amino acid substitutions along lineages especially when the coevolving sites are close in three-dimensional structure (e.g., Shim *et al.*, 2005; Wang and Pollock, 2007; Yeang and Haussler, 2007). Large part of these correlated amino acid substitutions could be due to compensatory evolution.

Another example of intermolecular compensatory evolution is the evolution of codon bias (Akashi, 1995). In many genomes, both in eukaryotes and prokaryotes, the preference of codon usage in degenerative codons has been observed and preferred codons often correspond to the most abundant tRNA in the genomes (Ikemura, 1981). The frequency of preferred codons would be different among genes and correlate with gene expression level (Duret and Mouchiroud, 1999). Because the selective effect of each codon is presumably weak and one gene harbors many degenerative codons, gain and loss of preferred codons within genes are considered to be evolving under compensatory weak selection evolution. Population genetics studies on codon usage bias in *Drosophila* showed that the strength of natural selection was indeed very weak and in the range of weak selection ($|Nes| \sim 1$) (Akashi, 1995). Similar argument could be applied to the evolution of nucleosome binding sites, where nucleotide A and T are preferred for nucleosome binding and GC content at genome-wide level is under compensatory weak selection (Kenigsberg *et al.*, 2010).

Intermolecular Compensatory Evolution

Intermolecular compensatory evolution is caused by a wide range of molecular systems. For example, transcription of genes is regulated by many transcription factors, which bind to promoter or enhancer regions of DNA adjacent to the target gene. Since both DNA-binding motif of proteins and target DNA sequence could be changed by mutations and the strength of binding is determined by their level of matching, the deleterious effect of changes could be compensated by other mutations in the partners. In addition, if gene expression is regulated by multiple regulatory sites, gain or loss of some binding sites may not largely affect the expression pattern of genes. For example, some genes with a function in important developmental process possess shadow (secondary) enhancers, which have redundant function with the other enhancers (Perry *et al.*, 2010). Such redundancy would produce the robustness of regulatory systems under environmental and stochastic perturbation during development. Gain and loss of regulatory sites would promote compensatory evolution, which leads the turnover of regulatory sites. The turnover of regulatory sites under compensatory evolution explains why gene expression pattern has been evolutionarily conserved while regulatory regions have been evolutionarily unconserved (Ludwig *et al.*, 2000; Doniger and Fay, 2007).

Because many proteins are often forming protein complex, compensatory evolution between different protein subunit is also possible. In addition, any interaction between proteins and between protein and DNA may be the target of compensatory evolution. However, the information on protein complex structure is much more scarce than that of protein three-dimensional structure. Compensatory evolution in such a complex system is mostly shown by indirect evidence using molecular phylogenetic methods, which is discussed later.

Inter-Genomic Compensation

Compensatory evolution could occur even between different genomes in an organism: between host and endosymbiont genomes. Because of their particular reproductive strategy, endosymbiont populations have experienced huge reduction of

effective population size, which could promote the accumulation of slightly deleterious mutations in their genomes. However, the effects could be compensated by mutations in host genomes. Compensatory evolution between host and endosymbiont genomes may explain the higher mutation rate and massive loss of essential genes in endosymbiont genomes after endosymbiosis event (Moran, 1996). In addition, experimental evidence supports that global loss of protein stability by the fixation of destabilizing mutations in endosymbiont genomes is compensated by the higher level of molecular chaperon expression in endosymbiont genomes (Fares *et al.*, 2002, 2004).

Organelles in eukaryotes such as mitochondria and chloroplasts are also originated from ancient endosymbionts, and compensatory evolution between host and organelle genomes are also possible (Rand *et al.*, 2004; Osada and Akashi, 2012). During the course of evolution, many protein-coding genes encoded in organelle genomes have been moved to host genomes. Those proteins are translated in nuclear, transported into organelle, and form protein complex or interact with each other. Like other endosymbiont genomes, organelle genomes often have many different evolutionary features such as mutation rate, recombination rate, and effective population size, which potentially accelerates compensatory evolution between nuclear and organelle genomes. Compensatory evolution between host and organelle genomes could initiate hybrid incompatibility, which is called cytonuclear incompatibility, and seems widespread across many taxa (Barr and Fishman, 2010). The genetic incompatibility between closely related species might be related to speciation processes.

Molecular Evolution Methods for Detecting Compensatory Evolution

Several molecular evolution analysis methods have been proposed to find the signature of compensatory evolution. One approach is to infer conditionally deleterious mutations in a particular species. Deleterious mutations in one species sometimes do not show any phenotypic defect in other species with different genetic background; these mutations are likely to be compensated by other mutations. The studies of mammalian and *Drosophila* genomes showed that ~10% of pathogenic mutations are not deleterious in the other species, suggesting that compensatory mutations would be widespread in genomes (Kondrashov *et al.*, 2002; Kulathinal *et al.*, 2004).

Another approach is to detect correlated changes of amino acid or nucleotide sites along lineages; however, the pattern of coevolution does not always show the evidence of compensatory evolution (but see Pollock *et al.* (1999) for particular implementation). For example, positive epistatic effect of mutations, which increase fitness more than the summation of the effect of independent mutations, would also result in such correlated substitution pattern. In addition, strong mutation bias or other substitution biases may have created similar pattern. The phylogenetic analysis of correlated changes in protein sequences started in 1970 by Fitch (Fitch and Markowitz, 1970), and later a number of computational methods have been intensively developed to identify coevolving sites within proteins or between proteins (reviewed in de Juan *et al.*, 2013). Those methods have identified many amino acid and nucleotide substitutions that are phylogentically correlated with one another, especially when correlated sites are in close proximity to each other in three-dimensional structure (Fukami-Kobayashi *et al.*, 2002; Dutheil and Galtier, 2007; Yeang and Haussler, 2007). Assuming the physical vicinity of coevolving sites, such coevolutionary information can be used to predict protein three-dimensional structures (Marks *et al.*, 2012).

Distinguishing compensatory neutral and compensatory weak evolution is often quite challenging. One of the expected differences between the two models is whether we could observe intermediate states, where fitness of the genotype is supposed to decline. For example, Meer *et al.* (2010) analyzed stem-loop regions of tRNA encoded in mtDNA and observed the excess of joint substitutions along lineages, but deficiency of intermediate states among lineages and within populations, which supports the compensatory neutral model. Analyzing temporal orders of substitutions with dense phylogenetic sampling may help to find the intermediate states and subsequent fixation of slightly advantageous mutations (Osada and Akashi, 2012). In addition to these viewpoints, growing data of protein structure and mutation experiments in laboratories would make it possible to understand the prevalence and mode of compensatory evolution in nature.

See also: Adaptive Landscapes. Adaptive Molecular Evolution: Detection Methods. Coevolutionary Fitness Landscapes. Coevolution, Introduction to. Effective Population Size. Gene Interactions in Evolution. Mitochondrial and Nuclear Genome Coevolution. Molecular Evolution, History of. Neutral Models of Genetic Drift and Mutation. Robustness and Evolvability in Molecular Evolution. Symbiosis, History of. Symbiosis, Introduction to. Waddington's Epigenetic Landscape, History of

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Complexity, the Role of Oxygen in Evolution of

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Glossary

Aerobic Aerobic organisms require oxygen for adenosine triphosphate (ATP) production.

Anaerobic Anaerobic organisms do not require oxygen for ATP production.

Cambrian explosion The Cambrian explosion describes the rapid radiation of major animal phyla during the Cambrian period 541–485 million years ago.

Eukaryote A eukaryote is an organism with a nucleus that contains the genetic material and organelles enclosed within membranes. The Eukaryotes are one of the three domains of life.

Great Oxidation Event (GOE) The GOE describes the appearance of free oxygen, released by photosynthetic cyanobacteria, in the atmosphere about 2.4 billion years ago.

Molecular clock The molecular clock allows the estimation of dates of divergence between taxonomic groups. A molecular clock assumes a fairly constant rate of nucleotide or amino acid substitutions over time and must be calibrated by known (mostly fossil) dates.

Mya Million years ago.

Prokaryote A prokaryote is an organism without a nucleus or any membrane-bound organelle. Prokaryotes are divided into two domains, Archaea and Bacteria. The third domain of life covers the Eukaryotes.

Romer's gap Romer's gap is an apparent gap in the fossil record of tetrapods in the early Carboniferous period from about 360 to 345 million years ago.

The Importance of Oxygen for Life

Oxygen (or dioxygen, chemical symbol: O_2) is a colorless gas. The air that surrounds us consists of 20.95% O_2, 78.09% nitrogen (N_2), 0.04% carbon dioxide (CO_2), 0.93% argon, and traces of other noble gases. O_2 also dissolves in the water body at variable amounts. Almost any O_2 is produced by photoautotrophic organisms, including cyanobacteria, algae, and plants (Figure 1).

O_2 drives the energy metabolism of 'aerobic' organisms (which include most animals, plants, fungi, unicellular organisms, and many bacteria). Humans and higher animals usually die within a few minutes without sufficient O_2 supply. In eukaryotes, O_2 serves as an electron acceptor in the electron transport chain of the mitochondria, which leads to the production of metabolic energy in the form of adenosine triphosphate (ATP) (Figure 2). Therefore, mitochondria are also called the 'power plants' of a cell. The electrons that fuel the electron transport chain derive from the oxidation of nutrients and are delivered as reduction equivalents ($NADH + H^+$ and $FADH_2$). The transfer of electrons to O_2 leads to the formation of (metabolic) H_2O. In prokaryotes, which lack mitochondria and other membrane-bound organelles, the electron transport chain is located at the plasma membrane. Some organisms dwell in O_2-free environments and are called 'anaerobic.' They either rely on fermentation or use other terminal electron acceptors than O_2. These alternative electron acceptors have smaller reduction potentials than O_2 and, therefore, in anaerobic metabolism much less metabolic energy (ATP) is extracted from the nutrients than in aerobic respiration. However, an efficient, O_2-driven energy supply is required to support higher life-forms. Therefore, most animals only thrive in a well-oxygenated atmosphere and changes in the ambient O_2 levels during the Earth's history were an important factor in evolution (Berner *et al.*, 2007; Hsia *et al.*, 2013; Ward, 2006). In this article, the development of the free O_2 level over the past 4.5 billion years and its relationship to the evolution of life is described.

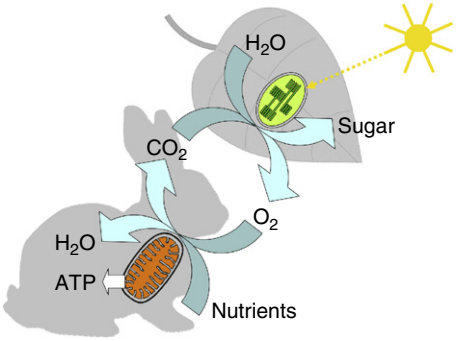

Figure 1 The oxygen cycle. Photoautotrophic organisms (cyanobacteria, algae, and plants) use sunlight to fixate CO_2; O_2 is released in photosynthesis as a by-product. O_2 is used by aerobic organisms (most animals, plants, fungi, unicellular organisms, and many bacteria) as an electron acceptor to produce metabolic energy (ATP). O_2 is reduced to H_2O; CO_2 is produced by oxidation of metabolites in the catabolic pathways.

The Rise of Oxygen in the Early Earth

Geochemical data suggest that the atmosphere of the early Earth was devoid of O_2 and mostly contained CO_2, N_2, methane, and water vapor. Thus, life on earth evolved in an O_2-free, highly reduced atmosphere (Figure 3).

The energy metabolism of the first, bacterium-like cells relied on the reduction of inorganic compounds (sulfur, iron, etc.) or free organic matter. Only with the emergence of oxygenic photosynthesis in the ancestors of the blue algae

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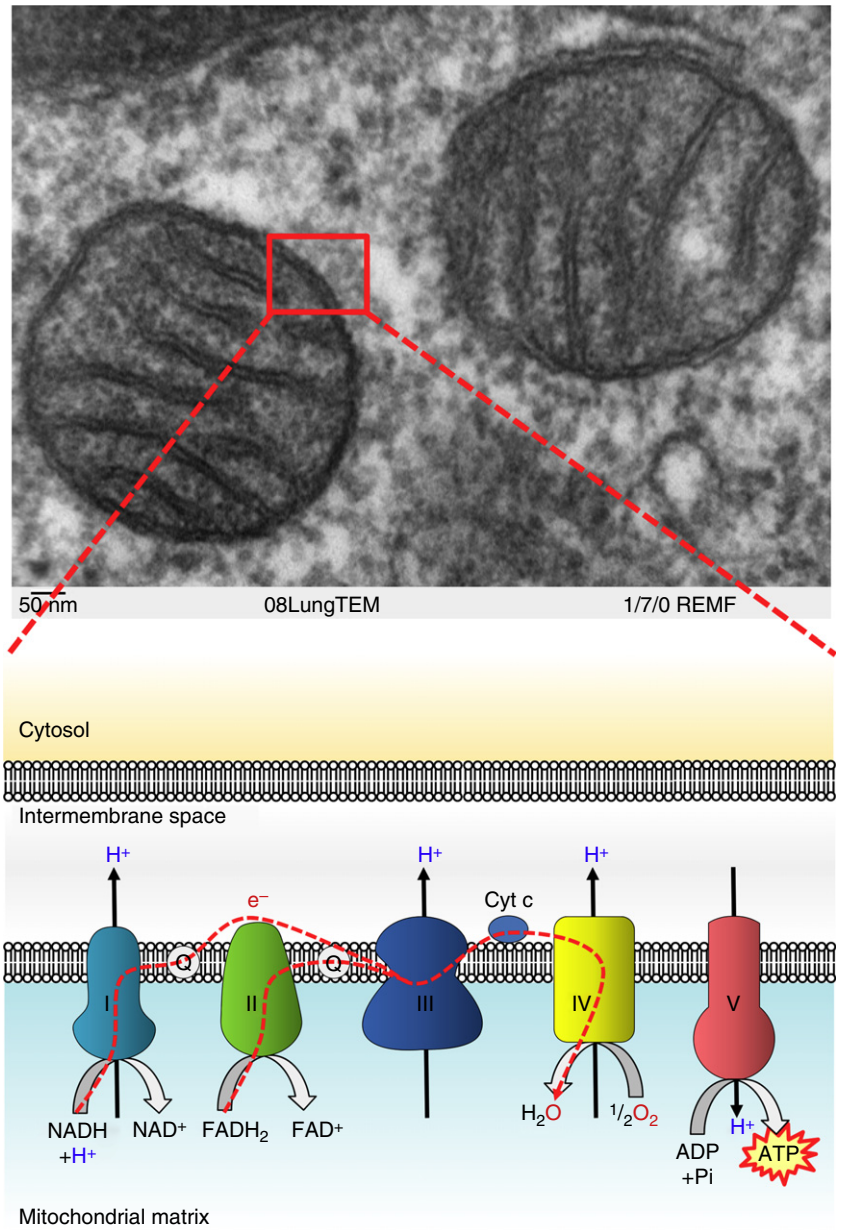

Figure 2 Mitochondria and the electron transport chain (also called respiratory chain). The mitochondria (upper panel; Louisa Howard – http://remf.dartmouth.edu/imagesindex.html http://remf.dartmouth.edu/images/mammalianLungTEM/source/8.html) are the site of aerobic energy metabolism, which is fueled by electrons that derive via NADH + H^+ and $FADH_2$ from the oxidation of food. The electrons are transported by a series of four complexes (I–IV) via redox reactions that pump protons (H^+) from the mitochondrial matrix to the intermembrane space between the inner and outer mitochondrial membrane. Ubiquinone (Q) acts as an electron carrier. O_2 is the final electron acceptor of the electron transport chain in complex IV. The proton gradient provides energy that drives the ATP synthase (complex V), which produces metabolic energy in the form of ATP.

(cyanobacteria) free O_2 appeared (Figure 4). The timing of this event is controversial, but there is evidence that photosynthetic organisms evolved more than 2.7 billion years ago (Brocks *et al.*, 1999; Lyons *et al.*, 2014). In photosynthesis, light energy is used to transfer electrons from chlorophyll to nicotinamide adenine dinucleotide phosphate (NADPH) in photosystem I and produces ATP (Figure 5). This metabolic energy is used to fixate CO_2 in the light-independent reactions of photosynthesis (Calvin cycle). Photosystem II replenishes the electrons in the chlorophyll by oxidation of various electron donors. In oxygenic photosynthesis, as it occurs today in plants, algae, and most cyanobacteria, H_2O is oxidized, and O_2 is released as a by-product (Figures 1 and 2). Early photosynthetic organisms, however, did not produce O_2 because they used free iron (Fe^{2+}), hydrogen sulfide (H_2S), or other reductants as electron donors, and even today some cyanobacteria retained the ability to switch to anoxic photosynthesis if no O_2 is available.

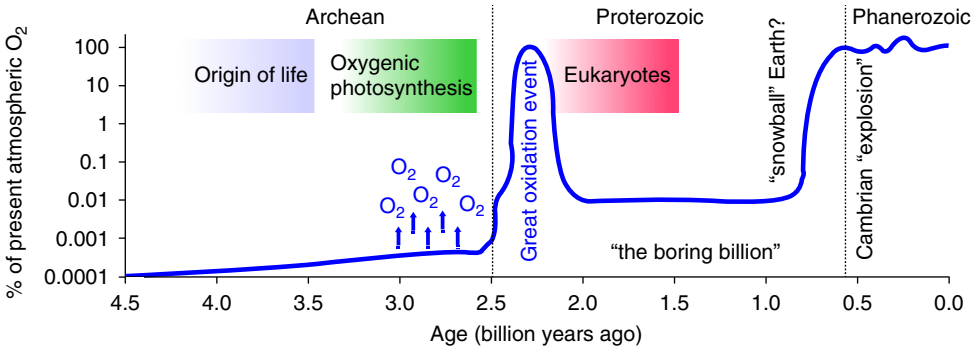

Figure 3 Evolution of atmospheric oxygen. The blue curve shows the estimated O_2 concentration over time in percent of the present atmospheric O_2 level (Berner, 2009; Kump, 2008; Lyons *et al.*, 2014). The early atmosphere was devoid of O_2, which accumulated only after the evolution of oxygenic photosynthesis. After the Great Oxidation Event 2.4 billion years ago, O_2 levels were low for a long period and only increased a few 100 million years before the Cambrian 'explosion.'

After the invention of oxygenic photosynthesis, O_2 did not significantly accumulate in the atmosphere for the next hundreds of million years because O_2 was taken up by sinks such as Fe^{2+}, H_2S, or organic matters. The oxidation of soluble Fe^{2+} to insoluble Fe^{3+} led to the massive deposition of banded-iron formations, which are today commercially important iron ore resources. The O_2 sinks in the ocean and atmosphere became gradually saturated, and first traces of free O_2 appeared. At that time, the concentration of O_2 in the atmosphere was still very low (probably less than 0.001% of today's levels: Lyons *et al.*, 2014) and only about 2.4 billion years ago the O_2 content of the atmosphere had notably increased, which is known as the 'Great Oxidation Event' (GOE: Holland, 2006; Lyons *et al.*, 2014). Two billion years ago, the sun was weaker, and the relatively warm climate on Earth mostly relied on methane, which is a powerful greenhouse gas. It has been speculated that during the GOE O_2 reacted with free methane, thereby reduced its concentration in the atmosphere and brought about the Huronian glaciation of the Earth, which lasted from about 2.4 to 2.1 billion years ago.

The First Oxygen-Breathers

O_2 is a double-edged sword: On the one hand, O_2 is a potent electron acceptor (thus having a highly negative reduction potential) that enables the efficient extraction of energy from the nutrients; on the other hand, O_2 is a dangerous toxin that causes oxidative stress and damages macromolecules, which may lead to cell death. The early prokaryotes 2.4 billion years ago were probably not adapted to high O_2 levels, and thus the GOE was likely the cause for one of the biggest extinction events in the Earth's history (Margulis and Sagan, 1986). Only species that evolved antioxidant mechanisms and that were able to eliminate O_2 survived. Other prokaryotes retreated into an O_2-free milieu, and some of their descendants still live today in anoxic environments.

The accumulation of O_2 also spurred the evolution of an electron transport chain that employs O_2 as the terminal electron acceptor. The electrons derived from the oxidation of food and the electron flux in the respiratory chain is used to pump protons (H^+) out of the cells; the resulting electrochemical gradient (pH and charge) drives a reverse proton pump that produces ATP (ATPase). It is not known whether the electron transport chain first evolved to eliminate toxic O_2, or whether the high affinity of O_2 to electrons and the resulting higher energy extraction provided a crucial evolutionary advantage.

The Emergence of Eukaryotes by Endosymbiosis

The electron transport chain first evolved in proto-bacteria, in which it is located in the cell membrane. Eukaryotes are characterized by the possession of a nucleus and various other organelles, which have specific functions and which are usually enclosed within membranes. Mitochondria and chloroplasts are unique among these organelles because they possess their own genomes and the associated machinery for gene expression. According to the endosymbiotic theory, mitochondria are descendants of ancient α-proteobacteria and were incorporated into a proto-eukaryote host (Esser and Martin, 2007; Pisani *et al.*, 2007; **Figure 6**). The origin of the putative eukaryote host is less clear. It has been suggested, for example, that it derived from ancient methanogen or sulfur-metabolizing Archaea (Pisani *et al.*, 2007). In a second endosymbiosis event, a eukaryote lineage took up a cyanobacterium, which gave rise to the chloroplasts (**Figure 5**; Elias and Archibald, 2009). These eukaryotes were the ancestors of algae and plants (**Figure 6**). Secondary endosymbiotic events, in which a chloroplast-possessing eukaryote was engulfed by another eukaryotic host, have occurred several times independently and gave rise to, for example, brown algae.

The timing of the origin of eukaryotes is notoriously difficult, with proposed dates ranging from 3.8 to 0.8 billion years ago (Roger and Hug, 2006). The first microfossils that have been attributed to the eukaryotes were found in rocks that are 1.8 billion years old (Knoll *et al.*, 2006), but biomarkers of putatively eukaryotic origin may be as old as 2.7 billion years (Brocks *et al.*, 1999). Recent molecular clock estimates (i.e., assuming an approximately constant rate of amino acid substitutions over time) calculated the age of the last common ancestor of extant eukaryotes about 1–1.9 billion years ago (Parfrey *et al.*, 2011). In any case, eukaryotes emerged in an environment with comparably low O_2 levels.

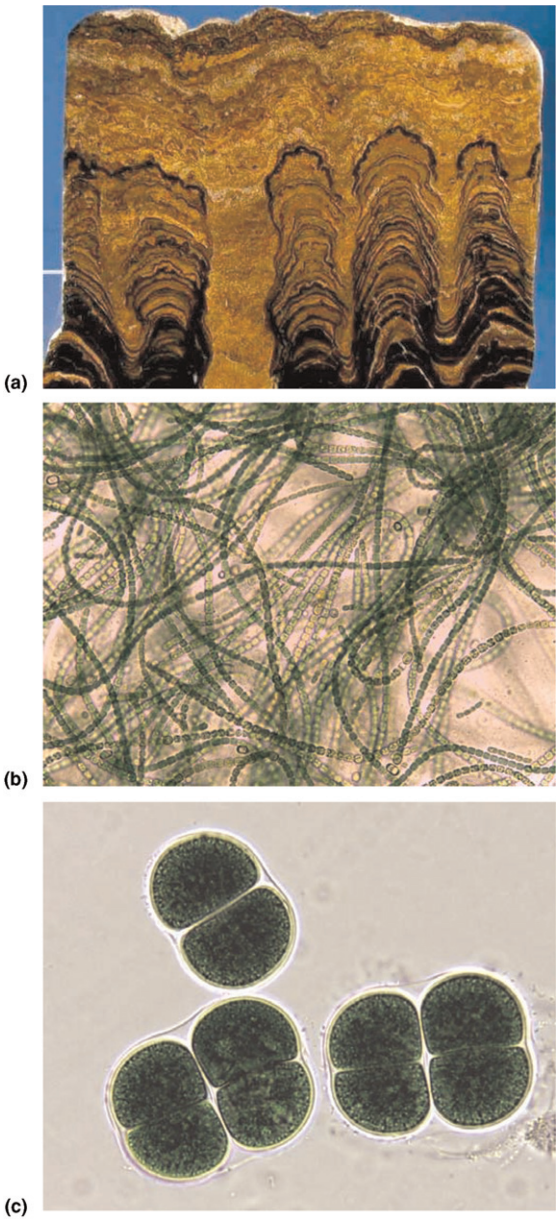

Figure 4 Fossil and extant cyanobacteria (blue algae). Stromatolites are fossil signs of cyanobacteria. These laminated rocks have been formed by biogenic processes due to the growth of cyanobacteria. The upper picture (a) shows stromatolites from the Proterozoic period from Eastern Andies South of Cochabamba, District of Cochabamba, Bolivia, South America (http://upload.wikimedia.org/wikipedia/commons/5/57/Proterozoic_Stromatolites.jpg, licensed under CC-BY 3.0). The lower pictures show extant cyanobacteria of the genera *Anabaena* (b) and *Chroococcus* (c). (b) and (c): Reproduced with the kind permission of Dieter Hanelt, University of Hamburg, Germany.

From the Rise of Oxygen to the Cambrian Explosion

Even after the GOE about 2.4 billion years ago, the concentration of O_2 remained low for a long time (**Figure 3**) and there was little progress in the complexity of life for a period that has been dubbed the 'boring billion' (Lyons *et al.*, 2014). Only the atmosphere and the surface waters were oxygenated while the deep oceans remained anoxic. However, there must have been sufficient O_2 to support relatively large, colonial life-forms 2.1 billion years ago (El Albani *et al.*, 2010). A second peak of O_2 causing persistent oxygenation of the water masses occurred much later, probably at the end of the Proterozoic period some 800–600 million years ago (mya) (Johnston *et al.*, 2009; Lyons *et al.*, 2014). Around that time, the first large, complex animals appeared, which diversified in the Cambrian period 541–485 mya. The relatively sudden appearance of most modern animal phyla within a – at the geological scale – short period of time has been named 'Cambrian explosion' (Conway Morris, 1993; Gould, 1989) and linked the rising oxygen levels (Canfield and Teske, 1996; Saltzman *et al.*, 2011; Shen *et al.*, 2008). Other factors, such as the enhanced availability of dissolved calcium, an increase in temperature after a global glaciation ('snowball earth' during the Cryogenian about 850–635 mya) or an arms race between predators and prey, may have also played a role in Cambrian explosion, but certainly O_2 was an essential prerequisite for the emergence of complex life-forms.

The size of the large animals that appeared in the Cambrian period exceeded anything that was before and required the evolution of morphological and physiological adaptations that ensured a sufficient supply with O_2. These adaptations include specialized respiratory surfaces (mostly gills in the aquatic environment) and a circulatory system with a heart that pumps oxygenated blood from the surface to the inner tissues (Schmidt-Nielsen, 1997). Respiratory proteins (hemoglobin, hemocyanin, or hemerythrin) enhance O_2 supply either by transporting it in the blood or by storing it in tissues (Burmester and Hankeln, 2014; Kurtz, 1999).

The Impact of Oxygen Fluctuations on Animal and Plant Evolution

In the Phanerozoic, which is the geologic eon in which complex animal and plant life has existed and which covers the past 541 million years, the atmospheric O_2 levels fluctuated between about 10% and 35% (**Figure 7**; Berner *et al.*, 2000, 2007). In the Early Cambrian period around 540 mya O_2 levels were probably about the same as they are today ($\sim$20%). The first plants originated from green algae and conquered land 500–470 mya (Steemans *et al.*, 2009; Wodniok *et al.*, 2011). They probably resembled modern liverwort. First vascular plants evolved around 30 million years later in the early Silurian period.

Oxygen is more abundant in the atmosphere than in the water body. However, various morphological adaptations were required that allowed animals to make use of the comparably high O_2 concentrations. These adaptations included, first of all, the respiratory organs, which are a tracheal system in arthropods or the lungs in vertebrates. Animals invaded land during two independent phases: First the ancestors of spiders, myriapods, and hexapods independently became terrestrial in the early Devonian period 420–410 mya. Between 380 and 365 mya vertebrates that resembled intermediates between fish and amphibians went on land. While in the arthropods trachea only evolved after terrestrialization, the ancestor of the terrestrial vertebrates (tetrapods) had lungs, similarly to the

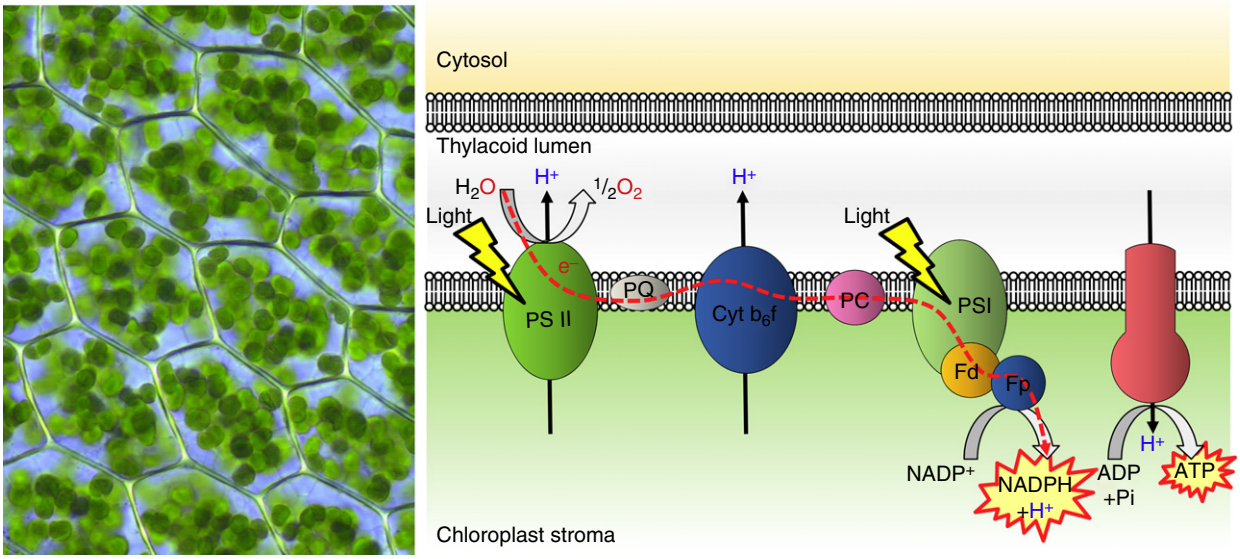

Figure 5 Chloroplasts and oxygenic photosynthesis. The picture on the left side shows chloroplasts inside leaf cells of the thyme-moss *Plagiomnium affine* (http://upload.wikimedia.org/wikipedia/commons/4/49/Plagiomnium_affine_laminazellen.jpeg; licensed under CC-BY 3.0). The picture on the right shows the light reaction of photosynthesis, in which light (photons) is converted into metabolic energy (NADPH and ATP). In plants, this reaction takes place in the thylakoid membranes of the chloroplasts, which contains four major complexes: photosystem II (PS II), cytochrome b6f (Cyt b6f), photosystem I (PS I), and ATP synthase. PS I and PS II absorb photons via pigments (mainly chlorophyll). The absorption of photons in PS II causes the oxidation of H_2O and the release of O_2. The electrons are transferred via plastoquinone (PQ), Cyt b6f and plastocyanin (PC) to PS I, in which (via ferredoxin [Fd] and ferredoxin-NADP reductase [Fp]), NADPH is produced after absorption of an additional photon. In addition, the electron transport chain generates a proton gradient, which is used by the ATP synthase to produce ATP.

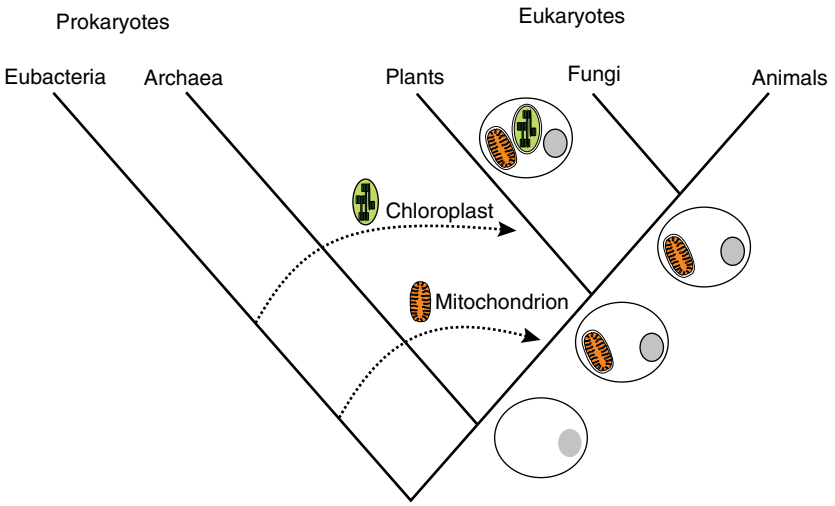

Figure 6 Origin of eukaryotes by endosymbiosis. According to the endosymbiotic theory, mitochondria of eukaryotes derive from eubacteria (probably α-proteobacteria). The host may be more closely related to the Archaea. A following endosymbiosis gave rise to the chloroplasts of algae and plants.

extant lungfish. It has been suggested that the rising O_2 levels during these phases were a main driver because terrestrial animals had to evolve respiratory systems that are adapted to the air, and first, primitive trachea and lungs were probably less efficient (Ward *et al.*, 2006). Between 360 and 345 mya there is a notable lack in the fossil record, which is known in paleontology as 'Romer's gap' (Figure 7). Romer's gap is preceded by a period of low atmospheric O_2 levels, which may have caused a reduction of terrestrial biodiversity (Ward *et al.*, 2006).

A great rise in O_2 level was observed in the Carboniferous period, commencing around 300 mya, and was likely caused by enhanced photosynthesis that accompanied the evolution of large vascular plants (Berner *et al.*, 2007). The appearance of trees on Earth was associated with an increased burial of organic matter, which can be found today as coal deposits. It has

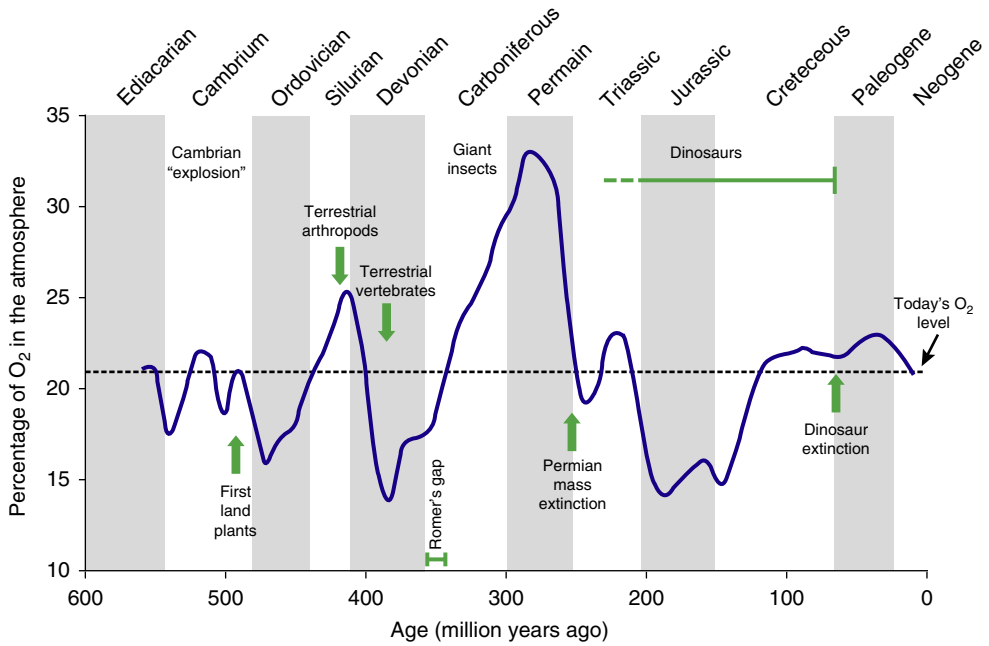

Figure 7 Changes in O_2 levels during the Phanerozoic period. The blue curve shows the estimated atmospheric O_2 concentration over time in volume-% (Berner, 1999, 2009; Ward *et al.*, 2006). Major events in plant and animal evolution are indicated (see also Hsia *et al.*, 2013); see text for details.

been speculated that the high O_2 levels during the Carboniferous period spurred insect gigantism (Graham *et al.*, 1995; Hsia *et al.*, 2013): For example, the late Carboniferous dragonfly *Meganeura* had a wingspan of up to 70 cm. However, there may be alternative explanations for the large insects during the Carboniferous period, such as the lack of other aerial predators (Clapham and Karr, 2012).

By the end of the Permian period about 250 mya the atmospheric O_2 levels had dropped dramatically. The boundary between the Permian and Triassic periods, which also marks the boundary between the Paleozoic and Mesozoic eras, is characterized by the largest known mass extinction event in the Phanerozoic that drastically reduced biodiversity. The causes of the late Permian extinction are still elusive and have been linked to enhanced volcanic activities, asteroid impact events, massive methane release into the atmosphere causing a greenhouse effect, etc. In any case, there is convincing evidence that large parts of the oceans became anoxic during that period, triggering the extinction of 80% to 96% of all marine species (Benton, 2005). Terrestrial animals suffered somewhat fewer losses, and about 70% of land vertebrate species disappeared.

Most of the Mesozoic period until about 110 mya is characterized by comparably low O_2 levels in the atmosphere. Therefore, dinosaurs, which are a synonym for animal gigantism, evolved and lived at low O_2 levels, at least until the middle Cretaceous period (Tappert *et al.*, 2013). Thus the large body size of many dinosaur species cannot be attributed to higher O_2 availability, but may be caused by other factors such as the high temperature in the Mesozoic period or enhanced plant growth augmented by high CO_2 levels. Additionally, physiological and anatomical adaptations, including air sacs and a bird-like respiratory system, may have supported an efficient extraction of O_2 from the thin air (Ward, 2006). In the past about 100 million years, which observed the extinction of dinosaurs at the end of the Cretaceous period 66 mya and the rise and radiation of mammals until today, the atmospheric O_2 levels remained comparably constant and in the range of today's levels.

Adaptations to Low and Changing Oxygen Environments

Today, an adequate supply with O_2 is essential for most animals. However, many species live in environments with low or changing O_2 levels, for example, in high altitudes or in poorly oxygenated waters. These organisms have evolved various adaptations that allow them to cope with unfavorable conditions. Such mechanisms include enlarged respiratory surfaces, better blood flow, higher hemoglobin concentrations, high-affinity hemoglobin, and a reduced metabolism. At the molecular level, a conserved pathway that involves a prolyl hydroxylase (PHD) and the hypoxia-inducible transcription factor (HIF) is essential for sensing the O_2 levels within an animal cell (Greer *et al.*, 2012). The PHD, which actually binds O_2, evolved already in early eukaryotes during the Proterozoic period (Place and Domann, 2013). The more sophisticated HIF pathway that transfers the signal and regulates gene expression came later with the evolution of multicellular animals and helps today to maintain cellular O_2 homeostasis, even under changing ambient environments.

See also: Bacterial Diversity, Introduction to. Cambrian Explosion: A Molecular Paleobiological Overview. Insects and Ecdysozoa,

Diversification of. Land Animals, Origins of. Land Vertebrates, the Origin and Evolution of. Metazoans, Origins of. Origin of Life, RNA World and. Origins of Life, History of. Vertebrates, the Origin of

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Consensus Methods, Phylogenetic

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Introduction

When evolutionary trees are estimated from different sources, biologists need to be able to describe the similarities between the trees. When two or more evolutionary trees are constructed for the same set of species, or taxa, a consensus tree can be used to visualize similarities between these different trees. In addition to answering questions about what a set of trees has in common, this approach summarizes a potentially large amount of information (in the form of many trees) with a single tree.

There are many applications for consensus trees. Consensus trees have often been used descriptively and as a visualization tool for understanding data in the form of trees. However, consensus trees can also be used inferentially as estimates of evolutionary trees.

An early application of consensus trees was when an evolutionary tree was inferred from two sources. For example, one tree is estimated from morphological data, while the other tree on the same species is from DNA data. Similarly, you could have one tree estimated from mitochondrial DNA and the other from nuclear DNA. Often the two trees would have many similarities, but also some disagreement. In this case, a consensus tree could indicate which evolutionary relationships were in agreement in the two trees. Disagreement between two or more trees can be represented in the consensus tree as an unresolved relationship.

The idea for the consensus tree approach is illustrated in Figure 1. In the figure, the three trees at the top can be considered as input, and the consensus method returns a single tree that summarizes relationships that the input trees have in common.

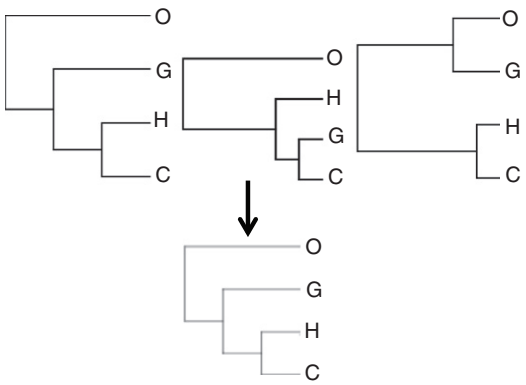

Figure 1 An example of a consensus tree. Here there are three input trees on the species chimpanzee (C), human (H), gorilla (G), and orangutan (O). A consensus method combines the three trees into an overall tree. In this case, the consensus tree happens to be the same as the left-most input tree. Some consensus methods will lead to this result, while others will lead to different consensus trees. See the text for details.

Consensus Tree Methods

Researchers in biology, computer science, mathematics, and statistics have devised dozens of consensus tree methods. The best method can depend on the data being used and the purpose for which the consensus tree is being constructed. In this section, some of the more common and historically earlier methods for consensus are described.

Consensus trees are often based on information that can be extracted from the input trees. Before illustrating consensus methods, we will first consider some of the ways of summarizing trees that are used for different types of consensus methods. Some of this summary information can also be distinguished by whether it applies to rooted versus unrooted trees. Examples of summary information used to construct consensus trees include clades, splits, rooted triples, and unrooted quartets, which are usually called just quartets.

For a rooted tree, a *clade* is a subset of taxa for which the most recent common ancestor does not have any descendant taxa in the study not included in the set. An example is shown in Figure 2. A *split* partitions the taxa of an unrooted tree into two non-overlapping sets, and there is a split associated with each branch of an unrooted tree (Figure 1(b)). A rooted triple is the tree on a subset of three taxa implied by a larger rooted tree. Similarly, a quartet is the four-taxon unrooted tree implied by a larger unrooted tree.

An important concept for both clades and rooted triples (and splits and quartets) is compatibility. Two clades are *compatible* if they can both occur on the same tree. The idea of compatibility also applies to splits, rooted triples, and quartets. For example, for a tree with taxa X, let X_1 and X_2 be mutually exclusive subsets of X with $X_1 \cup X_2 = X$. That is, every taxon belongs to one, and only one, of the subsets X_1 and X_2. Let Y_1 and Y_2 be another partition of X into two subsets of taxa. Then X_1 and Y_1 are compatible clades if (1) they have no taxa in common, or (2) either X_1 is a subset of Y_1 or Y_1 is a

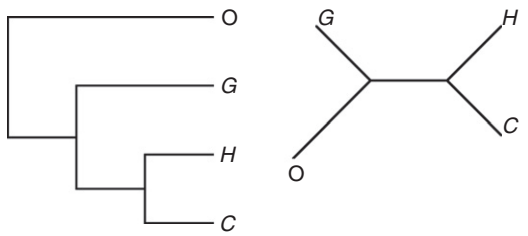

Figure 2 Rooted vs. unrooted trees. The tree on the left is rooted, where the most recent common ancestor of species *C*, *H*, *G*, and *O* is on the left, and time proceeds to the right. The tree on the right is the unrooted version of the tree on the left. The tree on the left includes {*C*,*H*} and {*C*,*G*,*H*} as clades, subsets of taxa which have a common ancestor that include no other taxa in the tree. For an unrooted tree, an analogous concept is a split, where removing one edge splits the taxa into two sets. For example, *CH*|*GO* is a split for the unrooted tree.

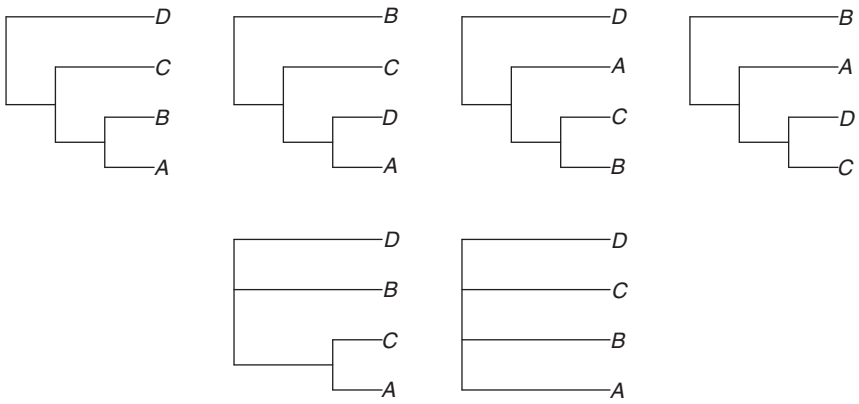

Figure 3 Example with four input trees. If the four trees on the top row are the input trees, then the first tree in the bottom row is the R^* consensus tree, and star tree on the bottom right is the Majority-Rule tree, and the Greedy consensus tree is any of the four input gene trees with equal probability. The example illustrates that the R^* consensus tree can include a clade that did not occur in any input tree.

subset of X_1. Similarly, splits $X_1|X_2$ and $Y_1|Y_2$ are compatible if either $Y_1 \subset X_1$, $Y_1 \subset X_2$, $X_1 \subset Y_1$ or $X_2 \subset Y_1$.

Compatibility of rooted triples is similar. The idea is that rooted triples are compatible if they can be displayed on the same tree. As an example, for trees on four species – *A*, *B*, *C*, and *D* – a triple can be represented by expressions such as *BC*|*D*, meaning that *B* and *C* share a common ancestor that is not ancestral to *D*. An example of a set of triples which is not compatible is *AB*|*C*, *BC*|*D*, and *AD*|*C* (Ranwez *et al.*, 2007). The only 4-taxon tree which has both rooted triples *AB*|*C* and *BC*|*D* is (((*A*,*B*),*C*),*D*), and this tree does not have the rooted triple *AD*|*C*.

Strict Consensus

A strict rooted consensus tree (Rohlf, 1982) consists of all clades that occur on all input trees. For unrooted trees, a strict consensus tree consists of all splits that occur on all input trees. Clades (or splits) that occur on some but not on all input trees lead to lack of resolution in the consensus tree (**Figure 3**, bottom row). The strict consensus tree can easily result in highly unresolved trees. It is particularly sensitive also to the problem of *rogue taxa*, in which several trees agree on relationships for most taxa, but one taxon with unpredictable placement in the trees makes strict agreement between the trees rare.

Semi-Strict or Combinable Component Consensus

The *semi-strict* consensus method (also called combinable component) generalizes the strict consensus method for the cases when input trees are not fully resolved (Bremer, 1990; Swofford, 1991). This is particularly useful in that trees estimated from DNA sequences are often not fully resolved and have polytomies (multifurcations) in which an ancestral node has three or more daughter nodes. In this case, a clade is on the rooted consensus tree if at least one input tree includes the clade, and no input tree contradicts that clade. Similarly, an unrooted semi-strict consensus tree has a split if at least one input tree has that split and no input tree contradicts that split.

Adams Consensus

Adams consensus (Adams, 1972) is somewhat similar to strict consensus in constructing a tree based on what all input trees have in common, but instead of using clades, the Adams consensus tree is based on the rooted triples that are not contradicted by any input tree. An appealing feature of the Adams consensus tree is that it is more robust to rogue taxa than strict consensus. It is therefore possible that a set of input trees would be completely unresolved using strict consensus but partially resolved using Adams consensus.

A surprising result due to Steel *et al.* (2000) is that an unrooted analogue to Adams consensus does not preserve all of the properties of the rooted Adams consensus if we wish to construct a tree from all quartets that occur in all input trees, then there are cases in which this procedure will not work. In their example, consider the input trees ((1,2),(3,4),(5,6)) and ((2,3),(4,5),(1,6)). A consensus tree must preserve the quartets ((1,2),(4,5)), ((1,6),(3,4)), and ((3,4),(5,6)). However, this means that the consensus tree must be one of the two input trees, and the choice is arbitrary. In particular, any particular choice could be reversed by relabeling the taxa. This problem does not arise for the rooted Adams consensus tree. This example illustrates that while many rooted consensus methods have unrooted analogues, at least some rooted consensus methods do not have satisfactory unrooted versions.

Majority-Rule consensus

Majority-Rule consensus includes all clades that occur in more than 50% of the input trees. The method can be generalized to only allow clades that occur in more than p% of the input trees, where p is any number strictly greater than 50%. The inequality is strict because it is possible for two clades to each occur on 50% of the input trees but that cannot coexist on the same tree.

Extended Majority-Rule or Greedy consensus

In Extended Majority-Rule, the clades on the consensus tree are determined one at a time, using the most frequently occurring clade first, then accepting the most frequently occurring clade compatible with clades already accepted. Any clade on the

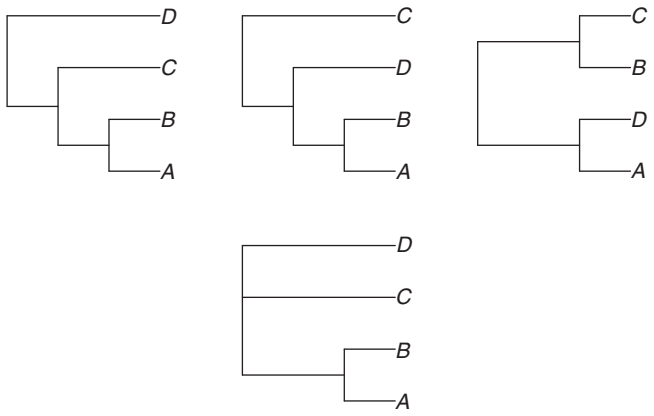

Figure 4 Example with three input trees. If the three trees on the top row are the input trees, then the tree in the bottom row is both the R^* consensus tree and the Majority-Rule tree. The Greedy consensus tree for this example is either of the first two trees in the top row with probability 50%.

Majority-Rule tree will also be on the Extended Majority-Rule tree, but this algorithm also allows the acceptance of clades that occur on less than 50% of the input trees. Because incompatible clades are eliminated, it is also possible for the Extended Majority-Rule tree to have clades that occur less frequently than other, incompatible clades. In the case of ties in frequency, the next clade accepted is either chosen arbitrarily (as is often implemented in software) or one of tied clades is chosen randomly. In practice, software packages might pick one clade arbitrarily (depending on alphabetical order or the input order of taxa in a file) rather than randomly.

Plurality consensus tree

The Plurarity consensus tree modifies the Extended Majority-Rule tree by stopping the algorithm if there are no clades more frequent than a clade that has been eliminated from consideration (Steel and Velasco, 2014). This leads to a tree that can be more resolved than the Majority-Rule consensus tree (and possibly have some clades that occur less than 50% of the time), but can also be less resolved than the Greedy consensus tree.

R^* consensus

The R^* consensus tree is obtained by determining, for each subset of three taxa, which is the most frequent rooted triple (Bryant, 2003). For example, if the taxa are the set X, then for taxa $x,y,z \in X$, if $((x,y),z)$ more often than $((x,z),y)$ and $((y,z),x)$, then we say that the triple $x,y|z$ is the favored rooted triple for that set of taxa. The R^* consensus tree consists of a tree that only displays these favored rooted triples. Some complications of the method are that (1) there might not be a favored triple for some subsets of three taxa due to ties for the most frequent triple, and (2) the set of favored triples might not be compatible. Examples of these cases are shown in Figures 3 and 4, respectively. In the case of ties, the resulting consensus tree should be unresolved with respect to the given triple. Favored triples that are incompatible will also lead to less resolution in the R^* consensus tree. Although the R^* consensus tree is based on the idea of rooted triples, an algorithm for constructing the R^* consensus tree can be based on determining the clades in the final tree on taxa X:

Rule for R^* consensus: The set of taxa $C \subset X$ forms a clade if for all $x,y \in C$ and all $z \in X \backslash C$, $xy|z$ is a favored triple.

The R^* consensus tree is necessarily a resolution of the Majority-Rule tree, meaning that any clade that is on the Majority-Rule tree is also on the R^* tree, even if the R^* tree is not completely resolved.

Q^* consensus

The Q^* consensus tree replaced by quartets is analogous to the R^* consensus with the role of rooted triples being (Bryant, 2003). That is, letting X_1 and X_2 be mutually exclusive subsets of the complete taxon set X with $X_1 \cup X_2 = X$, we can express the rule for Q^* consensus as:

Rule for Q^* consensus: The bipartition $X_1|X_2$ forms a split if for all $x,y \in X_1$ and all $w,z \in X_2$, $x,y|w,z$ is a favored quartet.

Consensus Trees for Maximum Likelihood and Bayesian Phylogenetics

Consensus of Bootstrapped Trees

When evolutionary trees are estimated using maximum likelihood or parsimony, a statistical technique called the bootstrap is often used to quantify uncertainty in the tree estimate (Felsenstein, 1985). The bootstrap resamples columns from the DNA alignment to make new DNA alignments, and evolutionary trees are estimated from these altered datasets. The technique produces one tree for every bootstrap replicate. Typically, between 100 and 1000 bootstrap replicates are used, and the resulting set of trees can be described using a consensus tree. Typically, Extended Majority-Rule, also called Greedy consensus, is used, and the counts of how many times each clade occurred in the final tree are used as a measure of bootstrap support.

Although a consensus tree is often reported from the bootstrap replicates, the resulting consensus tree might have a different topology from the tree inferred to be optimal and is not considered a more reliable estimate than the maximum likelihood or parsimony tree itself. The purpose of reporting the bootstrap estimates is to estimate the uncertainty

associated with particular clades in the inferred tree (Wheeler, 1991; Holder *et al.*, 2008).

Consensus of Estimated Posterior Distribution of Trees

Bayesian estimation of phylogenies usually uses Markov chain Monte Carlo (MCMC), in which trees are sampled in a Markov chain. The set of trees is used as an estimate of the posterior distribution of the tree being estimated. Typically a sample is taken from the trees visited in the MCMC run – for instance sampling every thousandth tree to avoid correlation between trees – and a consensus tree is made from the resulting sample. This consensus tree is then treated as the Bayesian estimate of the species tree, with posterior probabilities being placed on branches of the tree indicating the proportion of times each clade occurred in the sample. In this application, there might be hundreds to thousands of trees used as input for a consensus method. Like the bootstrap consensus tree, the Bayesian consensus tree requires a method that can deal with a lot of variation in the input gene trees. Holder *et al.* (2008) argue that although a Majority-Rule bootstrap consensus tree does not necessarily optimize the likelihood (and so is not preferable to the maximum likelihood tree), the Majority-Rule tree taken from the posterior distribution does minimize the Bayesian posterior expected loss, and therefore is an optimal tree to report for a Bayesian estimate of a phylogeny.

Summarizing bootstrap and posterior distributions of trees to quantify uncertainty in maximum likelihood and Bayesian trees has become one of the most common uses of consensus trees, although this was not an original application of consensus trees.

Median Trees

Median trees are trees that optimize some criterion with respect to set of input trees (Phillips and Warnow, 1996). For example, one might find the tree that minimizes the number of incompatible clades between the optimal tree and the input trees. The number of clades on one tree and not on the other is called the *Robinson–Foulds (RF) distance* (Robinson and Foulds, 1981) or *symmetric difference* between two trees. Thus, finding the tree that minimizes the sum of RF distances between the output tree and input trees is an example of a median tree. Other examples include finding the tree that minimizes the sum of rooted triple distances between the output tree and input trees, where the rooted triple distance is the number of triples on which two trees disagree (Critchlow *et al.*, 1996). The median tree approach extends naturally to unrooted trees using split or quartet distances.

The median tree approach introduces an optimality criterion for the output tree instead of directly computing the consensus tree from a set of input trees. This generally requires some sort of search over the space of possible trees, a problem which is often NP-hard and therefore computationally intensive, although heuristic approaches to get near-optimal solutions are often available that can run in polynomial time (Phillips and Warnow, 1996).

Consensus of Gene Trees to Estimate Species Trees

A natural reason to have multiple trees that need to be combined is when trees are estimated at independent orthologous loci for the same set of taxa. As an example, in a study of 23 species of yeast using 1070 loci, every estimated gene tree had a unique topology (Salichos and Rokas, 2013). There are several reasons that trees estimated at different loci often have different topologies. One reason is that due to short DNA sequences, trees are estimated with some uncertainty and often some degree of error. Interpreting the length of the DNA as analogous to sample size, this creates sampling error that leads to differences in estimated trees at different loci.

However, even if there were no sampling error (i.e., sequences were infinitely long or gene trees were known), there would still be variation in gene tree topologies due to biological causes. Commonly cited causes for such gene tree incongruence include hybridization between closely related species, recombination within loci, gene flow after speciation between non-sister taxa, horizontal gene transfer between non-sister taxa, gene duplication and loss, ancestral population structure, and incomplete lineage sorting.

Methods to combine gene trees into an overall tree for the purpose of estimating a species tree were developed early for the setting of gene duplication and loss (Page and Charleston, 1997). In this problem, a gene makes an additional copy of itself in a genome, and the two copies subsequently diverge in sequence and possibly also in function. The two gene copies are called *paralogs*. An example is the α-hemoglobin and β-hemoglobin genes, which are thought to have diverged several hundred million years ago in verterbrates (Jeffreys *et al.*, 1980; Hardison *et al.*, 2009). Comparing an α-hemoglobin gene from one species and two β-hemoglobin genes from two other species could lead to erroneously grouping taxa.

An early method for combining gene trees to produce an overall estimate of the species tree is called *gene tree parsimony* (Wehe *et al.*, 2008; Chaudhary *et al.*, 2010), where the idea is to find the species tree which minimizes the number of gene duplication events that could explain the discrepancies between the species tree and the gene trees. For this approach, for a given candidate species tree, a score is assigned to each gene tree based on the number of duplication and loss events required to explain the differences in the topologies. The overall score for a species tree is obtained by summing over the score for each gene tree. The species tree with the lowest total score is the preferred score. There is no guarantee that there is a unique minimum parsimony score, and in practice, because the search space is large, the tree that minimizes the parsimony score might have to be estimated with heuristics.

Many methods for estimating species trees from gene tree topologies in the context of incomplete lineage sorting have also been developed. These methods are only sometimes described as consensus methods, yet because they all take gene tree topologies as input and output a single tree, or a set of trees tied for some optimality criterion, they can all be considered consensus methods. An early approach was called *minimizing deep coalescence* (Maddison and Lacey Knowles, 2006; Than and Nakhleh, 2009), which scores the minimum number of times incomplete lineage sorting events must have occurred to explain discrepancies between the candidate species tree and the gene

trees, and an overall parsimony score is summed over gene tree topologies, similarly to the gene tree parsimony approach.

Several other consensus approaches for the incomplete lineage sorting setting have been tried. Properties of traditional consensus methods, where the consensus tree is interpreted as an estimate of the species tree (Degnan *et al.*, 2009), have been investigated. Converting input gene trees to sets of rooted triples and quartets have also been used. Here variations on R^* consensus – Rooted Triple consensus (Ewing *et al.*, 2008) and Q^* consensus – BUCKy (Larget *et al.*, 2010) can be used to combine rooted triples or quartets. Even if only using triples or quartets of the gene trees, branch lengths of the species tree can still be inferred, such as in the methods MP-EST (Liu *et al.*, 2009) and BUCKy (Larget *et al.*, 2010). This is because the frequencies of the most likely triple and quartet depend on the branch lengths in the species tree. Another set of approaches is to convert each gene tree into a distance matrix based on a pairwise distance for each pair of taxa based on how far apart the taxa are on the tree. These approaches are implemented in STAR for rooted trees (Liu *et al.*, 2009) and NJst for unrooted trees (Liu and Yu, 2011). One of the most recent approaches has been a median tree of quartets taken from the input gene trees (Mirarab *et al.*, 2014; Mirarab and Warnow, 2015).

Controversy around Consensus Trees

There has been debate around the use of consensus trees for inferring evolutionary trees. When there are several sources of data, for example both morphological data and DNA data, or DNA data from multiple genes, two strategies emerged: (1) make separate estimates of evolutionary trees on different datasets, then use a consensus tree as an overall estimate, and (2) combine all of the data and make an estimate of the best evolutionary tree to explain all of the data. There have been advocates for both approaches, with the combined data method sometimes being called a *total-evidence* approach (Barrett *et al.*, 1991; De Queiroz, 1993; Eernisse and Kluge, 1993).

The original debate had many papers in the 1990s, at which time gene trees and species trees were often not distinguished. However, the debate has resurfaced in the context of estimated species trees (Song *et al.*, 2012; Gatesy and Springer, 2013; Wu *et al.*, 2013) with some researchers advocating concatenating DNA alignments to form 'supergenes,' which are analyzed as if coming from a single tree topology, although often with partitions to allow different evolutionary processes for different genes (de Queiroz and Gatesy, 2007; Rokas *et al.*, 2003). Other researchers have preferred estimating gene trees separately, and combining these into an estimated species tree (Miyamoto and Fitch, 1995; Liu *et al.*, 2009). Some methods combine elements of both. For example, using multi-locus alignments to infer relationships on subsets of taxa and then using rooted triple or quartet methods (essentially supertree methods) to build the tree (DeGiorgio and Degnan, 2010; Chifman and Kubatko, 2014).

Generalizations: Multilabeled Trees, Supertrees, and Consensus Networks

The concept of a consensus tree has been generalized in many ways. For most applications of phylogenetics, each tip of the tree has a unique label, such as a taxon name. However, in some applications, it is useful to let tip labels not be unique, so that some labels are repeated. Such trees are called MUL-trees. Consensus methods for such trees have also been developed (Lott *et al.*, 2009). A special type of consensus reduces a single MUL tree into a tree with unique labels (Deepak *et al.*, 2013).

Another generalization of consensus methods is to allow different input trees to have different subsets of some larger taxon set. In this case, missing data, due for example, to some genes not being available for some taxa, lead to trees with overlapping but not identical taxon labels. Many methods for combining such trees have been developed, and the combined tree is called a *supertree* (Bininda-Emonds, 2004). Consensus trees are special case of supertrees in which each input tree happens to have the same taxon set. Therefore, a method motivated by the supertree context can be evaluated for its performance in the consensus setting, and this leads to many new potential consensus methods. One of the most popular is called Matrix Representation with Parsimony (Baum, 1992; Ragan, 1992). In this method, gene trees are converted into sequence data by recording, for each clade of the gene tree, which taxa are present or not present. Traditional parsimony, designed to infer trees from sequence data, can then be applied. Supertree methods have also been evaluated for their use in estimating species trees from gene trees (Kupczok *et al.*, 2010; Wang and Degnan, 2011).

A last generalization we mention is that instead of trees, one might have networks as input. Networks are generalizations of trees in which two edges might combine, corresponding to two species merging, in addition to edges splitting to represent speciation. Taking as input a set of networks defined on the same set of taxa, the goal for this problem is to find a network that summarizes the input networks (Holland *et al.*, 2004). A variation on this problem is to take as input a set of trees, and to find the network that best represents the disagreements between the trees (Chen and Wang, 2010; Yu *et al.*, 2011).

See also: Bayesian Phylogenetic Methods. Maximum Likelihood Phylogenetic Inference. Parsimony Methods in Phylogenetics. Phylogenetic Networks. Phylogenetic Tree. Species Trees, Inference of. Supertree Methods, Phylogenetic

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Conservation Biology, Evolution and

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Glossary

Heterozygosity A measure of the genetic variation within individuals in a population; mean observed heterozygosity refers to the proportion of individuals sampled carrying two different alleles at a given loci.

Demographic rates Per capita (per individual) birth and death rates, these determine the rate at which a population grows or declines over time.

Density-dependent population growth When the rate of increase of a population is not a constant, but varies as a function of population size.

Overdominance Also referred to as 'heterozygote advantage'; individuals with two different alleles at a loci have the highest fitness.

Quantitative trait A trait that is the result of the cumulative effects of many genes and the environment; quantitative traits (such as body size, and limb length) vary among individuals such that the range of phenotypes is continuous, rather than falling into discrete categories.

Stochastic Describes a process for which the outcome is determined at least by one random factor.

Introduction

Conservation biology as a discipline is deeply rooted in evolutionary biology. The overarching goal of conservation biology is to protect and maintain biodiversity, and biodiversity spans multiple levels of biological organization, including the diversity of genes, species, communities, and ecosystems. Evolutionary processes determine or influence diversity at all levels in this hierarchy. For example, loss of genetic diversity within a population can decrease population growth rate and persistence (Frankham *et al.*, 2002); genetic diversity within a population can also have important ramifications for community and ecosystem-level processes, such as the strength and stability of species interactions, community structure, primary productivity, and nutrient cycling (e.g., Lankau and Strauss, 2007; see review by Hughes *et al.*, 2008).

Within conservation biology, the subdiscipline of conservation genetics focuses on the application of genetic tools and methods pulled from a range of evolutionary fields (including systematics, phylogeography, population genetics, and increasingly genomics) to conservation problems. Conservation genetics is an active and growing field of study: a peer-reviewed journal of that name has been in print since 2000, in addition to the many papers exploring genetic topics in conservation published in journals with broader conservation or ecological themes. Conservation genetics has also received thoughtful attention in several textbooks (e.g., Groom *et al.*, 2005; Carroll and Fox, 2008); we recommend these sources and others listed at the end of this article for further reading.

Here we briefly discuss some of the areas where evolutionary biology and molecular genetics intersect with conservation biology. We divide our review into two broad topics: first, we consider some of the many ways in which molecular genetic patterns have been used to inform current conservation practice. Then we review the evolutionary processes most important for the persistence of small, demographically threatened populations, and how evolutionary principles might be incorporated into active management plans.

Pattern: What Can Genetic Patterns Tell Us about Biodiversity and Taxa of Conservation Concern?

The use of genetic techniques to answer ecological questions has exploded in frequency over the last decade as molecular techniques have become increasingly powerful, efficient, and inexpensive. Molecular markers such as microsatellites and DNA sequences have provided conservation biologists valuable insight into the basic biology (e.g., mating system, dispersal) and demographic history of species of concern. For example, molecular analyses have been used to illuminate the effect of kinship on social behavior of African elephants (Archie and Chiyo, 2012), and estimate the pre-whaling abundance of gray whales (Alter *et al.*, 2008). Molecular markers have also been utilized for forensic applications, such as identifying the geographic origins of captive chimpanzees (Goldberg, 1997), and determining what species of whale meat is being sold in Japanese markets (Baker and Palumbi, 1996).

Identification of Units of Biodiversity

'DNA barcoding,' the standardized use of short ($\sim$600 bp), highly conserved regions of the genome to distinguish species-level taxonomy (see review by Kress *et al.*, 2015), can be used to identify cryptic species and resolve taxonomic disputes. For example, analysis of the mitochondrial gene encoding cytochrome *c* oxidase subunit 1 (CO1; the most common 'barcode' marker used for animal taxa) revealed that the neotropical skipper butterfly *Astraptes fulgerator* represents a complex of at least 10 well-resolved species (Hebert *et al.*, 2004). Molecular genetic analysis has also been applied extensively to document variation within species, and to identify populations that are sufficiently differentiated that they require separate management. This is the idea behind evolutionarily significant units, or ESUs (Ryder, 1986). ESUs are recognized by the Endangered Species Act and have been broadly embraced by management agencies since first proposed in the mid-1980s, in spite of the fact that there is ongoing debate about the practical

application of the label, and what, exactly, counts as evolutionary significance (see review by Crandall *et al.*, 2000). Of key concern is the relative importance of adaptive divergence versus longstanding reproductive isolation. Neutral or nearly neutral molecular markers (i.e., noncoding DNA) are most often used to evaluate population divergence, and these may not reflect the same patterns as quantitative traits or adaptive differences among populations (McKay and Latta, 2002).

Conservation Planning

Most terrestrial conservation efforts are based upon a framework of protecting habitat; thus an active field of conservation biology (systematic conservation planning) involves developing methods for prioritizing different areas for conservation. These assessments typically use information on species distributions or their proxies (e.g., vegetation type, environmental parameters) and maximize representation of diversity based on different criteria, such as within-site species richness and complementarity of species assemblages, patterns of endemism or restricted-range species, and representation of threatened species. An ongoing challenge is to better incorporate genetic and trait diversity below the species level into conservation assessment. There are theoretical reasons why genetic diversity and species diversity might be broadly correlated across space, since they are influenced by processes that act in parallel at both scales (e.g., connectivity, habitat heterogeneity; see review by Vellend and Geber, 2005). However, empirical evidence thus far is mixed; thus conservation planning based on species-level patterns may not adequately protect within-species patterns of divergence (e.g., Thomassen *et al.*, 2011; Rissler *et al.*, 2006; Moritz, 2002).

Another way that evolutionary biology has been used to inform spatial conservation planning is by incorporating species' evolutionary history into the 'ranking' process. Some species are more important than others for maintenance of diversity from an ecological perspective (e.g., keystone species, ecosystem engineers); other species are more important from an evolutionary perspective, arguably because they represent greater evolutionary depth (e.g., basal taxa), or because they are more evolutionarily labile and thus (may) have higher potential for future diversification (e.g., species-rich clades). A variety of metrics have been proposed to integrate taxonomic diversity into conservation planning (e.g., Steel *et al.*, 2007; Graham and Fine, 2008; Rosauer *et al.*, 2009). The most commonly used metric currently is phylogenetic diversity, or PD (Faith, 1992), which incorporates both the topology of the phylogenetic tree as well as branch lengths for the species in question. A recent example of this approach is the Zoological Society of London's Evolutionarily Distinct and Globally Endangered (EDGE) program, which pairs PD with information on extinction risk, as assessed by the International Union for Conservation of Nature (IUCN) Red List criteria (Isaac *et al.*, 2007).

The primary challenge facing conservation planning is to preserve not just the existing patterns of genetic and species diversity, but also the evolutionary processes that create and maintain these patterns (Moritz, 2002; Hendry *et al.*, 2011; Brooks *et al.*, 2015). Cowling and Pressey (2001) tackle this problem for a major biodiversity hotspot, the Cape Floristic Region in southwestern Africa. They identified key landscape components thought to contribute to the evolutionary process, especially gradients (fine scale, edaphic gradients as well as macrogeographic climatic gradients), and corridors of movement allowing exchange between inland and coastal areas. This example is exceptional in that the Cape Floristic Region is better studied than many other regions of conservation concern; however, the basic message – preserve gradients, species interactions, and dispersal – is broadly applicable.

Process: The Problem of Small Populations

Much of conservation genetics has focused on the challenges facing small, fragmented populations, for obvious reasons. Of central concern is the loss of genetic diversity as populations decline in size, and the resulting interplay between genetic diversity and population demography. Preservation of genetic diversity is considered critical for two reasons. First, genetic diversity within individuals (e.g., heterozygosity) can directly influence individual fitness, and thus have immediate impacts on demographic rates in small, inbred populations. Second, loss of genetic diversity at the scale of the population broadly equates to a loss of evolutionary potential. This idea can be traced back to Fisher's Fundamental Theorem: under selection, the rate of change of mean fitness is equal to the additive genetic variance in fitness (Fisher, 1958). As the environment shifts, small populations may lack the heritable variation in functional traits necessary to respond adaptively.

Adaptive genetic change that allows a declining population to avoid extinction is called 'evolutionary rescue' (reviewed by Gonzalez *et al.*, 2013; Carlson *et al.*, 2014). Both theory (e.g., Holt and Gomulkiewicz, 1997) and increasing experimental evidence (Bell and Gonzalez, 2009) show that the likelihood of evolutionary rescue depends on the direct and interactive effects of three factors: the degree of mismatch between mean phenotype and environment, the supply of genetic variation, and population size. In general, population size is positively correlated with genetic diversity, at least as measured by neutral or nearly neutral molecular markers (Frankham, 1996). Spielman *et al.* (2004) conducted a meta-analysis of 170 taxonomically paired comparisons of threatened and nonthreatened species; they found that in 77% of cases, heterozygosity (measured at microsatellite or allozyme loci) was lower in the threatened species (**Figure 1**). However, Mendelian molecular markers are often a poor predictor of quantitative diversity (Reed and Frankham, 2001), and adaptive variation may be maintained by selection while neutral variation is lost (McKay and Latta, 2002). Empirical data collected thus far suggest that only the smallest populations show reduced evolutionary potential (see review by Willi *et al.*, 2006). Nevertheless, the trajectory of evolutionary rescue is a race with extinction – even if small populations do contain individuals with appropriate traits (or if these appear by mutation or gene flow), small populations will have a more difficult time achieving rescue than larger ones, both because the rate at which adaptation must occur is faster and because stochastic processes (like genetic drift, discussed below) dominate in small populations.

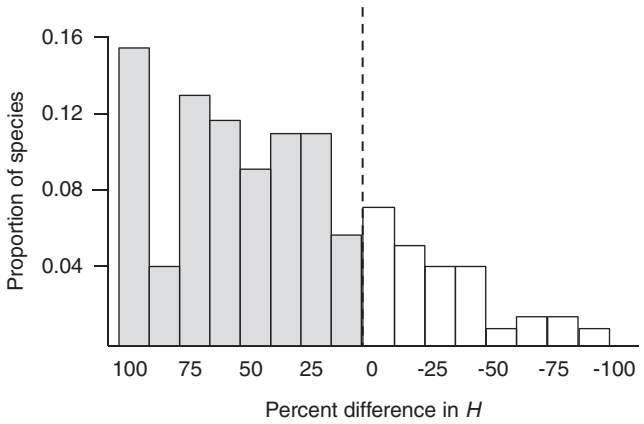

Figure 1 Distribution of percent differences in heterozygosity (*H*) between threatened and taxonomically related non-threatened taxa. Shaded bars (77% of all comparisons) show cases where *H* is lower in the threatened taxa. Data from Spielman, D., Brook, B.W., Frankham, R., 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America 101, 15261–15264.

Genetic Drift

Genetic drift is the change in allele frequencies in a population over time due to random sampling events (e.g., differences among individuals in survival or fecundity that are unrelated to their phenotype/genotype). Although the specific genetic consequences of genetic drift during a given demographic bottleneck are unpredictable, the overall effect of drift is to erode genetic diversity.

Effective population size, or N_e, is a measure of how sensitive a population is to genetic drift. N_e is defined as the size of a hypothetical, theoretically ideal population that would experience the same level of inbreeding, loss of heterozygosity, and genetic drift per generation as the real population in question (Kimura and Crow, 1963). Other factors besides the census size of a population will influence the change in allele frequencies over time (e.g., an uneven sex ratio, past fluctuations in population size, nonrandom variation in family size); by excluding these factors, N_e makes it possible to evaluate and compare measurements of drift across species with very different life histories. There are different ways to empirically estimate N_e over both short- and long-term time scales (see review by Hare *et al.*, 2011), but N_e is virtually always smaller, and often much smaller, than the census size of a population. Frankham (1995) reviewed published estimates of N_e/N for wildlife species, and found that N_e averaged only 10–11% of total census size.

In large (unthreatened) populations, it takes a long time to see a major effect of genetic drift on allele frequencies; genetic diversity represents a balance between mutation and natural selection. However, when $N_e s \ll 1$, where s is the selection coefficient describing the difference in fitness between two alleles, drift can counter selection, and the alleles will behave as if they are neutral (Wright, 1931). Thus through this mechanism, small populations may show greater maladaptation (i.e., mismatch between environment and mean phenotype) than larger ones. By similar logic, mildly deleterious mutations will tend to accumulate in small populations, because selection is ineffective at removing them. This can lead to 'mutational meltdown': as deleterious mutations become fixed, they drive down population growth rate (and size), making the population progressively more susceptible to fixation of future mutations (Lynch *et al.*, 1995).

Inbreeding and Inbreeding Depression

Small populations are often subject to inbreeding, or the mating of closely related individuals. Inbreeding per se does not affect allele frequencies; however, because closely related individuals are likely to share alleles that are identical by descent, this increases the likelihood that progeny will inherit the same allele from both parents. Thus inbreeding causes a decline in mean heterozygosity. This in turn can lead to inbreeding depression, typically defined as the relative reduction in fitness of inbred progeny compared to outbred progeny. This fitness differential varies with environment: the expression of inbreeding depression is often greatest in physiologically stressful environments (reviewed by Reed *et al.*, 2012). For example, inbreed white-footed mice (*Peromyscus leucopus*) displayed equivalent survival to outbreed mice in the lab but significantly reduced survival when released into the wild (Jimenez *et al.*, 1994). Similarly, Keller *et al.* (1994) showed that a severe winter selected against relatively inbred individuals in a wild population of song sparrows (*Melospiza melodia*).

Inbreeding depression can be caused by several different genetic mechanisms: the expression of deleterious recessive alleles (i.e., alleles that were 'masked' in the heterozygous state, but expressed in homozygotes), or the loss of overdominance at loci where heterozygotes have the highest fitness. Reduction in fitness caused by the first mechanism can be alleviated over time by selection, assuming that inbreeding occurs gradually and selection against homozygous recessive individuals is not overwhelmed by genetic drift. This process is referred to as purging the genetic load. Support for the role of purging in declining populations is mixed; some reviews suggest that purging only occurs in some cases and does not completely remove fitness costs associated with inbreeding (Ballou, 1997; Byers and Waller, 1999), which underscores the

need to better understand the genetic mechanism of inbreeding depression across taxa (Crnokrak and Barrett, 2002).

Interplay between Genetic versus Nongenetic Processes Affecting Demography

In addition to genetic drift and inbreeding, there are also critical nongenetic processes that influence the viability and persistence of small populations. For example, small populations may be subject to positive density-dependent population growth (called an Allee effect, or depensation in fisheries science). Any mechanism that causes a particular component of individual fitness (e.g., individual survival, growth rate or fecundity) to increase with increasing population size or density is considered a 'component Allee effect'; if the net result of all component Allee effect (plus possible compensatory changes in other components of fitness) is an increase in population growth rate, this is a 'demographic Allee effect' (Stephens *et al.*, 1999). The three most commonly reported mechanisms leading to an Allee effect are mate limitation, cooperative defense behavior, and predator satiation (see review by Kramer *et al.*, 2009). For example, groups of bighorn sheep (*Ovis canadensis*) smaller than five individuals show reduced group vigilance in spite of the fact that individuals in these small groups increase their vigilance behavior; further, the per capita risk of predation is greater in small groups if a predator attack does occur (Mooring *et al.*, 2004).

Small populations are also vulnerable to environmental and demographic stochasticity. Year-to-year variation in population growth rate, as well as larger environmental perturbations ('catastrophes'; e.g., storms, fires, etc.), will have a greater impact on small populations than larger ones, simply because larger populations have a greater numerical buffer between the number of individuals at any given time and extinction. Demographic stochasticity is the variation in realized population growth rate that occurs even when the underlying vital rates (e.g., birth and death rates) that determine population growth are constant – the result of applying a rate to real (whole) numbers. Like genetic drift, the relative magnitude of this effect increases as population size declines.

The relative importance of genetic versus nongenetic factors for population persistence has been debated since the inception of conservation genetics (see review by Brook, 2008). Demographic processes are often thought to impact small populations before genetic effects become important; this idea dates back to an influential paper by Lande (1988). However, there is no doubt that inbreeding depression can be an important cause of decline for wild populations (Keller and Waller, 2002). A direct link between inbreeding and extinction risk has been shown in experimental populations of *Drosophila* (Wright *et al.*, 2008), the whitefly *Bemisia tabaci* (Hufbauer *et al.*, 2013), the plant *Clarkia pulchella* (Newman and Pilson, 1997), and in both experimental and wild populations of the Glanville fritillary butterfly *Melitaea cinixi* (Saccheri *et al.*, 1998; Nieminen *et al.*, 2001). Further, the processes described above do not act in isolation: any negative consequences of inbreeding for population growth rate will have implications for all other processes mediated by population size. Gilpin and Soule (1986) first coined the term 'extinction vortex' to describe the sequence of events and synergy between direct and indirect (genetic) demographic consequences of small population size (**Figure 2**). If an ecological impact (e.g., harvest, pollution, or an invasive species) reduces population size, this exposes the population to demographic stochasticity and possible Allee effects; these in turn reduce population growth rate and size, increasing the likelihood of inbreeding and the magnitude of genetic drift, further reducing population growth rate and size.

Dispersal and Gene Flow

One of the fundamental processes that affects population dynamics and persistence is the movement of individuals (dispersal) and their genes (gene flow) from one population to another. This movement may either help or hinder the recovery of threatened populations, depending on the particular circumstances. First consider dispersal alone: on the

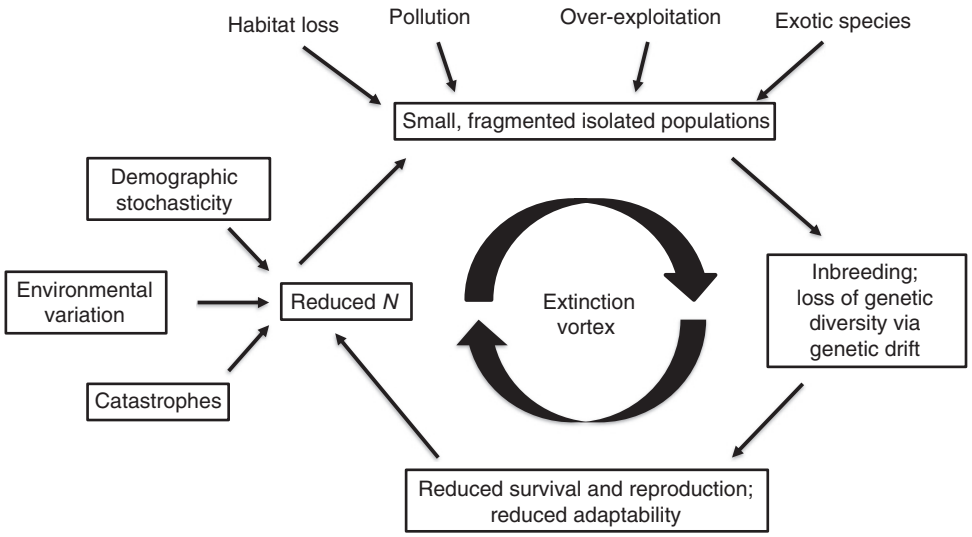

Figure 2 The interplay between genetic and demographic consequences of small population size can lead to an 'extinction vortex.' Reproduced from Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge: Cambrige University Press.

one hand, the movement of individuals into a declining population serves to pump up population size, and thus can reduce the impact of Allee affects, demographic stochasticity, and genetic drift. Thus, dispersal can have a positive impact on adaptive evolution and population persistence, even in the absence of corresponding gene flow, by potentially maintaining a population in decline (i.e., a demographic sink) long enough for adaptation to occur (Holt *et al.*, 2003). However, in theory, dispersal could also increase population size past its local carrying capacity, thus reducing the fitness of all genotypes; this would make it harder for a new beneficial allele to increase in frequency (Gomulkiewicz *et al.*, 1999).

The effect of gene flow on population dynamics and adaptation is equally complicated (see review by Garant *et al.*, 2007). Gene flow typically acts to increase the genetic diversity within populations; if gene flow increases additive genetic variation for ecologically relevant traits, this can increase the adaptive potential of small populations. New alleles brought in via gene flow can alleviate inbreeding depression and cause population growth rate to rebound – this phenomenon is called genetic rescue (reviews by Tallmon *et al.*, 2004; Whiteley *et al.*, 2015). However, gene flow is not a panacea: gene flow can increase within-population diversity, but it homogenizes genetic differences among populations – if these differences are adaptive, then gene flow will have a negative effect on mean fitness. This is called outbreeding depression, defined as a reduction in fitness of progeny produced by the matings of genetically divergent individuals. When gene flow links populations or groups of individuals experiencing different selection pressures, gene flow can counter the effect of natural selection on allele frequencies, and 'swamp' local adaptation. Outbreeding depression may also occur even if populations have experienced similar selective environments, if the populations have evolved different genetic 'solutions' involving multiple interacting loci (i.e., epistasis). Gene flow between populations can break up these coadapted gene complexes, leading to low fitness in the progeny.

There have been several high profile examples of successful genetic rescue in the last decade, perhaps the most famous of which is the Florida panther (*Puma concolor coryi*). The Florida panther is the last surviving subspecies of the puma in eastern North America. By the early 1990s, Florida panthers were reduced to a single population of ~25 individuals suffering from physical complications linked to inbreeding, including heart defects, high loads of infectious diseases, kinked tails, and adult males with low sperm quality and undescended testes (Johnson *et al.*, 2010). After eight female panthers were translocated from Texas into Southern Florida, the frequency of these traits declined, the population tripled in size, and population growth rate went from 5% annual decline to 4% growth (Benson *et al.*, 2011).

There are far fewer empirical examples of outbreeding depression than of inbreeding depression, but the fitness consequences for declining populations can be equally damaging (review by Edmands, 2007); thus active translocation of individuals by humans, or 'assisted gene flow' (Aitken and Whitlock, 2013), must be approached with some caution. This is one of several areas in which genomic methods (i.e., high-throughput, massively parallel sequencing) offer tremendous promise for conservation. Genomics allows the screening of thousands of markers across the entire genome, providing a powerful tool for exploring the genetics of adaptation (Ouborg *et al.*, 2010; Harrisson *et al.*, 2014). Genomic methods can be used to select appropriate source populations for assisted gene flow, and even to identify individuals within those populations that are likely to have the greatest 'rescuing' effect, either because they have highest genome-wide genetic diversity, or because they possess particular alleles associated with fitness (Whiteley *et al.*, 2015).

Concluding Thoughts

There is now broad recognition that the distinction between so-called ecological time scales (i.e., days to years) and evolutionary time scales (i.e., thousands of years) is a false one – microevolutionary changes can occur rapidly, within human lifespans. Thus, effective conservation must consider both the evolutionary and ecological consequences of any management action or inaction (Ashley *et al.*, 2003; Stockwell *et al.*, 2003). Latta (2008) points out two ironies that challenge our ability to control our evolutionary impact on nature: first, humans select against the very traits that we most value in the wild species that we harvest. For example, fisheries typically select against the largest and fastest-growing individuals, removing those genotypes from the gene pool (Biro and Post, 2008), just as trophy hunting selects against the traits that hunters most prize (Coltman *et al.*, 2003). The second irony is that the species and populations we often wish would remain static (such as insect pests, weedy plants, and pathogens) usually have the greatest potential to evolve resistance to our actions, while the species we hope would adapt to environmental change (i.e., rare, endangered taxa) are least likely to be able to do so. Thus the challenge facing conservation biology is to find ways to proactively manipulate evolutionary processes to achieve conservation goals. To thwart unintended anthropogenic selection, this might mean establishing reserves to act as reservoirs of particular genotypes (e.g., Baskett *et al.*, 2005); to promote adaptation to climate change, this could mean translocating more drought- or heat-tolerant genotypes from a lower latitude to a higher one (Aitken and Whitlock, 2013). Applied evolution has been successfully used in agriculture and medicine; although there are inherent risks associated with attempting to harness and shape evolutionary processes, the need for applied or prescriptive evolution in conservation and resource management has never been greater (see reviews by Hendry *et al.*, 2011; Lankau *et al.*, 2011; Smith *et al.*, 2014).

See also: Biogeography, Conservation. Conservation Biology, Quantitative Genetics in. Invasive Species, Evolution and. Responses to Climate Change, Evolution and

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Conservation Biology, Quantitative Genetics in

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Glossary

Effective population size (N_e) The number of individuals required to account for the actual rate of increase in inbreeding or genetic drift in a real population. Real populations violate the assumptions of the models used to predict the rate of increase in inbreeding or genetic drift, and therefore N_e will differ from the actual population size (N).

Inbreeding depression Change in the mean of a quantitative trait because of inbreeding. It implies a deterioration of fitness traits.

Mutational meltdown Accelerated decay in population size driven by fixation of deleterious mutations occurring in small populations, where genetic drift overcomes the purifying effect of natural selection. The decrease in population size exacerbates the power of genetic drift over that of selection, further increasing the fixation of deleterious alleles in a recurrent cycle leading the population toward extinction.

Nondirectional dominance Situation where the alleles with decreasing (or increasing) effect on the trait have not a tendency to be either recessive or dominant. Fitness traits usually show directional dominance in the sense that alleles decreasing fitness tend to be recessive.

Outbreeding depression Decline in mean population fitness due to crossing of individuals from populations adapted to different local conditions.

Purging Reduction in frequency of deleterious mutations due to selection against recessive effects that are expressed because of inbreeding.

Q_{ST} Index of quantitative genetic differentiation between populations, analogous to Wright´s fixation index, F_{ST}.

Recessive lethal equivalent A group of deleterious mutations that, combined additively, account for the same inbreeding depression as a single recessive lethal. Its average number is typically estimated from the regression slope of fitness measures on the inbreeding coefficient.

Synergistic (reinforcing) epistasis Type of gene interaction in which the joint effect on a quantitative trait of the alleles of two (or more) loci is higher than the sum of the individual effects of the corresponding alleles. The contrasting type, or antagonistic epistasis, results when the joint effect of the alleles is lower than the sum of their individual allelic effects.

Introduction

The maintenance of biodiversity is one of the main worries of our societies, as the extinction of species continues at a dramatic rate and the number of those requiring human intervention to secure their viability is becoming increasingly large (Frankham *et al.*, 2010; Allendorf *et al.*, 2013). Conservation biology is not only focused on wild animal and plant species, but also on rare animal breeds associated with local production systems of cultural interest or supplying quality products of economic demand (Oldenbroek, 2007). Environmental deterioration and pollution, human structural developments, commercial overexploitation, and introduction of exotic species are some of the main factors responsible for the increasing rates of biodiversity loss.

The vast majority of traits related to the survival and adaptation of species, as well as those pertaining to production systems of economic value, are quantitative traits. Thus, most of the main concerns associated with endangered species and conservation programs are related to quantitative trait issues (Frankham, 1999). Unfortunately, estimation of genetic components of variation for quantitative traits is usually difficult, except in controlled situations where genealogical information or genotypic data from molecular markers is available, such as in the main commercial animal breeds and plant strains, some wild species where genetic monitoring is undertaken, or some plant species where common garden experiments can be carried out with relative ease.

Species or breeds at the brink of extinction, or those which are threatened or require human intervention, are almost invariably characterized by permanent or temporal low census sizes, geographical fragmentation, and high levels of isolation. The main genetic consequences of these factors are (Allendorf *et al.*, 2013): (1) The loss of genetic variation through the loss of alleles by genetic drift. For quantitative traits, this implies a reduced potential of populations for adaptation to new environmental threats, such as climate change or competition with introduced foreign species. (2) The increase in inbreeding rates, leading to inbreeding depression, the deterioration of fitness traits because of inbreeding. (3) Finally, for *ex-situ* conservation programs focused on the maintenance of genetic variation under captive conditions, the adverse collateral effects on future survival, due to adaptation to captivity and relaxation of natural selection, with possible consequences on the genetic architecture of quantitative traits. These aspects are explained more deeply in what follows.

Fitness Decline in Small Populations

One of the most important consequences of the small population sizes of endangered species is the decline in the mean of quantitative traits associated with fitness (such as viability, fecundity, or mating success). This decline occurs through two highly related processes. First, inbreeding depression, which is mostly due to the expression of recessive deleterious

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mutations in homozygosis as a consequence of the unavoidable mating of relatives (Charlesworth and Willis, 2009). Second, the eventual random fixation of deleterious mutations, because of the prevalence of random genetic drift over natural selection. This latter process, called mutational meltdown (Lynch *et al.*, 1995), implies an increasing accumulation of fitness-reducing mutations which further lowers the population size in a recurrent cycle leading the population toward extinction. Note that although the two above processes are a consequence of inbred mating and low population sizes, they can be considered separately. Inbreeding depression regards to the concealed deleterious genetic load (that due to recessive deleterious mutations segregating in heterozygous condition), whereas mutation meltdown refers to the eventual fixation of deleterious mutations and these, once fixed, do not contribute to inbreeding depression. In addition, additive deleterious mutations contribute to mutation meltdown but not to inbreeding depression. The magnitude of these processes depend on the rate, mean effect, dominance, and distribution of effects of deleterious mutations, becoming generally enhanced in stressful environments (Reed *et al.*, 2012).

The estimations of the deleterious mutation rate and mutational effects arise from mutation-accumulation experiments or molecular-based inferences. Mutation-accumulation experiments have been carried out in a number of species using populations of small census size, initially devoid of genetic variation, where natural selection is minimized to allow random fixation of deleterious mutations (Mukai, 1964). Estimates of the genomic haploid mutation rate from these experiments vary largely between species but the mean across 31 estimates reviewed by Halligan and Keightley (2009) in higher eukaryotic species is 0.08 (median of 0.04), with a mean deleterious homozygous effect on fitness traits of 0.22 (median of 0.16). This suggests that mutations detected in mutation-accumulation experiments are relatively rare and have very substantial effects on fitness. Different lines of evidence indicate that the majority of these mutations show partial recessivity, with an average heterozygous effect of about 20% of the homozygous effect (García-Dorado and Caballero, 2000).

The necessarily short temporal scale of mutation-accumulation experiments, the lack of power associated with phenotypic measures of fitness components and the problems of finding appropriate non-mutated control lines imply that these experiments are only able to detect mutations of relatively large effect. Analyses of sequence data making assumptions about the fraction of the genome constrained by selection yield much higher estimates of the genomic mutation rate. For example, the estimated haploid genome-wide deleterious mutation rate using this approach is as large as 0.6 in *Drosophila* and 1.1 in humans, with a distribution of effects dominated by large-effect mutations (Halligan and Keightley, 2009; Keightley, 2012). The discrepancy between both kinds of estimates is likely to be that most deleterious mutations accounted by molecular-based approaches have tiny effects which are relevant in evolutionary timescales but cannot be detected in mutation-accumulation experiments (García-Dorado *et al.*, 2004).

From a conservation point of view, where the timescale of the pertinent programs is usually short (dozens or hundreds of generations), the relevant deleterious mutations would be those with mild to severe effects and substantial recessivity, i.e., those typically detected in mutation-accumulation experiments. These mutations will rarely cause mutational meltdown but, in large populations, can account for substantial genetic load due to recessive effects concealed in heterozygotes. Therefore, they are responsible for inbreeding depression after a reduction in population size. This is one of the main issues in conservation biology, as inbreeding depression is virtually ubiquitous in endangered and captive species. In fact, in a survey of 119 zoo populations, Boakes *et al.* (2007) found that inbreeding depression for neonatal survival was significant across all populations, although its extent varied widely among taxa.

On the other hand, inbreeding depression can be opposed by purging, as inbreeding produces homozygous individuals in which recessive deleterious effects are exposed to natural selection (see review by Leberg and Firmin, 2008). It has been theoretically shown that purging can restrain to a large extent the decline in fitness in moderate size populations (García-Dorado, 2012). For example, simulations assuming a model with 0.05 partially recessive deleterious mutations per haploid genome per generation, with average effect 0.2 in homozygosis (mean effect of 0.04 in heterozygotes), show a decline in fitness of only about 10% in a 100 generation period for populations of size $N=10$, instead of the 40% decline expected in the absence of purging (**Figure 1**; Pérez-Figueroa *et al.*, 2009). Purging, however, is not too evident in captive populations, and in the survey of Boakes *et al.* (2007) it was significant in some of the populations studied but produced an average increase in fitness lower than 1%. However, it must be kept in mind that zoo populations may have been subjected to previous substantial purging, whose consequences pass undetected in this type of studies. Deliberate inbreeding has been proposed as a way to eliminate deleterious recessive mutations, but the results from Boakes *et al.* (2007) suggest

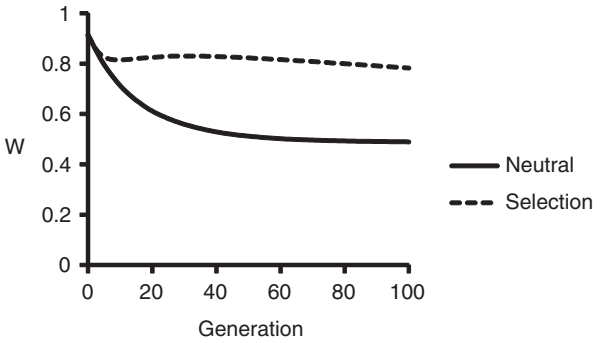

Figure 1 Simulated mean change in fitness (W) in a population of census size $N=10$, assuming deleterious mutations appearing at a rate of 0.05 mutations per haploid genome and generation with effects obtained from an exponential distribution with homozygous mean effect 0.2 and heterozygous effects, negatively correlated with homozygous effects, and mean 0.04. The continuous line assumes a model without selection, whereas the broken line assumes purging selection. Data from Pérez-Figueroa, A., Caballero, A., García-Dorado, A., López-Fanjul, C., 2009. The action of purifying selection, mutation and drift on fitness epistatic systems. Genetics 183, 299–313.

that the efficiency of this procedure is very questionable (see also de Cara *et al.*, 2013).

Consequences of Bottlenecking and Fragmentation on the Genetic Variance for Quantitative Traits

Endangered populations are characterized by permanent low census sizes or frequent populations shrinks (bottlenecks) and population fragmentation. Immediate consequences are the loss of genetic variation and the increase in between-population differentiation. Under neutrality, the contribution of additive loci to the additive variance of a quantitative trait after bottlenecks is expected to decline linearly with the inbreeding coefficient (F) (Wright, 1951), but that of dominant or epistatic loci may initially increase until a critical F value is reached, followed by a final decline (Robertson, 1952; López-Fanjul *et al.*, 2002). Thus, it has been suggested that an excess of additive variance after some inbreeding may enhance the adaptive potential of populations through an assumed conversion of nonadditive into additive variance (Wade and Goodnight, 1998). Laboratory experiments indicate that an increase in additive variance after bottlenecks was small or absent for morphological traits but large for life-history traits (Van Buskirk and Willi, 2006; Taft and Roff, 2012). Both results are compatible with the general assumption of morphological traits being controlled by genes with little or nondirectional dominance (i.e., where the alleles with decreasing, or increasing, effect on the trait do not show a tendency either to be recessive or to be dominant) and showing no inbreeding depression, and fitness component traits with an additive variance determined by deleterious recessive alleles segregating at low frequencies and strongly depressed by inbreeding.

Neutral theoretical models predict that the contribution of epistasis to the increase in genetic variance after bottlenecks can be substantial (Wade and Goodnight, 1998). However, simulations accounting for purifying natural selection strongly constrain the boost in additive variance for fitness, mainly through purging of deleterious recessive alleles, rendering small increases in variance after bottlenecks with or without epistasis (Pérez-Figueroa *et al.*, 2009). Thus, whereas synergistic (reinforcing) epistasis for fitness implies an excess of increase in variance with bottlenecks over that expected without epistasis (due just to dominance; Robertson, 1952; black lines in **Figure 2**), purging selection reduces considerably the magnitude of the increase in additive variance and almost completely removes the excess due to epistasis (red lines). On the other hand, for metric traits subject to stabilizing selection, an excess in additive variance following bottlenecks could not be attributed to simple dominance and was only observed when synergistic epistasis was present, although its magnitude was again considerably constrained by selection (Ávila *et al.*, 2014). Thus, for both selection regimes, a drift-induced excess in additive variance is unlikely to produce a net increase in the adaptive potential of populations. In addition, observed increases in additive variance could not be attributed to a depletion of nonadditive variance through a conversion process.

Fragmentation of populations and further isolation is usually an undesirable phenomenon from the conservation point of view. However, between-group differentiation for quantitative traits can also be considered a desirable property in some circumstances. For example, it has been reckoned that about half of the total genetic variation of importance in animal breeds is between-breed variation (Oldenbroek, 2007). This variation could become useful if livestock production needs to be quickly adapted to new challenges, and conserving breeds with particular characteristics is a rational and important strategic objective. In the wild species context, the possibility of a given subpopulation to adapt to a given environmental change may largely depend on the possibility of receiving rare advantageous alleles by migration from other subpopulations (Blanquart and Gandon, 2011). In fact, quantitative genetics theory predicts that, at low levels of inbreeding, the total amount of genetic variance in a subdivided population is $(1 + F_{ST})$ times larger than that of an undivided population (Wright, 1969), where F_{ST}, essentially a scaled measure of the variance in allele frequency differences among subpopulations, is a measure of the level of differentiation among them. Thus, a certain differentiation among subpopulations might be considered as a way of keeping reservoirs of variation.

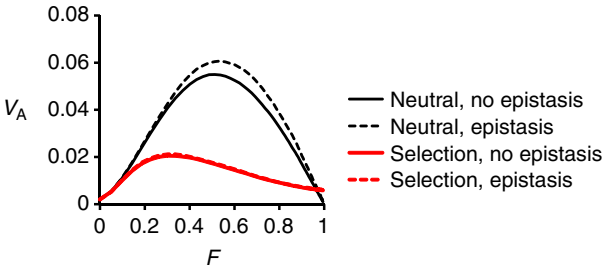

Figure 2 Simulated change in additive genetic variance (V_A) after consecutive bottlenecks of size $N = 10$ during 100 generations, plotted against the inbreeding coefficient. The initial bottlenecked population is taken from a large base population at mutation-selection-drift equilibrium where deleterious mutations appear at a rate of 0.05 mutations per haploid genome and generation with homozygous effects obtained from an exponential distribution with mean 0.2. Heterozygous effects have mean 0.04 and are negatively correlated with homozygous effects. The continuous lines assume non-epistatic gene action and the broken lines assume strong synergistic epistasis between pairs of loci. Black lines assume a model without selection, whereas red lines assume purging selection. Data from Pérez-Figueroa, A., Caballero, A., García-Dorado, A., López-Fanjul, C., 2009. The action of purifying selection, mutation and drift on fitness epistatic systems. Genetics 183, 299–313.

Minimum Viable Population Size

A question of utmost interest in conservation biology is the minimum effective size enabling a population to persist over a certain time under the threats of inbreeding depression and loss of variation by genetic drift. There are several arguments to answer this question based on quantitative genetic reasoning (reviewed by Frankham *et al.*, 2014). A first issue is the minimum viable population size needed for persistence in the short term. Because typical animal breeding programs aim to a

maximum rate of increase in inbreeding ($\Delta F = 1/2N_e$, where N_e is the effective population size; Wright, 1931) about 1% per generation, a minimum value of $N_e = 50$ is deduced (Soulé, 1980; Franklin, 1980). Frankham *et al.* (2014) suggest a more specific proposal. Considering a maximum decline in fitness of 10% over a short-term period of five generations, and assuming a total genetic load for vertebrates of about six recessive lethal equivalents, the necessary N_e would be 142. Following this argument, Frankham *et al.* (2014) suggest a minimum viable population size of at least $N_e = 100$ for minimizing inbreeding depression in the short term.

The second issue is to determine the minimum viable effective population size sufficient to retain evolutionary potential in the long term. Assuming a mutation-drift model of variation, the equilibrium additive genetic variance (V_A) for a quantitative trait is about $V_A = 2N_eV_M$ (Lynch and Hill, 1986), where V_M is the mutational variance, i.e., the input of per-generation additive variance due to neutral mutation. Because the mutational variance takes values around $10^{-3}\ V_E$, where V_E is the environmental variance, this means that for a typical quantitative trait of heritability $h^2 = 0.5$ (so that $V_A = V_E$), the critical effective size must be around 500 individuals (Franklin, 1980; Lynch and Lande, 1998). The incorporation of selection to the model indicates that it should be at least doubled (Frankham *et al.*, 2014), although it also shows that, at some point, selection becomes the factor limiting genetic variance, so that no further increase in V_A is expected by further increases in N_e (Amador *et al.* 2010). Simulations considering other factors such as the mutational meltdown process explained above have also concluded that populations of N_e at least 1000 individuals are necessary to guarantee population survival for periods of about 100 years (Lynch *et al.*, 1995; Lynch and Lande, 1998).

For captive breeding programs, it has been proposed that the minimum long-term viable population size should be that required to maintain at least 90% of the genetic diversity during a period of 100 years (Frankham *et al.*, 2010). A review of 188 published studies of *ex-situ* conservation programs where different estimates of variation are available (most from neutral markers but also from studbook data) concludes that to accomplish this criterion a minimum number of 15 founders and a minimum size of the captive population of 100 individuals are sufficient (Witzenberger and Hochkirch, 2011).

The above theoretical arguments suggest that minimum viable effective population sizes should be of the order of 100 to avoid a substantial impact of inbreeding depression in the short term and of 1000 to avoid a substantial loss of variation and fitness in the long run. Because the ratio between the effective size, estimated with demographic or molecular marker data, and the census size has been found to be about 10–20% (Frankham, 1995; Palstra and Fraser, 2012), this would imply that viable populations should include a number of breeding individuals, an order of magnitude higher than their N_e estimates. However, the considerable variation across populations and species for the ratio between effective size and census size questions the applicability of this relationship in a general way (Jamieson and Allendorf, 2012). The above arguments are also constrained by the limitations of the model assumed and ignore many other factors such as demographic stochasticity and the structure of the populations. Population viability analyses (see meta-analysis by Traill *et al.*, 2007) may consider these and other factors to provide more specific recommendations for a given probability of persistence over a specified period of time. Frankham *et al.* (2014) propose to standardize a 99% probability of persistence for 40 generations for a typical outbreeding species. Nevertheless, the recommended minimum viable population sizes should be taken only as rough indications, as extinction risks may arise from a complex interaction of factors and the uncertainty and contingency of the data may impede proposing any universally applied figure (Flather *et al.*, 2011). Furthermore, since purging operates on the basis of previously accumulated inbreeding, it should be taken into account to predict the expected declines in the medium-long term, when it may restrain fitness losses to a large extent (Pérez-Figueroa *et al.*, 2009; García-Dorado, 2012; see **Figure 1**).

Neutral Molecular Variation as a Surrogate or Complementation to Quantitative Genetic Variation

Theoretical studies show that estimates of quantitative genetic variation can be more informative than molecular markers to detect human-induced impacts on genetic diversity, particularly when these involve changes in environmental variance, shifts in local optimal conditions due to environmental contaminants, or fluctuations in the migration rate between populations adapted to different optima (Carvajal-Rodríguez *et al.*, 2005). However, because estimating genetic components of variation for quantitative traits is generally difficult or even unfeasible, estimates from neutral molecular markers of gene diversity (expected heterozygosity, H; Nei, 1987) and allelic richness (El Mousadik and Petit, 1996) are used as surrogates of heritability and additive genetic variance. Likewise, gene frequency differentiation (F_{ST}; Wright, 1951) and its derivatives such as G_{ST} (Nei, 1987) or allelic type differentiation statistics (El Mousadik and Petit, 1996; Jost, 2008; Caballero and Rodríguez-Ramilo, 2010) are used as proxies for quantitative trait genetic differentiation, usually quantified by the statistic Q_{ST} (Spitze, 1993). This is defined as $Q_{ST} = V_B/(V_B + 2V_W)$, where V_W and V_B are the additive within- and between-population components of the genetic variance for the quantitative trait. As in many situations, such as those for most endangered species, genetic components of variance cannot be obtained and a proxy of Q_{ST} (called P_{ST}) has been proposed, based on using phenotypic rather than genetic variance components and making assumptions about the proportion of the total phenotypic variance due to additive genetic effects. This method is obviously not recommended, because phenotypic plasticity is very common (Pujol *et al.*, 2008), but should not be completely dismissed as a first approximation in some instances (Brommer, 2011).

The comparison between statistics of gene frequency differentiation and quantitative genetic differentiation has useful applications in conservation biology. For example, because F_{ST} for neutral markers should be approximately equal to Q_{ST} for neutral additive traits, the comparison between both statistics gives information on the presence of divergent selection for the pertinent quantitative trait among populations, implying

$Q_{ST} > F_{ST}$ (see e.g., Kremer and Le Corre, 2012). Uniform selection across populations ($Q_{ST} < F_{ST}$) is more difficult to prove, as both dominance and epistatic gene action usually imply $Q_{ST} < F_{ST}$ even in the absence of selection (López-Fanjul *et al.*, 2003). The empirical evidence indicates that Q_{ST} is generally larger than F_{ST} (Leinonen *et al.*, 2013), which suggests that a considerable part of the observed population divergence for quantitative traits should be attributed to differential selection pressures imposed by local environmental conditions. This has important implications in conservation management as hybridization of different populations may cause outbreeding depression (Edmands, 2007), i.e., the decline in fitness when highly different populations are mixed, arguing against forced migration policies.

The empirical relationship between molecular variability and morphological, behavioral, or life-history variability seems to be generally low to moderate. The mean correlation between molecular heterozygosities and estimates of genetic variation for a range of quantitative traits across 71 studies was about 0.22 (Reed and Frankham, 2001), whereas the correlation between variation at neutral markers and fitness traits was 0.43 in another meta-analysis of 34 studies (Reed and Frankham, 2003). Moreover, the relationship between individual genetic diversity and fitness-related traits (heterozygosity-fitness correlation) is generally low (Chapman *et al.*, 2009), although can be relevant in small populations or those recently affected by bottlenecks (Szulkin *et al.*, 2010). However, direct associations between neutral marker variation and adaptive changes for quantitative traits have been found experimentally. For example, Santos *et al.* (2012) found significant associations between variation for nine microsatellite markers and changes in life-history traits as a result of adaptation to laboratory conditions for 21 generations of six populations sampled from two natural locations. In addition, simulation studies have shown that neutral molecular markers are reasonable predictors of the global genetic potential for adaptation of subdivided populations. In particular, gene frequency measures of molecular variability are good predictors of short-term response to selection for quantitative traits, whereas allelic number measures are better predictors of long-term and total response (Caballero and García-Dorado, 2013). This is in concordant with theoretical arguments which suggest that short-term response to selection mostly depends on heterozygosity, whereas long-term selection is highly dependent on the number of alleles (Allendorf 1986; Allendorf *et al.*, 2013), as deduced from the fact that population bottlenecking, which has a large impact on allelic diversity, constrains the response to selection (James, 1970). Simulation and empirical data with *Drosophila melanogaster* confirms that total selection response for quantitative traits is higher for populations where the number of alleles for molecular markers have been maximized than for analogous populations where the marker heterozygosity has been maximized (**Figure 3**; Vilas *et al.*, unpublished).

The possibility of applying next-generation sequencing techniques to an increasing number of non-model species is enabling a huge improvement in the precision of genetic estimates, the assessment of the levels of introgression and differentiation among populations, the identification of regions that have been subject to selection, and the mapping of genetic variants for important traits through Genome Wide Association Studies (Steiner *et al.*, 2013). Thus, fine scale analyses from conservation genomics are opening a large number of possibilities for improvement of conservation programs.

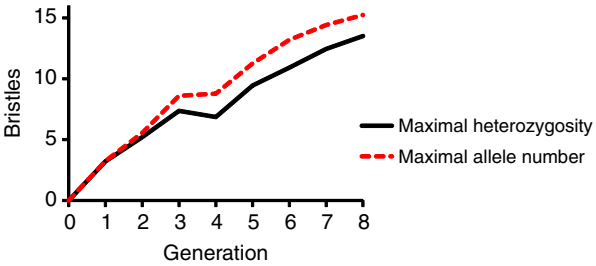

Figure 3 Divergence between the means of lines selected for increased and decreased sternopleural bristle number during eight generations. The two scenarios differ in that the starting population was obtained maximizing the expected heterozygosity for nine microsatellite markers (continuous black line), or maximizing the number of alleles for the same markers (broken red line). In each generation truncation selection was performed in which 50 individuals of each sex were scored and the 10 individuals of each sex with the highest or the lowest bristle number were used as breeding individuals. Data from Vilas *et al.*, unpublished.

Implications of Conservation Management on Quantitative Trait Variation

From a genetic perspective, the objective of any conservation program is to maintain the largest possible levels of genetic variation in the populations so as to keep their evolutionary potential, to avoid inbreeding depression and to restrict adaptation to captivity to a minimum (Frankham *et al.*, 2010; Allendorf *et al.*, 2013). For some captive species or plant reservoirs in *ex-situ* conservation programs, manipulation of breeding schemes can be at least partially undertaken and simple strategies followed to maintain genetic diversity. These strategies, based on maintaining the largest possible N_e, are basic procedures to avoid changes in population size over generations, keeping a balance sex ratio and avoiding inbred matings. In addition, equalizing contributions from parents to progeny, i.e., each pair or individual contributes the same number of progeny to the next generation, is a well-known method to double N_e, thus reducing to one-half the rate of increase in inbreeding and genetic drift (Gowe *et al.*, 1959; Wang, 1997). This method can be generalized using genealogical or molecular marker information to find the contributions from parents such that the average coancestry (kinship) of the progeny is minimized. This method is expected to maximize gene diversity and N_e (Caballero and Toro, 2000, 2002). When individuals are unrelated or uniformly related, the method simply reduces to the equalization of contributions.

Equalization or minimum coancestry contributions maintain the maximum possible gene diversity, but has also a number of collateral consequences on adaptive variation. If individuals or pairs are forced to contribute the same number of progeny, natural selection on fecundity is avoided, except

for complete mating failures, and selection for viability occurs only within families. This implies an overall relaxation of natural selection with both positive and negative consequences. On the positive side, selection for adaptation to captivity is reduced, a desirable aspect if reintroduction of the species in the wild is an intended objective (Frankham, 2008; Williams and Hoffman, 2009). On the negative, relaxation of selection may enhance the fixation of fecundity deleterious mutations over generations, implying a reduction in fitness over time. Despite this disadvantage, analytical and simulation studies indicate that equalization of contributions is expected to produce higher fitness than random contributions for small populations (below about 50 individuals) in the short term (up to 10–20 generations) (Schoen *et al.*, 1998; Fernández and Caballero, 2001; Theodorou and Couvet, 2003; García-Dorado, 2012). The available experimental data confirm these theoretical expectations. Empirical comparisons involving a range of population sizes (from 8 to 100) and generation periods (from 4 to 38 generations) either did not find evidence to suggest that equalization of contributions entailed an important disadvantage in the fitness of the population or even produced evidence for some fitness advantage (Loebel *et al.*, 1992; Borlase *et al.*, 1993; Montgomery *et al.*, 1997; Rodríguez-Ramilo *et al.*, 2006; Sánchez-Molano and García-Dorado, 2011; Ávila *et al.*, 2013).

Most endangered species, both in natural settings and captive conditions, are characterized by being structured in different breeding groups showing some degree of isolation. Thus, management methods have to consider this structuring of the populations. Isolation between subpopulations implies an excessive increase in local inbreeding and, therefore, some degree of migration among subpopulations is advised in conservation programs. A classical rule, based on the island model derived by Wright (1951), is to allow for one migrant per generation and subpopulation (Mills and Allendorf, 1996). More sophisticated methods, making use of demographic (Wang, 2004) or genealogical or molecular marker information (Fernández *et al.*, 2008; Ávila *et al.*, 2011), can provide a more effective control of the relative levels of diversity between and within subpopulations. Migration, however, has to be kept to a minimum when different subpopulations may be adapted to different local optima for one or more quantitative traits subject to selection (Storfer, 1999; Hereford, 2009; Sánchez-Molano *et al.*, 2013), since hybridization of different populations may cause outbreeding depression (Edmands, 2007).

One of the main consequences of *ex-situ* conservation programs is the genetic adaptation to captive conditions caused by both natural and artificial selection (Frankham, 2008; Williams and Hoffman, 2009). Because of its benign characteristics, captivity implies selection for variants possibly deleterious in wild conditions. Quantitative trait analysis can be particularly useful in the study of adaptation to captivity through the use of the animal model for zoological records (Pelletier *et al.*, 2009). Regarding management methods, both empirical (Frankham *et al.*, 2000) and simulation studies (Fernández and Caballero, 2001; Saura *et al.*, 2008) have shown that minimum coancestry or equalization of contributions procedures are effective in reducing adaptation to captivity. However, there is little direct evidence to support an increase in fitness after reintroduction by implementing some of these conservation methods (Frankham, 2008; Williams and Hoffman, 2009). Other recommendations have been made with this purpose, such as reducing the number of generations in captivity and fragmenting the populations, if possible, allowing for some migration between them as a way to control local inbreeding (Frankham, 2008; Williams and Hoffman, 2009).

See also: Conservation Biology, Evolution and. Inbreeding and Nonrandom Mating. Quantitative Genetics in Natural Populations

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Convergent Evolution, Adaptive Radiation, and Species Diversification in Plants

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Glossary

Adaptive radiation It is the rise of a diversity of ecological roles and associated adaptations within a lineage. Component species of an adaptive radiation often show convergence with ecologically similar members of other lineages.

C_4 photosynthesis C_4 photosynthesis is one of three biochemical pathways that plants use to fix CO_2 as reduced carbon (sugar) as food. It has evolved dozens of times from the more common pattern of C_3 carbon fixation, especially in plants of hot, sunny, seasonally dry habitats. C_4 photosynthesis uses PEP carboxylase to capture (carboxylate) CO_2 initially as an organic acid throughout the leaf mesophyll, then ships the acid to cells around each vein bundle, where the CO_2 is released and fixed via light capture and ordinary C_3 photosynthesis in those cells. This spatial separation of CO_2 capture and light capture reduces the inefficiency of photosynthesis caused by the C_3 carboxylating enzyme RUBISCO running photosynthesis in reverse when CO_2 is at low levels inside the leaf. C_3 photosynthesis is often more productive in moist or shady habitats.

CAM (crassulacean acid metabolism) photosynthesis It is an unusual photosynthetic variant seen in succulent plants of deserts, as well as in some epiphytes or plants of salt marshes, in which the stomata open only at night, and CO_2 is initially captured as an organic acid; CO_2 is then released from the acid by day and fixed using ordinary C_3 photosynthesis. It greatly reduces water loss but also reduces the total potential amount of carbon fixation.

Convergent evolution (evolutionary convergence) It is the rise of similarities in form, physiology, and behavior among distantly related organisms that inhabit similar ecological conditions, despite phenotypic differences among their ancestors.

Cordilleras Cordilleras are extensive chain of mountains, such as the Andes or the New Guinea Highlands.

Epiphytes Plants that grow on the branches or trunks of woody plants are called epiphytes; the main groups of vascular epiphytes are orchids, bromeliads, ferns, and members of the philodendron family.

Giant rosette shrubs They are unbranched or sparsely branched woody plants with large leaves ($>$ 20 cm long) clustered around the single terminal bud of each stem; often a dominant growth above treeline in equatorial regions.

Marcescence It is the retention of dead organs (e.g., leaves) that would otherwise be shed from stems.

Mycorrhizae Mycorrhizae are mutualistic associations between plant roots and fungi; the mycorrhizal fungi aid in obtaining water and mineral nutrients, especially phosphorus, from the soil, based the very small diameters and great surface-to-volume ratio of the fungal threads.

Krummholz Krummholz (crooked or twisted wood in German) is a short, mat-like growth form assumed by some tree species above treeline. These mats can be one to a few tens of centimeters in depth, shelter in the ground boundary layer, and have aerodynamically smooth canopies. In essence, they are gigantic cushion plants. Often they are sheltered under snow during the winter.

Paramo It is a distinctive vegetation-type found in the equatorial alpine zone of the Andes in South America, marked by dominance of giant rosette shrubs of *Espeletia* of the daisy family (Asteraceae) and occasionally *Puya* of the bromeliad family (Bromeliaceae). Evergreen, small-leaved shrubs and grasses and sedges are usually also present. A convergent vegetation-type occurs above treeline on the high volcanoes of equatorial East Africa, in the so-called Afro-alpine zone.

Phreatophyte It is a plant in a dry region that taps a more or less permanent source of moisture in the underlying water table. Such water tables can be shallow in desert washes, where water carried by floods associated with rainstorms infiltrates the ground, or in low areas in a semi-arid landscape where groundwater can accumulate after flowing from other areas nearby.

Pith It is soft, spongy tissue inside plant stems that can act as a storehouse for moisture and nutrients.

Pollinia (singular, pollinium) They are discrete packets of hundreds to tens of thousands of pollen grains that are transferred between plants as intact units, attached to pollinators by sticky points of attachment or mechanical yokes. Pollinia have evolved in some orchids and in milkweeds.

Sheet flow It is a surface movement of water across nearly flat, relatively smooth soil or rock surfaces in the form of a thin, continuous film or deeper layer or sheet.

Species sorting It is the emergence of different distributions of species along an ecological gradient that results from their context-specific advantages in competition, avoiding predation, and/or interacting with mutualists under particular conditions, based on differences in their biological equipment.

Stomatal conductance It is a measure of the permeability of the stomata of a leaf to the diffusive movement of water vapor and, thus, of carbon dioxide as well. At a given difference between the humidity (or CO_2 concentration) inside and just outside a leaf, the rate of water loss (or CO_2 uptake) will increase proportionally with stomatal conductance. The latter reflects the density, size, and degree of opening of stomata in a complex fashion.

Vascular plants They are plants with specialized cells (xylem) that die when mature and act as pipes to conduct water efficiently along the length of a plant, a stem, or a leaf. Vascular plants include the angiosperms (flowering plants), gymnosperms (conifers, cycads, *Gingko*, and their allies), and ferns.

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Figure 1 Convergent evolution, exemplified by **alpine cushion plants** ((a) *Diapensia lapponica*, family Diapensiaceae, Mount Chokai, Japan; (b) *Donatia novae-zeelandica*, family Stylidiaceae, Mount Ossa, Tasmania; (c) *Silene acaulis*, family Caryophyllaceae, near sea level in arctic tundra, Norway); **desert succulent plants** ((d) saguaro (*Carnegiea gigantean*), family Cactaceae, Arizona; (e) *Euphorbia virosa*, family Euphorbiaceae, Namibia; (f) *Pachypodium lamerei*, family Apocynaceae, Madagascar; (g) *Alluaudia procera*, family Didieraceae, Madagascar); **hummingbird-pollinated flowers** ((h) *Lobelia cardinalis*, family Campanulaceae, eastern North America; (i) *Psittacanthus robustus*, family Loranthaceae, Bahia, Brazil; (j) *Costus pulverulentus*, family Costaceae, Costa Rica; (k) *Columnea glabra*, Gesneriaceae, Central America); and **pitfall-trap carnivorous plants** ((l) close-up of small pitcher leaves of *Cephalotus follicularis*, family Cephalotaccaceae, Western Australia; (m) *Nepenthes ephippiata*, Nepenthaceae, Sarawak; (n) *Sarracenia oreophila*, family Sarraceniaceae, southeastern United States; (o) *Brocchinia hechtioides*, family Bromeliaceae, southern Venezuela).

Evolutionary convergence is the rise of phenotypic similarities among distantly related organisms that inhabit similar ecological conditions. Similar habitats, microsites, or ways of making a living should generate similar selective pressures that, in turn, favor morphological and physiological traits that maximize fitness and competitive ability under those conditions, and lead to convergence among species despite differences among their ancestors. Presumably, this is why alpine plants in so many lineages have a cushion growth form and small, thick leaves; why annuals and succulents with small, silvery, or spiny photosynthetic surfaces dominate so many deserts; and why plants pollinated by hummingbirds tend to have reddish tubular flowers that secrete large amounts of dilute, hexose-rich nectars (Figure 1).

Fitness and competitive ability are context-dependent (Givnish, 1986). The growth forms and traits that maximize success in certain environments (e.g., cushion shrubs with small, thick leaves in alpine and arctic tundra) will almost inevitably be disadvantageous under other conditions (e.g., tropical rain forests). Differential adaptation of plant species to one or another set of conditions – and convergent evolution in traits that determine that adaptation – are thus important drivers of plant diversification at large spatial scales. Where physical conditions are key prime determinants of survival and reproduction – as might be expected in extreme environments, in which one or a few conditions are severe or highly unusual and limit the growth and survival of most species – then convergence over evolutionary timescales should be marked. Over shorter periods, local conditions can also allow species to sort themselves along ecological gradients based on their differential ability to survive and compete successfully under different conditions, reflecting differences in their biological equipment. As a result, species sorting – a process operating over relatively short, ecological timescales – can also generate convergence among species at specific points along ecological gradients (Weiher and Keddy, 1995).

Convergence in a particular kind of environment suggests that the trait in question is adaptive there, but functional

studies are required to understand why that trait enhances fitness under those conditions (Givnish, 1997). Concerted convergence – involving two or more genetically, developmentally, and functionally independent traits – can arise through selection pressures imposed by different, correlated features of a given environment. For example, selection for thin broad leaves in forest understories favors net-like venation for biomechanical reasons, while selection for effective seed dispersal in windless understories favors the rise of fleshy fruits. Both traits have arisen, almost always together and associated with the invasion of forest understories, more than 20 times in monocots (Givnish *et al.*, 2005).

Similarities in form and physiology can often lead plants to compete intensely with each other. Consequently, selection and competitive sorting may also favor divergence among locally coexisting species (Stubbs and Wilson, 2004), creating a tension between the amounts of convergence versus divergence expected within a habitat or species assemblage. Divergence may be especially favored under less extreme conditions in which interactions with other plants, rather than the external environment, play a more important role in determining plant survival and reproduction.

Close relatives can often be each other's most potent competitors, based on their phenotypic similarity with each other (e.g., see Burns and Strauss, 2011). Divergent selection among such relatives can lead to adaptive radiation, the rise of a diversity of ecological roles, and attendant adaptations within a lineage (Givnish, 1995; Schluter, 2000). Adaptive radiation is more likely where alternative resources are underutilized by members of other lineages, perhaps as a result of mass extinction, the colonization of isolated islands, mountains, or lakes by few other groups, or the evolution of a 'key innovation' (e.g., the epiphytic habit) that allows a lineage to invade and ecologically partition a new range of habitats or resources (Givnish, 2015). Adaptive radiation and evolutionary convergence are often two sides of the same evolutionary coin, with species within a radiation adapted to a particular habitat or pollinator class exhibiting convergence with members of other lineages adapted to the same conditions.

Adaptive radiation is often conspicuous in plants on oceanic islands and archipelagoes, with the extent of variation in habitat, growth form, leaf shape, and pollinators being extraordinary in such groups as the Hawaiian lobeliads (**Figure 2**), mints, silverswords, and *Schiedea* (pink family); *Sonchus* (daisy family), *Echium* (viper bugloss family) and other groups on the Azores, Canary Islands, Madeira, and Cape Verde Islands; and *Scalesia* (daisy family) and *Opuntia* (cactus family) on the Galápagos (Carlquist, 1965; Givnish, 1998; Lindqvist and Albert, 2002; Carlquist *et al.*, 2003; Sakai *et al.*, 2006; Kim *et al.*, 2008; Givnish *et al.*, 2009; Stocklin, 2009). But adaptive radiation can also arise on continents, as exemplified by such groups as mariposa lilies (*Calochortus*, lily family), beardtongues (*Penstemon*, plantain family), and rein-orchids (*Platanthera*, orchid family) in North America (Hapeman and Inouye, 1997; Patterson and Givnish, 2004; Wilson *et al.*, 2007), *Brocchinia* (bromeliad family) in the Guayana Shield (Givnish *et al.*, 1995), lupines (*Lupinus*, legume family) in the Andes, the Brazilian Highlands, and western North America (Drummond *et al.*, 2012), and *Eucalyptus* (myrtle family) and *Banksia* (protea family) in Australia (Williams and Woinarski, 1997; Mast and Givnish,

Figure 2 Adaptive radiation in growth form and habitat in the Hawaiian lobeliads: (a) Giant rosette shrubs in alpine bogs in *Lobelia* sect. *Galeatella*; (b) treelets of wet subalpine openings in *Trematolobelia*; (c) sea cliff succulents in *Brighamia*; (d) canopy trees, exemplified by *Cyanea hamatiflora* (long leaves) in cloud forests on Maui; (e) understory treelets under intact canopies, exemplified by *Cyanea floribunda* on Hawaii; (f) multi-stemmed shrubs of rain- and cloud-forest edges, canopies, and canopies in *Clermontia*; and (g) treelets of mesic scrub in *Delissea* (Givnish *et al.*, 2009).

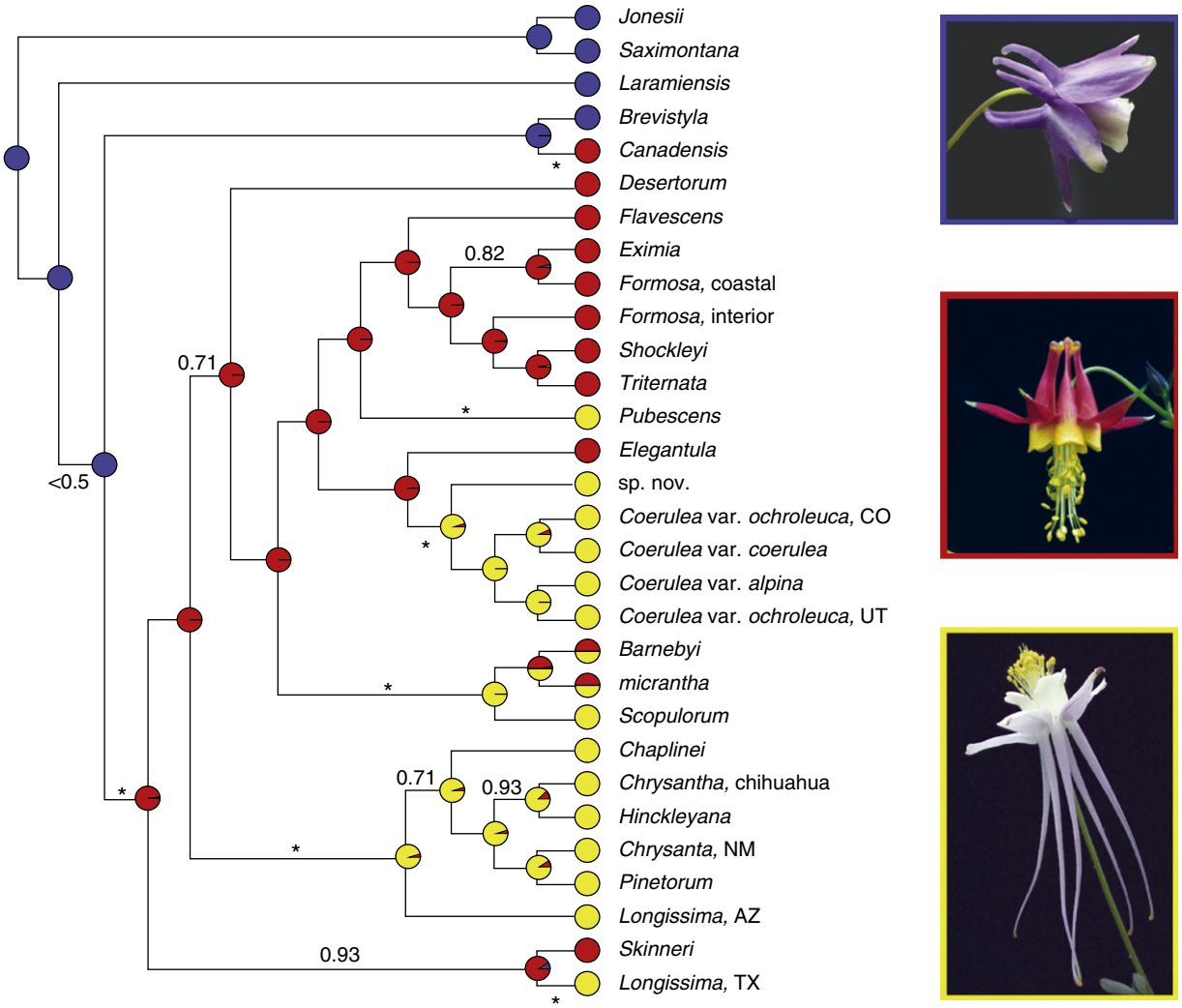

Figure 3 Adaptive radiation involving the sequential evolution of floral adaptations to bumblebees (blue), hummingbirds (red), and hawkmoths (yellow) in North American *Aquilegia*. The probability of each syndrome at each ancestral node is shown by the width of slices in the pie charts. Asterisks indicate inferred shifts in pollinators, including two from bumblebees to hummingbirds, and five from hummingbirds to hawkmoths. All inferred shifts involved an increase in the lengths of floral spurs (containing the nectar) and pollinator mouthparts. Figure from Givnish (2010a), redrawn from Whittall and Hodges (2007).

2002). Given the much wider range of parent materials present on continents versus volcanic or uplifted limestone islands, it is not surprising that adaptive radiation by soil type or bedrock is essentially a continental phenomenon (e.g., invasion of serpentine, gypsum, alkali, clay, loamy, and sandy soils by *Calochortus*, and invasion of serpentine and vernal pools by the pincushion plants (*Navarretia*, phlox family) (Givnish, 2010a)). In the California Floristic Province, over 200 species have become specialized entirely on serpentine soils with aberrant Mg:Ca ratios and high levels of heavy metals (Brady *et al.*, 2005).

Adaptive radiation can lead to increased rates of species diversification within a lineage. Whittall and Hodges (2007) inferred that adaptive radiation in floral form and associated pollinators was a central force driving diversification in columbines (*Aquilegia*) of the Ranunculaceae (buttercup family) (**Figure 3**). Carlquist (1970) argued that adaptive radiation in habitat, growth form, and pollinators was a key driver of plant speciation on islands, and Stebbins (1974) made a similar argument for adaptive radiation helping drive the global diversification of angiosperms at the level of families and genera. Set against these ideas, however, is the fact that many lineages exhibit phylogenetic niche conservatism, in which close relatives remain ecologically quite similar and do not appear to undergo adaptive radiation. Crisp *et al.* (2009), for example, showed that stasis within biomes in plants of the Southern Hemisphere appeared to outweigh biome shifts by more than 25:1 – although this does not exclude the possibility that lineages that remained within a biome have instead radiated with respect to finer-scale variation in habitat, pollinators, seed dispersers, or mycorrhizal fungi. More importantly, there are striking cases of adaptive radiation – for example, Darwin's finches with their great diversity in beak form and diet, and bromeliads in the genus *Brocchinia*, with

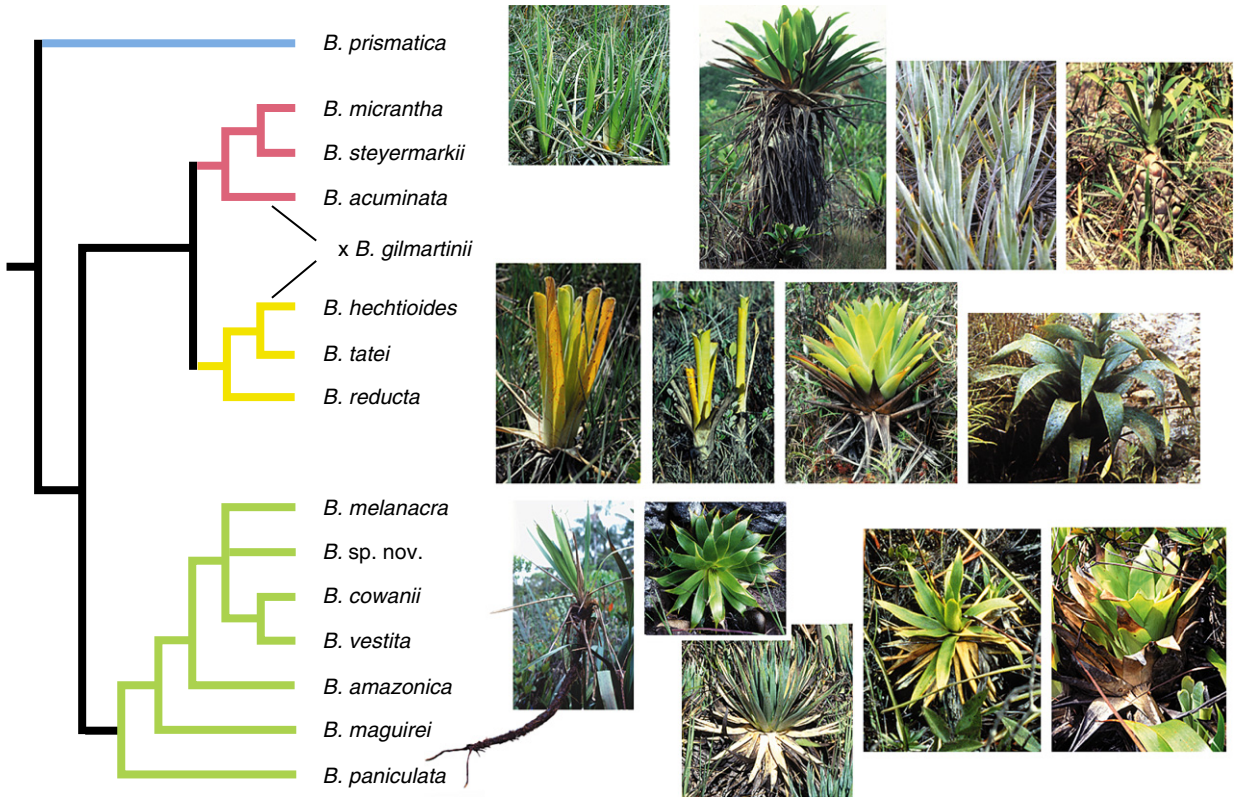

Figure 4 Adaptive radiation in growth form and nutrient capture strategy in *Brocchinia* of the bromeliad family (Bromeliaceae). *B. prismatica* (blue) and all members of the Melanacra Clade (green) except for *B. paniculata* have loosely overlapping leaves, impound no rainwater, have small and sparse leaf hairs (trichomes), and apparently obtain all of their nutrients from bog peats and damp sands and sandstone surfaces. *B. paniculata*, *B. micrantha*, and *B. acuminata* of the Acuminata Clade (red), and all members of the Reducta clade (yellow) have tightly overlapping leaves that form tanks in which rainwater accumulates, and have larger or denser arrays of leaf trichomes. In the Acuminata clade, *B. acuminata* (first image, second row) is a gigantic, unbranched tree-like plant that impounds several liters of water among its leaf bases, in which frogs often live; *B. acuminata* (third image) is an ant-fed plant, with ants living among the swollen, tough, chlorophyll-lacking leaf bases; and *B. steyermarkii* (second image) has lost the tank habit and apparently relies on nutrient absorption via roots from the soil. In the Reducta clade, *B. hechtioides* and *B. reducta* (first and second images, third row) are carnivorous plants, specialized on bees and wasps vs. ants, respectively. *B. tatei* can grow on the ground (as shown in the third image) or as an epiphyte, and catches falling plant debris; terrestrial populations have been seen with N-fixing cyanobacteria growing in their tanks. *B. gilmartinii* (last image, third row) apparently is a hybrid between *B. acuminata* and *B. hechtioides*; its ecology remains uninvestigated. *B. paniculata* (last image, last row)has a growth form similar to that of *B. micrantha*; the remaining species obtain mineral nutrients from the soil. The tough leaf tips of *B. melanacra* (first image, last row), a bog species, are non-functional in fully expanded leaves but protect the terminal bud from fire when young. Phylogeny and images from Givnish *et al.* (1997).

their greater range of strategies for nutrient capture than any other plant genus (**Figure 4**) – that show either the same rate of species diversification as close relatives, or a much lower rate (Givnish, 2015). Other factors – notably short generation times, and limited seed dispersal leading to genetic differentiation within species at small spatial scales and, ultimately, speciation – can have a profound effect on species diversification independent of ecological divergence among relatives.

Based on the phylogenies (family trees) of various plant lineages derived from DNA sequences and calibrated against time using fossils, comparisons show that the rates of net species diversification tend to be higher in herbs (especially annuals) than in woody plants, in animal- versus wind-pollinated species, in families with a greater diversity of growth forms, pollination mechanisms, and species distributions, in families at lower latitudes, in clades with hermaphroditic versus unisexual flowers, in plants with bilateral versus radial symmetry of flowers, in plants with hummingbird pollination or with spurred or tubular flowers, in epiphytic lineages, and in young lineages (see review by Givnish, 2010a, as well as Ricklefs and Renner, 1994, 2000; Whittall and Hodges, 2007; Magallón and Castillo, 2009; Smith and Beaulieu, 2009; Givnish *et al.*, 2014). High rates of species diversification in the grass family (Poaceae) are correlated with the evolution of C_4 photosynthesis and climatic aridification in the Miocene (Spriggs *et al.*, 2014). High rates of diversification in orchids, the largest angiosperm family, are correlated with the evolution of pollinia, epiphytism, CAM photosynthesis, pollination by butterflies, moths, and euglossine bees, and (especially) life in extensive tropical cordilleras (Givnish *et al.*, 2015). As predicted long ago by Ehrlich and Raven (1964), speciation in mustards (order Brassicales) appears to have increased as a result of a coevolutionary arms race involving their chemical defenses and the detoxification abilities of the cabbage butterflies (family Pieridae) whose larvae feed on them. Edger *et al.* (2015) showed that species diversification in the

mustards accelerated with their evolution in sequence of indolic glucosinolates (mustard oils), then methionine-derived glucosinolates, then novel glucosinolate variants. Species diversification in pierids similarly accelerated with evolution of abilities to detoxify indolic, then methionine-derived, then structurally novel glucosinolates. Gene and genomic duplication events appear to have been involved on both sides of this arms race each time it escalated.

Evolutionary Convergence, Divergence, and Diversification within Plant Communities

Arid environments – Deserts are extreme environments, with very low mean rainfall ($< 25\,\mathrm{cm\,y^{-1}}$), high to very high temperatures and low humidity in summer, cool to cold temperatures at night and during winter, and often thin, sandy or rocky soils. The great deserts of the world are mostly centered at 30° N and 30° S latitude, where large masses of hot, dry air descend to the surface after having ascended and lost their moisture via convective thunderstorms near the equator (Allaby, 2006). Dry conditions are exacerbated by the rain shadows created by mountains upwind, and by cold water offshore the western edge of continents. Rainfall is infrequent and highly variable from year to year, especially in deserts receiving the lowest mean rainfall. Where cold water is offshore and upwind (e.g., in the Coloradan Desert of California), almost no rainfall falls in winter; when warmer water is offshore (e.g., in the Sonoran Desert of Mexico and Arizona), monsoons can also bring heavier rainfall during the summer months.

Convergence and divergence are both hallmarks of desert floras (Orians and Solbrig, 1977a). Desert plants include *drought avoiders*, *drought evaders*, and *drought tolerators*. *Drought avoiders* include desert annual herbs that germinate after rains, and grow, flower, and set seed while the soil is relatively moist. In most deserts, these annuals are active almost exclusively after winter rainfall. In deserts with summer and winter rains, there are different groups of species that are summer and winter annuals. Annuals are short in stature, and allocate heavily to flowering and seed production. Species vary substantially in leaf thickness, reflectance, stomatal conductance, and photosynthetic capacity, from thick, hairy, silvery leaves with low rates of photosynthesis and transpiration, to thin, dark green leaves with high rates of photosynthesis and transpiration. *Drought evaders* include deciduous shrubs and herbs in the open desert, which shed their leaves when faced with drought, as well as phreatophytic trees, shrubs, and perennial herbs that live along washes and can tap a constant source of water. Washes serve as watercourses after rains, and substantial amounts of water brought by surface flow infiltrate their soils. Phreatophytes can tap a relatively permanent water table that is often several meters below the surface. *Drought tolerators* include evergreen shrubs like creosote bush (*Larrea*) with small, thick, tough leaves, and leaf or stem succulents like *Agave* and *Opuntia* that have CAM photosynthesis; these groups can also tolerate prolonged and intense droughts while retaining live leaves (**Figure 5**).

Among these growth forms, there is an inverse relationship between maximum photosynthetic rate per unit leaf mass and ability to maintain high photosynthetic rates as soil water content and water potential drop (Orians and Solbrig, 1977a,b). Desert annuals have the highest maximum rates of photosynthesis, but those rates are achieved with high stomatal conductance and rates of transpirational water loss, and so their photosynthetic rates drop rapidly as soils dry. Evergreen shrubs and especially succulents have much lower maximum rates of photosynthesis, but can remain active with

Figure 5 Examples of plant growth forms characteristic of the Sonoran desert of North America: (a) Stem succulent (saguaro, organ pipe, and cholla cacti (*Carnegiea, Stenocereus, Opuntia*) are visible) and drought-deciduous shrub (brittlebush (*Encelia farinosa* of the daisy family, with conspicuous yellow flowers and silvery leaves); (b) evergreen shrub (creosote bush (*Larrea divaricata*, family Zygophyllaceae) with narrow, tough leaves); (c) stem succulent with photosynthetic bark and drought-deciduous leaves (ocotillo (*Fouquieria splendens*), family Fouquieriaceae); (d) leaf succulent (*Agave palmeri*); (e) winter annual (*Phacelia calthifolia*, phlox family); and (f) summer annual (*Pectis papposa*, daisy family). All succulents shown have CAM photosynthesis except ocotillo, which is C_3.

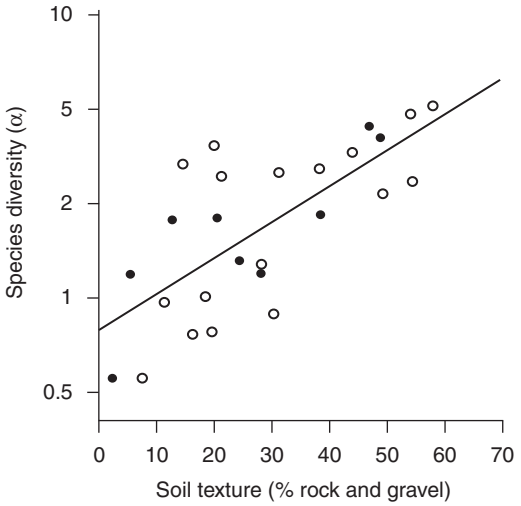

Figure 6 In the caption, use hollow and solid circles to refer to the North and South American deserts. Species diversity (Fisher's α index) vs. soil texture in North American (○) and South American (•) deserts (redrawn from Orians and Solbrig, 1977a,b). Note the convergence in the relationship of diversity to soil texture; more species are found per unit area on coarser soils with more rock and gravel.

relatively little fall-off in carbon capture even when soils are very dry (predawn water potential approximately -6 MPa). As a result of the inverse relationship between photosynthesis and its sensitivity to water potential, as soils dry after the winter (or summer) rains cease, there will be periods when each growth form – annuals, then deciduous herbs and shrubs, then evergreen shrubs, then succulents – has a photosynthetic advantage. The greater the range of moisture conditions present during a year, the greater the diversity of growth forms should thus be able to coexist, via a seasonal partitioning of photosynthetic advantage (Orians and Solbrig, 1977b). In deserts with sparse rainfall, coarse sandy or gravelly soils store the most moisture because they have high infiltration rates and little water is lost through evaporation at the surface after a storm; finer-grained soils have low infiltration rates and wind up storing less moisture. Consequently, we would expect the diversity of growth forms – and of plant species – to be higher on coarser soils. Indeed, Orians and Solbrig (1977b) found remarkably tight and similar relationships between species diversity and percentage of sand and gravel in deserts in Arizona and Argentina (**Figure 6**). Based on the alluvial sorting of soil texture, sites high on bajadas (desert slopes) have coarse soils and high diversities of both life forms and plant species, while those low on bajadas and alluvial flats have finer soils and lower diversities of life forms and species. In addition, in areas with lower rainfall – and thus, greater proportional variation in rainfall from year to year – deserts become increasingly dominated by annuals (Schaffer and Gadgil, 1975).

High rates of diversification are expected in desert annuals, based on their short life cycles (Lewis, 1966), tendency toward inbreeding (Raven and Axelrod, 1978), and dearth of effective means of long-distance seed dispersal (Givnish, 2010a). Little is known quantitatively on the actual rates of diversification, however. Over 2000 species of desert and vernal-pool annuals comprise 26% of the vascular flora in the California Floristic Province, and most of these most likely arose in the last 15 million years following the uplift of the Sierra Nevada and the origin of winter rainfall (Raven and Axelrod, 1978). Evans *et al.* (2009) showed that a clade of *Oenothera* (Onagraceae, evening primrose family) composed almost exclusively of desert winter annuals spawned at least 11 species in 250 000 years, which would correspond to a net rate of species diversification D ($= \ln(S)\, T^{-1}$, where S is the number of species in a clade, and T is the stem age of that clade) of 9.6 sp sp^{-1} My^{-1} – an extraordinarily high rate that exceeds that of all plant groups studied to date (e.g., $D < 7.6$ My^{-1} for European *Dianthus* in the pink family (Valente *et al.*, 2010); $D < 5.2$ My^{-1} for Andean *Lupinus* (lupine; Drummond *et al.*, 2012)). Succulents in the Aizoaceae (ice plant family) of the South African Karoo also have a high rate of species diversification ($D < 1.75$ My^{-1}), but this appears related to their exceptionally poor powers of long-distance seed dispersal (splashed from capsules by raindrops) rather than their growth form (Klak *et al.*, 2004; Ellis *et al.*, 2006).

Another mechanism that might promote high rates of diversification in desert winter annuals, and maintain high levels of species richness, involves competition across years based on their differential responses of germination, survival, growth, and seed production in response to variation in rainfall within a growing season (Angert *et al.*, 2009). Annuals vary greatly in leaf thickness, reflectance, stomatal conductance, and photosynthetic capacity. Those with thin, green leaves have high photosynthetic rates and can produce large numbers of seeds in a rainy year, but are sensitive to drought; those with thicker, more silvery leaves have lower rates of photosynthesis and seed production but can survive in years with less rainfall. Angert *et al.* (2009) demonstrate that this tradeoff, combined with the high incidence of multi-year dormancy in winter annuals and their persistence in the seed bank, can permit a large diversity of annuals to coexist locally over long periods of time.

Across life forms, thicker, more reflective, and more steeply inclined leaves and lower stomatal conductance reduce water loss per unit leaf mass while reducing photosynthesis to a lesser extent. The high cost of water loss in dry environments, in terms of roots required to replace transpirational water losses, thus favors the evolution of thick, reflective leaves with low stomatal conductance. Narrow leaves reduce water loss per unit leaf area and increase convective cooling, and thus should also be favored in deserts (Givnish, 1986). Seasonal water shortage prevents perennial herbs or shrubs from covering the entire ground surface, allowing annuals – which perforce are short over much of their life cycle – to compete successfully with such taller plants. Low coverage, even after rains, favors short stature throughout the life cycle in desert annuals, and limited stature in perennial dominants (Givnish, 1982; Tilman, 1988).

C_4 photosynthesis – while much less common that the C_3 photosynthetic pathway worldwide – often results in higher photosynthetic rates at low rates of transpirational water loss under warm, dry, sunny conditions. In deserts, C_4 photosynthesis is seen in many summer-active annuals and perennials, especially in grasses, sedges, amaranths, chenopods, and knotweeds, and is especially common in salt flats, where

reductions in water lost result in less energy being expended to excrete salt from the leaves, or to exclude it from being absorbed in the first place.

CAM photosynthesis reduces water loss even more than C_4 photosynthesis, although at the cost of greatly reduced carbon uptake. Not surprisingly, succulent plants with CAM photosynthesis are common in many deserts and semi-arid areas, including cacti and *Agave* species in New World deserts, and morphologically convergent *Aloe* and *Euphorbia* species in the Old World. Both C_4 and CAM involve CO_2 concentrating mechanisms that yield an advantage when atmospheric CO_2 levels are low. Cacti and Agave in the New World, and Aizoaceae in the Old World, underwent rapid species diversification starting 15 million years ago, suggesting that aridity and a drop in atmospheric CO_2 levels may have been drivers of a global diversification of succulent CAM plants (Arakaki *et al.*, 2011). A more detailed study, however, also suggests that the invasion of Central and North America as well as the rise of pollination by birds, bats, and hawkmoths may also have driven diversification in the cactus family (Hernández-Hernández *et al.*, 2014).

Among succulents generally, stature increases with the density of competing vegetation in which they occur: stone plants (*Lithops*, ice plant family) occur on nearly bare ground and are just a centimeter tall, while saguaro cacti – the tallest members of the cactus family – often grow in fairly dense desert woodlands on sites receiving abundant sheet flow, and can grow up to 12 m in height. Even so, desert succulents on different continents and in different families often show striking convergence, involving stem succulents in the cactus and spurge families (Cactaceae and Euphorbiaceae) in the New versus Old World, leaf succulents in the agave and aloe families (Agavaceae and Aloeaceae) in the New World versus Africa, and bark succulents in the ocotillo and Madagascar succulent families (Fouquieriaceae and Didieraceae). Slow-growing but water-rich succulents defend themselves from herbivores with spines, stinging hairs, and caustic sap, and in some cases (e.g., the famous stone plants) may have avoided being eaten by ostriches through visual mimicry of the stony soil on which they grow. Some cacti (e.g., *Pediocactus* and *Sclerocactus*) have flattened, brown spines that appear to mimic the dried leaves of grasses among which they grow.

Alpine tundra – Alpine habitats above treeline on mountains are another kind of extreme environment, with low temperatures ($< 10\ ^\circ C$ mean during summer), short growing seasons, frequent freeze–thaw cycles, cold winters, heavy UV irradiation, and in many localities, high winds, heavy snows, and thin soils (Körner, 2003). Treeline elevation varies from sea level at the continental limits to tree growth in the Arctic (ca. 60° N) and Antarctic (ca. 70° S) to 3800–4500 m in the tropics and subtropics. In areas with adequate moisture and soil, areas of continuous tree cover correspond roughly to areas with at least 100 days with mean temperatures $\geq 6.5\ ^\circ C$ (Körner, 2003). The mean ground temperature at 46 treeline sites between 68° N and 42° S is $6.7^\circ \pm 0.8\ ^\circ C$ (Körner and Paulsen, 2004). The strong correlation of treelines with thermal conditions strongly suggests that temperature is a key determinant of their position.

Although the limits of tree growth along moisture and light gradients has long been viewed to result from limited carbon capture (Boysen-Jensen, 1949), some authors have stated that carbon limitation does not set the position of alpine treelines, despite the negative effects of lower temperatures and shorter growing seasons on carbon capture. Körner (1998, 2003) argued that the position of alpine treelines is set not by carbon limitation but by limitations of cell growth – especially of the roots – by cold temperatures. Trees roots should be especially vulnerable to this limitation because even short saplings would create cold soil conditions by shading the ground and insulating it with a thick layer of air; sunlit compact herbs and cushion shrubs should, on the other hand, warm the soil immediately below them well above air temperature. But the hypothetical limitation of root growth under tall plants would still leave carbon balance as a prime determinant of treeline position: taller plants would simply be unable to continue aboveground growth without matching root growth to enable absorption of water and nutrients, with negative effects on photosynthesis likely. In fact, tree height decreases smoothly and linearly with elevation in the European Alps, far below the range at which the hypothesized limitation of trees by cold soil would operate, with tree height at treeline of 5 m in Norway spruce (*Picea abies*) and 8 m in Swiss stone pine (*Pinus cembra*) (Paulsen *et al.*, 2000).

So an abrupt decline in maximum height by a few meters near treeline may, as argued by Körner, be driven by cold soils under taller trees and by an elevation of leaf temperature and hence photosynthesis by assuming a krummholz cushion form (**Figure 7**) within the ground boundary layer, but carbon limitation is important in setting treeline position. Recent CO_2-addition experiments have confirmed an increase in growth by European larch (*Larix decidua*) but not Swiss mountain pine (*Pinus uncinata*) near treeline – clearly implicating carbon limitation – although the strength of this effect weakened after 6 years (Dawes *et al.*, 2015). Experimental warming of soils in the same experiment elevated growth by pine (but not larch), with the expected increase in root allocation.

In non-equatorial alpine environments, the dominant growth forms are krummholz, cushion shrubs, and compact to tall herbs (Körner, 2003). The aerodynamically smooth canopies of krummholz and cushion shrubs allow air to move past them without much mixing, permitting their leaves to warm 5–15 °C above the air only a meter or two above the ground boundary layer. Elevated leaf temperatures in a thermally limited environment can clearly be advantageous; such a benefit, as well as increased root temperatures, should also accrue to short herbs. In addition, smooth canopies of krummholz and cushion shrubs should divert winds around rather than through canopies, greatly reducing transpirational water loss under windy conditions, and decreasing sand- and ice-blasting of exposed plant parts. Desiccation or mechanical abrasion may indeed help shape the canopies of krummholz and cushion plants by eliminating projecting parts. Plants in many different families (e.g., Apiaceae, Asteraceae, Caryophyllaceae, Diapensiaceae, Ericaceae, Rubiaceae, Saxifragaceae, Stylidiaceae – the carrot, daisy, pink, diapensia, heath, coffee, saxifrage, and triggerplant families) have evolved this habit in alpine and arctic tundra communities across the Northern and Southern hemisphere. The warmth and sheltering influence of cushion plants, together with the moist

Figure 7 Krummholz of balsam fir (*Abies balsamea*) on Mt. Washington in northern New Hampshire.

soil they shelter, can permit them to have a beneficial effect on nearby plants in harsh alpine habitats (Badano and Lohengrin, 2006).

Narrow, thick leaves reduce water loss per unit leaf mass with small decreases to photosynthetic rate (see above), and so should be advantageous in alpine habitats with cold soils that restrict water uptake. Short-statured plants with streamlined canopies have advantages in elevating leaf temperature and reducing transpiration and mechanical damage in cold, windy alpine environments, but should be at a disadvantage in competing for light against taller competitors. Therefore, we expect taller competitors with looser canopies to replace short, cushion-form plants at lower elevations and in less exposed microsites, as can be frequently observed in many areas (e.g., see Billings and Mooney, 1968; Slack and Bell, 2014). Small changes in microtopographic position – involving shift of only a few centimeters – can have dramatic effects on wind exposure and snow accumulation, and on the plants inhabiting a microsite; such variation may partly account for the large numbers of species that co-occur in many alpine habitats. The short growing season in such habitats is, no doubt, responsible for their brief but spectacular period of flowering.

Frosts occur often throughout the growing season in many alpine environments, and increasingly so at higher elevations and more northerly exposures (Körner, 2003). Many species – including several cushion plants – have evolved the ability to supercool (that, chill below freezing without ice nucleation) or have internal ice barriers to prevent crystals from growing and damaging other parts of a plant once nucleation has begun (Kuprian *et al.*, 2014). Thermal gradients resulting from heating of the underlying soil by day can also prevent freezing damage to vegetative parts in cushion plants (Hacker *et al.*, 2011). The inflorescences of some plants project above the vegetative body and are therefore more exposed to both radiative and convective cooling, and somewhat decoupled from thermal inputs from the underlying soil. Reproductive shoots are often less frost resistant than vegetative shoots (which frequently can tolerate ice formation), but some species can supercool to at least $-22\ ^{\circ}C$ without damage (Kuprian *et al.*, 2014).

Frequent freeze–thaw cycles during the growing season can heave seedlings and small plants from the soil. This should strongly favor plants which spread vegetatively and that do not depend as strongly on seedling establishment. Not surprisingly, 87% of vascular plant species in the Swiss alpine zone exhibit vegetative spread (Hartmann, 1957). Freeze–thaw cycles should hit seedlings of trees especially hard, given that they are non-clonal and have no vegetative connection to deep-rooted individuals.

Above treeline on equatorial mountains, there is essentially no thermal seasonality, but summer every day and winter every night (Hedberg, 1964; Smith and Young, 1987). Such communities are dominated by unbranched, giant rosette shrubs with marcescent leaves (i.e., those that persist on the stem after they die) (**Figure 8**). This highly unusual growth form has evolved convergently in *Espeletia* (daisy family) and *Puya* (bromeliad family) in Andean paramo and puna; in *Dendrosenecio* (daisy family) and *Lobelia* (lobelia family) in the Afro-alpine zone; and in *Agyroxiphium* (daisy family) and *Lobelia* in Hawaii. Different lineages evolved the giant rosette habit on different continents within the daisy and lobelia families (Knox, 2014; Givnish, 2010b).

The unbranched habit in equatorial alpine rosette shrubs permits concentration of foliage around a single terminal bud; in many species of *Espeletia* and *Dendrosenecio*, the heavily pubescent leaves wrap around the bud at night and prevent it from freezing and dying (Meinzer and Goldstein, 1986). In *Lobelia keniensis* of the wet Rwenzori Mountains in equatorial East Africa, rainwater collects among the tightly packed terminal leaf rosettes; its terminal buds apparently survive nightly frosts by sitting at the bottom of a small pool formed by the plant itself (Hedberg, 1964). The unbranched habit should also increase the rate of height growth, taking plants from the thermal extremes (frost by night, warm conditions by day) near the ground surface to more buffered conditions a meter or two above the ground (Smith and Young, 1987). The massive stems of the rosette shrubs enclose a large volume of pith; plants withdraw water from the pith early in the morning, when photosynthesis becomes possible after the sun rises but the plants are unable to withdraw water from the still-frozen ground (Meinzer and Goldstein, 1986). Species of *Espeletia* at higher elevations have a higher ratio of pith volume to leaf area, based either on a reversed elevational cline in plant height, or an increase in pith diameter. Leaf marcescence

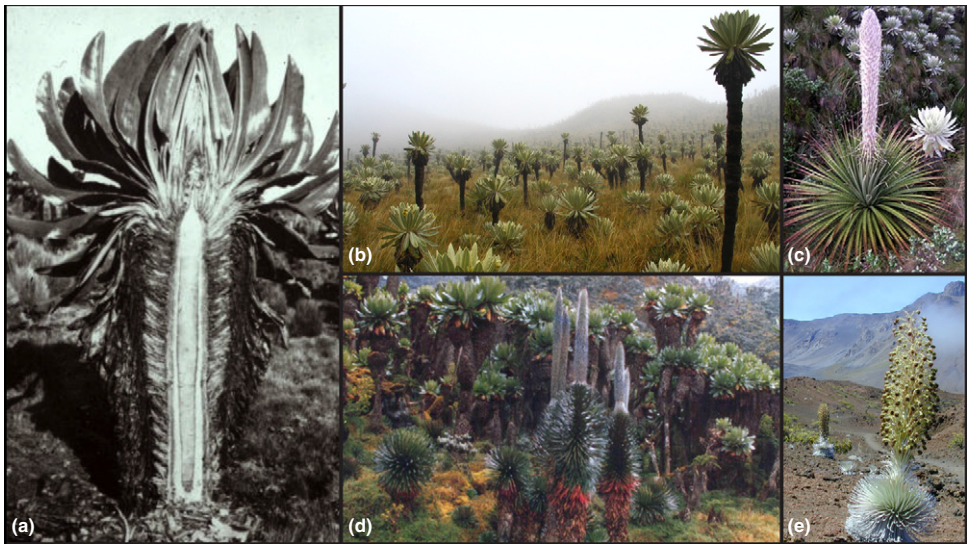

Figure 8 Giant rosette shrubs of the equatorial alpine zone. (a) Section through a *Dendrosenecio* (Asteraceae (daisy family) from the equatorial alpine zone in East Africa; (b) stand of *Espeletia pycnophylla* in an Ecuadorean paramo; (c) *Puya clava-herculis* in an Ecuadorean paramo; (d) *Lobelia wollastonii* (with elongate, silvery inflorescences) and *Dendrosenecio adnivalis* growing in the Afro-alpine; and (e) Haleakela silversword (*Agryoxiphium sandwichense*, daisy family) growing near 3000 m elevation on east Maui.

produces an insulating blanket around the stem that prevents the pith from freezing and dying, and facilitates morning photosynthesis (Meinzer and Goldstein, 1986).

Alpine plants have undergone high rates of diversification around the globe, with rates D from 0.37 to 0.81 My^{-1} in Himalayan larkspurs (*Delphinium* subg. *Delphiniastrum*), 0.35 to 2.19 My^{-1} in New Zealand rock-cresses (*Pachycladon*), and 0.60 to 1.60 My^{-1} in Andean groundsels (Espelitiinae), 1.48 to 3.21 My^{-1} in Andean gentians (*Gentianella*), and 1.56 to 5.21 My^{-1} in Andean lupines (*Lupinus*) (Hughes and Atchison, 2015). Doubtless these high rates reflect the recent formation of many alpine regions through glacial retreat and orogeny, the archipelago-like nature of many mountain ranges, and the short life cycles of some of the plants involved.

Summary

Alpine habitats and deserts exemplify extreme environments, where plant growth and survival are strongly limited by one or a few factors, and where strong similarities in plant form or physiology often arise through convergent evolution and through species sorting along environmental gradients based on the traits with which different plants are endowed. Phenotypic divergence among species – reflecting divergent evolution, or species sorting based on dissimilarities among species – arises via competition. Closely related species are often very similar phenotypically and thus are each other's most intense competitors. Selection for divergence among species in habitat, form, physiology, or mutualists within a lineage leads to adaptive radiation; species sorting can lead to community assembly based partly on advantages accruing to species with similar traits adapted to environmental conditions, and based partly on advantages accruing to species with divergent traits that reduce competition among them. Under certain circumstances, adaptive radiation can lead to accelerated rates of speciation and net species diversification. But limited dispersal, and differences among lineages in growth form, generation length, and extrinsic barriers to dispersal and gene flow within the habitats they occupy, may be as or more important in driving different rates of net species diversification.

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See also: C_4 and CAM Photosynthesis in Land Plants, Evolution and Diversification of. Parallel and Convergent Molecular Evolution

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Cooperation and Public Goods, Bacterial

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Social behaviors are those that have fitness consequences for both actor (the individual performing the behavior) and recipient (West *et al.*, 2007). Hamilton (1964a) first classified social interactions according to whether the fitness consequences for the actor and recipient are beneficial or costly (**Table 1**). If a behavior increases the fitness of the recipient, it is deemed either 'mutually beneficial' (when the actor also gains a fitness advantage), or 'altruistic' if the behavior is costly to the actor. Alternatively, 'selfish' behavior increases the fitness of the actor at a cost to the recipient, while 'spiteful' behavior is costly to both.

Definition of Cooperation

A cooperative behavior is generally described as an investment in resources that benefits an individual (the recipient) other than the actor (Chase, 1980). Two important features of cooperation are:

1. that the behavior exhibited by the actor is costly relative to a nonactor, and
2. that the behavior increases the fitness of other individuals, regardless of whether or not they adopt the same behavioral strategy.

Under this definition, both mutualism and altruism (**Table 1**) are considered to be cooperative interactions because they both increase the fitness of the recipient. Velicer (2003) emphasizes that altruism is a subset of cooperation in which there is no direct benefit to the actor, cautioning that semantic confusion arises when the use of the terms 'cooperation' and 'altruism' are used interchangeably.

There are two categories of explanation for the evolution of cooperation (Calcott, 2011). First, there is a need to explain how cooperation generates a benefit, and secondly, there is a need to explain how individually costly behaviors can continue to exist.

Benefits of Bacterial Cooperation

Cooperative groups in many lineages may have obtained advantage from colonization of new niches, or from the advantage of increased size afforded by the ever-present open niche at the top of the size scale (Bonner, 1988, 2000). One proposed advantage of increased size is that larger assemblages of cells avoid predation by filter feeders (Bell, 1985; Boraas *et al.*, 1998). The argument may be reversed such that increased size enhances feeding efficiency. For example, a low sucrose environment selects for multicellular clumps in experimental populations of the yeast *Saccharomyces cerevisiae* because this nutrient is digested extracellularly by a secreted enzyme (invertase), preventing solitary cells from growing at low cell and sucrose concentrations (Koschwanez *et al.*, 2013). Primitive flagellated or ciliated cells may be able to swim faster as part of a group, enabling the group to catch prey (Bonner, 1998). Both the eukaryotic myxomycetes (true slime molds) and the prokaryotic myxobacteria can feed more effectively by increasing the size of the feeding mass (Bonner, 1998). These species produce extracellular enzymes to digest large food, which they subsequently absorb directly. This form of feeding was originally termed 'pack' feeding in the case of myxobacteria (Dworkin, 1972) and is now more commonly referred to as 'wolf-pack' feeding. Cooperative feeding may have arisen in heterotrophs because the formation of multicellular groups allowed cells to benefit from the efficiency of aerobic respiration (Pfeiffer *et al.*, 2001). The higher yield of ATP (chemical energy) resulting from cooperative resource consumption during respiration is produced at a lower rate than from fermentation and therefore may be advantageous in the presence of a slowly diffusing food source.

Other advantages of cellular cooperation include benefits associated with both fixed surface attachment and enhanced dispersal. Single cells located in an ideal position for growth may be swept away by currents or wind, whereas an increased ability to adhere to surfaces by cell clusters might be selectively advantageous (Bonner, 1998). Conversely, primitive clustering aggregates may enhance dispersal of spores (Gross, 1994).

Table 1 Classification of social interactions

		Effect on recipient	
		+	−
Effect on actor	+	Mutualism	Selfishness
	−	Altruism	Spite

Source: Reproduced from Hamilton, W.D., 1964a. The genetical evolution of social behaviour. I. Journal of Theoretical Biology 7, 1–16.

Explanations for Cooperation

Cooperation exists in all kingdoms of life, and yet the phenomenon has sparked debate in the field of evolutionary biology for the greater part of the last century (Wright, 1945; Wynne-Edwards, 1962; Hamilton, 1963; Maynard Smith, 1964; Williams, 1966; Trivers, 1971; Wilson, 1975; Dawkins, 1976; Frank, 1998). The challenge is to explain how individually costly behaviors can continue to exist. Natural selection favors types that are relatively more fit than others, and therefore the evolution of cooperative behaviors seems paradoxical because such behaviors are, by definition, costly to actors relative to recipients.

Various methods have been proposed that can, in principle, explain how cooperative behaviors can exist in an evolutionary

context. These explanations can be viewed as mechanisms by which the cost of a cooperative behavior can be offset by benefits gained at different levels of the biological hierarchy. Individually costly behaviors have been explained at the level of selection below the individual (e.g., genes), at the level of the individual (e.g., reciprocal interactions between individuals), and at levels above the individual (e.g., interactions between groups). These three categories of explanation for the evolution of cooperation are discussed in the following subsections.

Shared Genes

Inclusive fitness theory (Hamilton, 1964a,b), kin selection (Maynard Smith, 1964), and selfish gene theory (Dawkins, 1976) are all variations of explanations for cooperation involving shared genes. According to these theories, cooperation can be maintained in a population when one individual benefits another individual with whom it shares genetic material through descent from a common ancestor. By definition, this mechanism can only explain cases of cooperation between members of the same species (Sachs *et al.*, 2004). Inclusive fitness theory posits that if cooperative behaviors are directed toward relatives, the 'inclusive fitness' (a measure of an individual's fitness that includes the reproductive success of its kin) of the actor can increase because other copies of its genes receive the benefit of the cooperative action. In other words, cooperation increases the reproductive success of the actor's genetic material (which encodes the cooperative trait), regardless of the reproductive fate of the actor. This phenomenon was mathematically formalized by Hamilton's inequality, $rb - c > 0$, where r is the coefficient of relatedness between the recipient and the actor, b is the additional reproductive benefit gained by the recipient, and c is the reproductive cost to the actor. Hamilton's rule predicts that cooperation will be favored by natural selection when the inequality is satisfied, and that cooperation is more likely to be directed toward relatives.

Kin selection can be partitioned into the categories of kin fidelity and kin choice, which differ in the mechanism by which they satisfy Hamilton's rule (Sachs *et al.*, 2004). Kin fidelity is contingent on the natural spatial distribution of related individuals (e.g., bacterial colonies). Cooperators do not actively direct cooperative actions toward kin, but toward physically close individuals who will likely be more closely related to the actor than the average member of the population. Many bacterial populations naturally exist in static environments, resulting in any cooperative behavior to be passively directed toward close relatives. Alternatively, if individuals exhibit a distinguishing phenotype that enables recognition of relatives, cooperative actions may be actively directed toward kin, even in unstructured environments.

The shared gene framework cannot be applied to those instances of cooperation between unrelated individuals or to cooperation between members of different species (Nowak, 2006). Evidence shows that relatedness is not an essential requirement for the evolution of cooperation and, particularly in the case of kin fidelity, may often be a spurious by-product of cooperation by other mechanisms (Kaushik and Nanjundiah, 2003; Smukalla *et al.*, 2008; Nowak *et al.*, 2010). Even kin choice may be achieved through the 'green beard' effect (Dawkins, 1976), which requires a single genetic locus to encode both the cooperative trait itself and a distinguishing feature (a 'green beard') by which to recognize and direct cooperative behavior toward other individuals carrying this locus. The green beard effect is also effective between nonrelatives who share a green beard gene(s). For example, flocculation in the yeast *S. cerevisiae* is induced by expression of the green beard gene *FLO1*, which causes cells to preferentially stick to other cells expressing *FLO1* regardless of genetic relatedness across the rest of the genome (Smukalla *et al.*, 2008). Similarly, the *csA* gene in the social amoeba *Dictyostelium discoideum* encodes a cell adhesion protein that interacts by homophilic binding to proteins anchored in the cell membrane of other cells, thereby facilitating preferential aggregation with other *csA*-encoding cells during fruiting body formation (Queller *et al.*, 2003).

To summarize, formal descriptions of the essential features of kin selection reveal that relatedness is optional and the essence of cooperation can be captured by any mechanism that promotes assortment between cooperating types (Godfrey-Smith, 2009). Although inclusive fitness theory is an empirically well-supported accounting method that is often invoked to explain the evolution of cooperation, it provides little insight into ecological mechanisms underpinning assortment (Doebeli, 2010).

Reciprocity

Reciprocal interactions between individuals can offset the cost of cooperation with fitness benefits gained at the same level, i.e., the level of the cooperating individuals themselves. Reciprocity, or 'reciprocal altruism' (Trivers, 1971), is classified as a mutually beneficial form of social interaction (**Table 1**) because both the actor and the recipient benefit from the cooperative interaction. While the evolution of a trait that directly benefits the actor may appear to be straightforward selection at the level of the individual, the challenge, as with all cooperation, is to explain how cooperation is maintained amongst the ever-present possibility of defection.

The paradox of cooperation is often examined within the framework of evolutionary game theory (Lewontin, 1961; Maynard Smith and Price, 1973), and its application to the 'Prisoner's Dilemma' game – a game that tests the consequences of cooperation and defection (Trivers, 1971). In the Prisoner's Dilemma game, defection will spread in a population made up of two 'players,' a cooperator and a defector, despite the cooperative strategy providing the best pay-off when both players cooperate. The Prisoner's Dilemma has been observed in many areas of ecology, including simple organisms such as viruses (Turner and Chao, 1999).

Reciprocity can evolve if there are repeated encounters between the same individuals (in an iterated Prisoner's Dilemma game), and if an individual has the ability to vary its strategy according to its partner's previous actions. The maintenance of a cooperative bacteriophage evolving at a low multiplicity of infection (ensuring clonal populations) during long-term selection experiments (Turner and Chao, 2003) can be interpreted as a player changing strategy to dominate the game (e.g., Chen *et al.*, 2012).

One successful game strategy is 'tit-for-tat,' in which an individual responds toward its partner with the same action the other player performed in the previous round (Axelrod and Hamilton, 1981). In *Pseudomonas aeruginosa* colonies growing on solid substrate, the costly secretion of pyoverdin, an iron chelator, is maintained in the population by tit-for-tat trafficking between contacting cells (Julou *et al.*, 2013). The local concentration varies according to the pyoverdin secreted by neighboring cells, feeding into a positive feedback loop that induces pyoverdin synthesis in the focal cell. Furthermore, this local exchange modulates the growth of individual cells thereby preventing a long sequence of retaliation because local patches of non-producers are outcompeted by the faster growth of producers.

Group Selection

Individually costly behaviors can be offset by benefits gained at levels above the individual – the level of the group. While cooperative actions may be selectively disadvantageous during competition between lower level entities within a group, they can be maintained by enhancing the competitive ability of the group in those instances where groups compete against groups. Darwin himself recognized that natural selection between groups might explain the evolution of self-sacrificial behavior among humans (Darwin, 1871). Michod and Roze (1999) even define cooperation in terms of the group-level fitness benefit: "an interaction that possibly decreases the fitness of the individual while increasing the fitness of the group".

The evolution of cooperation as a group-level adaptation has been (and still remains) one of the most debated areas in evolutionary biology, largely due to confusion over different modes of group selection. The debate was sparked in the 1960s when Wynne-Edwards (1962) argued that reproductive constraint evolved 'for the good of the group' because groups that do not exhibit restraint go extinct due to overexploitation of resources. The process of differential proliferation and extinction of groups leads to the evolution of cooperative behaviors that are adaptations of the group, rather than adaptations of the individual.

David Sloan Wilson's 'trait-group' model of group selection explicitly demonstrated that the cost of cooperation within groups could be offset by the differential productivity of groups (Wilson, 1975). However, Wilson's model was based on a different conception of the group, leading to a widespread misunderstanding of group selection. In Wilson's model the global population repeatedly separates into temporarily interacting 'trait-groups' with varying compositions of the group-beneficial cooperative trait. Despite the individual cost of cooperation, groups with a higher proportion of cooperators contribute more individuals to the total population than groups with a lower proportion of cooperators. The differences between this model of group selection, and one that requires differential reproduction of groups (*sensu* Wynne-Edwards and Wade) are illustrated in **Figure 1**. Progressively, group selection theory has developed into Multilevel Selection theory (Heisler and Damuth, 1987), which distinguishes between the type of group selection in which groups are part

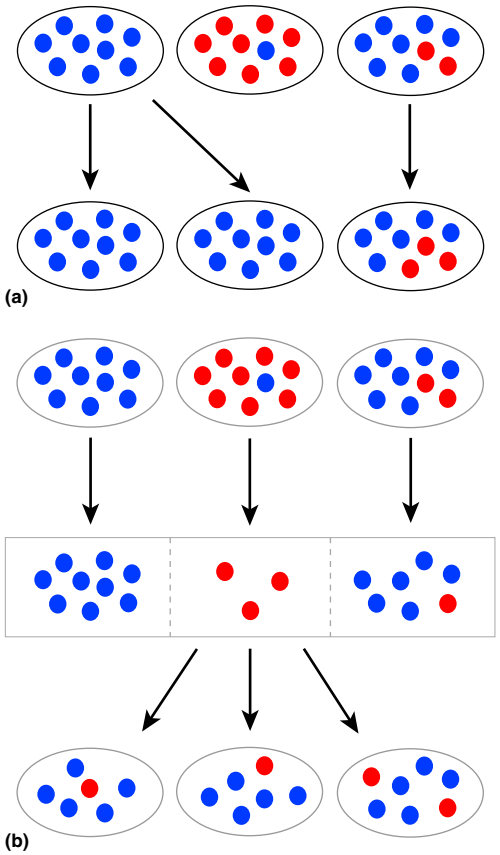

Figure 1 Two models of group selection. (a) Wynne-Edwards type of group selection. Selection is between groups via differential extinction and reproduction of groups, with well-defined groups and little gene flow between them (solid boundary lines). Blue circles represent cooperators, whereas red circles represent selfish types. Selfish types spread within groups, however groups with more cooperators leave more group offspring. (b) Trait-group model of group selection with arbitrarily defined groups (gray boundary lines). Groups with more cooperators make a higher contribution to a shared reproductive pool, from which new groups are formed. Reproduced from West, S.A., Griffin, A.S., Gardner, A., 2007. Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. Journal of Evolutionary Biology 20, 415–432, with permission from Wiley.

of the environment of individual, and the type in which groups themselves are units of selection (Hammerschmidt *et al.*, 2014).

The trait-group model of group selection can be alternatively conceptualized as individual selection with fitness-affecting interactions between neighbors – as is the case for kin selection. Group selection can effect the evolution of cooperation when variation between groups is increased relative to variation within groups – a situation that can be achieved by increasing the coefficient of relatedness, *r*, between interacting individuals (Frank, 1998). Indeed, it has since been argued that the two models are identical (Wade, 1985; Queller, 1992; Frank, 1998; Nowak *et al.*, 2010), although Traulsen (2010) has demonstrated that this mathematical equivalence is in fact only found in special cases. Similarly, reciprocity can drive assortative interactions between cooperators, decreasing the variation within groups of interacting individuals, and

increasing variation (and selection) between groups. In fact from a theoretic perspective, groups of a particular structure are unnecessary for the evolution of cooperation in those instances where the population is structured so that individuals interact with their neighbors (Maynard Smith, 1976; Godfrey-Smith, 2009). Cooperators can persist in a population if they are more likely to interact with each other than by chance (Godfrey-Smith, 2009). Therefore, the key principle common to the three classes of explanation for the evolution of cooperation is 'correlated interaction' between individuals (Godfrey-Smith, 2009). Preferential interactions among kin, reciprocity, and group structure are all different mechanisms for achieving correlation between traits expressed in a population.

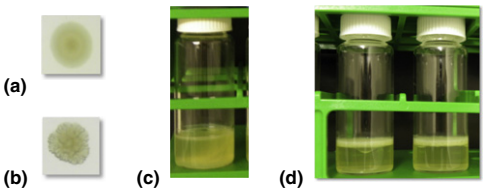

Figure 2 *Pseudomonas fluorescens* grown on solid and liquid media. (a) SM colony on solid agar. (b) WS colony on solid agar. (c) SM cells colonize the broth phase of liquid media in a microcosm. (d) WS cells form a visible mat at the air–liquid interface in a microcosm. SM and WS phenotypes (a,b) are heritable and correlate with both genotype and niche specificity (c,d). SM, smooth; WS, wrinkly spreader (Bantinaki *et al.*, 2007; Rainey and Travisano, 1998; Spiers *et al.*, 2002).

Examples of Bacterial Cooperation

Myxobacteria

The myxobacteria (to which the model species *Myxococcus xanthus* belongs) exhibit some of the most sophisticated cooperative interactions in the bacterial kingdom. Social swarming of *M. xanthus* allows the coordinated 'wolf-pack' predation of many other species of microbes, which they kill and degrade with costly extracellular digestive enzymes (Reichenbach, 1999). Furthermore, when they encounter starvation conditions, individuals aggregate into groups of ~100 000 cells and construct elevated fruiting bodies in which stress-resistant spores are formed and are capable of dispersing to unexploited resource patches. Only a portion (10%) of cells within the fruiting bodies differentiate into spores, while 30% of cells make up the exterior of the fruiting body, and the remaining cells undergo programmed cell death (Wireman and Dworkin, 1977; Dworkin, 1996), which is thought to provide nutrients to the other cell types. Developmental 'cheaters' evolved from an ancestral *M. xanthus* strain propagated in the laboratory under optimal growth conditions (nutrient-rich medium) suggest that this behavior is individually costly (or maladaptive in rich shaken laboratory culture), and an adaptation to fluctuating nutrient availability (Velicer *et al.*, 2000). When mixed with their ancestor, clones from several evolved lines cheated by being overrepresented in the spores of the fruiting body relative to their initial frequency in the mixture. Moreover, Fiegna and Velicer (2005) observed the re-evolution of social proficiency by an obligate cheater in a fluctuating environment with alternating cycles of starvation and growth. After four cycles the obligate cheater, which was introduced as a rare (1%) invader of a population of the marked ancestral strain, increased in frequency by re-evolving the ability to undergo social development.

Pseudomonas fluorescens

The evolution of cooperation has been observed in laboratory populations of *Pseudomonas fluorescens*, a free-living motile bacterium that is commonly found in the plant rhizosphere. The wild-type phenotype of the strain SBW25 is termed 'smooth' (SM) because of the appearance of its colony morphology when grown on solid agar plates (**Figure 2(a)**). In spatially structured 'microcosms' containing the ancestral SM genotype (**Figure 2(c)**), surface biofilms arise from single mutant cells that overproduce acetylated cellulose, which acts as a cell–cell glue (Spiers *et al.*, 2003). The failure of the mutant 'wrinkly spreader' (WS) cells (so called because of their distinctive colony morphology, **Figure 2(b)**) to separate at cell division leads to the formation of a 'mat' at the air–liquid interface (**Figure 2(d)**). Natural selection favors WS cells because they gain access to an abundance of oxygen denied to SM cells due to the vertical oxygen gradient that develops rapidly within the medium.

The WS phenotype meets the criteria of a cooperative trait outlined above (Chase, 1980). First, while WS cooperators invade SM populations by occupying a vacant niche, cellulose production by actors (WS cells) is costly relative to nonactors (SM cells) (Rainey and Rainey, 2003). Secondly, cellulose production increases the fitness of other individuals: SM cells not only increase in frequency in populations founded with WS, but SM are fitter in the presence of WS than in their absence (**Figure 3**; Rainey and Rainey, 2003). Therefore, cooperating WS collectives are inevitably short-lived. Selection continues to favor mutant SM types that cheat by no longer producing cellulose, yet reap the benefits of cooperation (access to oxygen). If the number of defecting SM cells increases to too high a frequency to maintain integrity of the mat, this emergent group phenotype is destroyed, i.e., the mat collapses.

A Cautionary Note on Microbial Sociobiology

It has become increasingly fashionable to caste all microbial interactions as inherently social, however cooperation is not an inevitable outcome of communal living. Nevertheless, concerns over the potential misclassification of cooperation in cases of incidental by-products has led to many descriptions of cooperation requiring that a cooperative behavior is selected to enhance the fitness of others, and can therefore only be a target for selection if there is a fitness feedback from the recipient to the actor. West *et al.* (2007) illustrate this point with two examples, both involving the excretion of waste products that bestow a fitness benefit upon (a) dung beetles, in the case of elephant waste products; and (b) waste-utilizing microbes, in the case of bacterial waste products. Under a very broad definition of cooperation, these examples might be considered

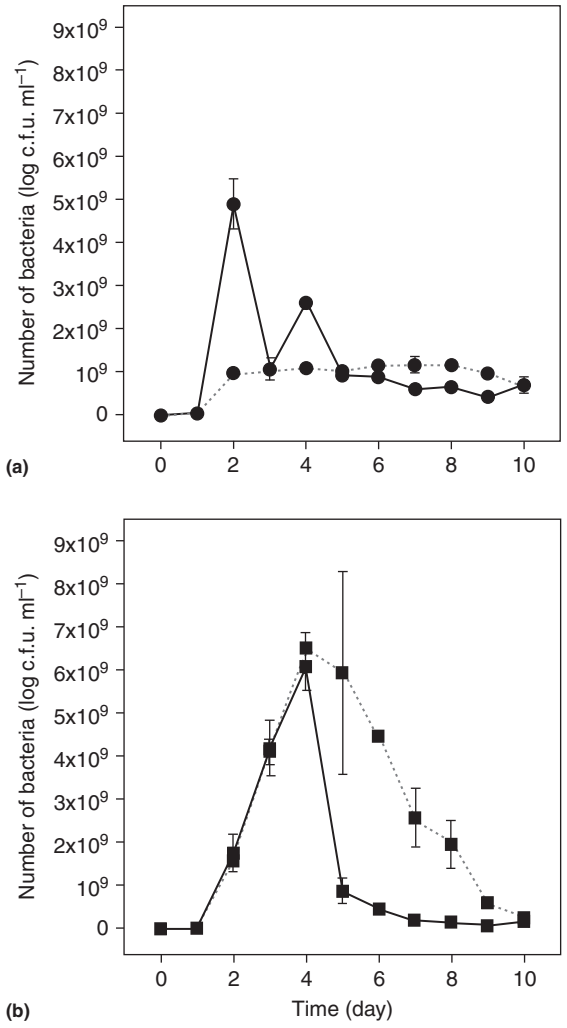

Figure 3 Evidence for cooperation in *Pseudomonas fluorescens*: population dynamics of WS and SM genotypes in the presence and absence of competition. (a) The effect of WS on the fitness of SM genotypes is the difference between dotted (absence of WS) and solid (presence of competing WS) lines. (b) The effect of SM genotypes on the fitness of WS genotypes is the difference between the dotted (absence of competition) and solid (presence of competing SM genotype) lines. SM, smooth; WS, wrinkly spreader. Reproduced with permission from Rainey, P.B., Rainey, K., 2003. Evolution of cooperation and conflict in experimental bacterial populations. Nature 425, 72–74.

'mutually beneficial cooperation,' because both the actors and recipients receive a benefit from the waste produced by the actors. To prevent the misclassification of biological phenomena such as by-products, the authors add the criterion that a cooperative behavior must be selected because of its beneficial effect on the recipient. However, under the definition of cooperation described above by Chase (1980), Velicer (2003), and many others, a cooperative behavior is costly relative to nonactors. The production of waste products by elephants and bacteria is not costly relative to those who do not produce waste; in fact waste disposal is individually beneficial, therefore these are not examples of cooperative interactions. Secondly, mutually beneficial interactions, by definition, benefit the actor, and therefore may originate and be maintained in a population because of the benefit incurred by actors. While nonactors may arise in the population and receive a benefit, it does not follow that the cooperative behavior was selected because of the benefit received by the recipients.

Syntrophy

Syntrophic (cross-feeding) interactions, which are due to the nutritional interdependence that underpins the symbiotic relationship between many species of bacteria, are common in the microbial world, yet they present no dilemma in terms of the evolution of cooperation because they are not costly.

The de novo evolution of syntrophy in bacteria was observed by Julian Adams *et al.* in experiments with *Escherichia coli* growing in an unstructured environment with a single limiting resource (glucose) (Helling *et al.*, 1987). The continuous culture evolved to become stably polymorphic after ~100 generations due to adaptations that allowed them to coexist by exploiting different niches. The less dominant strain (CV101) was maintained in the population because of adaptations that enabled it to utilize a metabolic by-product (acetate) excreted by the dominant strain (CV103) (Rosenzweig *et al.*, 1994). While the maintenance of CV101 was entirely dependent on the success of CV103, this mutually beneficial interaction is not cooperative because there is no obvious cost to the producer.

Cross-feeding interactions are common between different species in bacterial biofilm communities. The experimental work of Hansen *et al.* (2007) showed that simple mutations can lead to a stable symbiotic association between two unrelated soil-inhabiting bacteria, *Acinetobacter* sp. and *Pseudomonas putida*. When the two species were propagated in a spatially structured environment with benzyl alcohol as the sole carbon source, the persistence of *P. putida* was dependent on *Acinetobacter* for provision of a metabolizable form of carbon, benzoate. Mutations in the lipopolysaccharide (LPS) biosynthesis gene *wapH* gave rise to a 'rough' morphological variant of *P. putida*, which led to a more intimate and specialized association between the two species. No individually costly behavior underpinned the evolution and maintenance of this interaction between the two species; symbiotic relationships between species within bacterial biofilm communities are therefore not dependent on cooperation.

Public Goods

Public goods are extracellularly secreted compounds that are equally available to be used by the producer cell and all other ('public') cells in the population. By this definition, the production of public goods is a cooperative behavior because it is costly, and it increases the fitness of other cells in the population. However, some bacterial functions are 'leaky' – many bacteria unavoidably produce publicly available resources, yet this cooperative behavior can be automatic and based on selfish interests (Morris *et al.*, 2012). Confusion arises because there is a tendency to assign all extracellular products as public goods, however evidence is lacking.

Bacteriocins are extracellular toxins released by some bacteria that kill members of their own species, except those that

carry a linked immunity function. The production of bacteriocins is lethal to the small proportion of cells that do so, because the cells must lyse to release the molecule, and in so doing release nutrients that can be utilized by other cells in the population. In a series of elegant experiments, Chao and Levin (1981) sought to understand the evolutionary origins and maintenance colicin production, a bacteriocin produced by some strains of *E. coli* following terminal DNA damage (Salles *et al.*, 1987). In a well-mixed environment, a colicin-producing strain was able to invade a non-producing sensitive strain, but only when the initial frequency of producers in the population was higher than ~2%. Below this frequency, the colicin concentration was not sufficient to wipe out the sensitive population because the death rate of the producer was greater than the kill rate of the sensitive strain. The opposite was observed in a physically structured environment. When propagated on semisolid agar, the unstable equilibrium disappeared and the colicin-producing strain was able to invade from rare because the extra resources released from the lysed cells were disproportionately available to other producer cells within the colony. Colicin production must therefore have evolutionary origins in unstructured habitats that prevent the 'public' availability of the molecule.

Siderophores are small iron-scavenging molecules secreted by bacteria in iron-limiting environments, and are often considered 'public goods' without necessarily assessing both the cost of production and the availability of the molecule in the population. The generality of the conclusion that siderophore (pyoverdin) production in the bacterium *P. aeruginosa* is a cooperative public good (e.g., Griffin *et al.*, 2004) has recently been questioned on the basis that only under limited laboratory conditions can pyoverdin producers be considered cooperators, and non-producers 'cheaters.' Moreover, pyoverdin production can be personalized (i.e., it is not 'publicly' available) and there is inadequate evidence for a cost to pyoverdin production in the environment under which it evolved or is maintained outside of the laboratory. See Zhang and Rainey (2013), Kümmerli and Ross-Gillespie (2013), and Rainey *et al.* (2014) for further reading on this debate.

See also: Natural Selection, Introduction to. Sociobiology, History of

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Cospeciation

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Glossary

Codivergence The process in which a lineage diverges into two new phylogenetic lineages, be they species, populations, strains or genotypes, as a result of the host lineage also diverging into separate lineages.
Cospeciation The process in which a lineage speciates as a result of another speciation event: more specific than codivergence, it is concerned only with species.
Definitive host That host species of a parasite or pathogen that is required by the parasite or pathogen for its reproductive stage.
Duplication The coevolutionary event where a parasite or similarly dependent taxonomic unit speciates independently of its host species, and both new parasite species remain on that host.
Host With respect to a parasite or pathogen, a species that is infected or occupied by that parasite or pathogen as a major part of its life cycle (see parasite, pathogen, definitive host).
Lineage The path through evolutionary time of a species, disregarding any speciation events that may have occurred on that path. The simplest such path is that from one speciation event to the next speciation one, as represented on a phylogenetic tree as a single edge.
Lineage sorting The coevolutionary event where a parasite currently infecting a host species that has itself speciated, only persists on one nascent host species.
Loss When comparing dependent and independent evolutionary trees, the situation where a parasite is unexpectedly absent from a host species, either having undergone a linear sorting event, an extinction, or from having simply not been observed by chance.
Parasite A species that uses another species as an integral part of its life cycle.
Preferential host switching The observation that parasites tend, when infecting new host species, to switch to more closely related host than to more distantly related ones.
Species In the context of this article, a phylogenetically distinct group of individual organisms which have as a collection diverged from another such group.

Article Outline

This article will begin with some definitions, and then discuss some of the causes and effects of cospeciation in general. Next there are a few particular cases, with no claim that this is a comprehensive list. After that there is a brief overview of methods of detecting cospeciation, and a short summary.

A Few Examples

There are many examples of cospeciation that have been studied to a greater or lesser degree. Among 'coevolutionists' perhaps the most famous is the case of the pocket gophers and their obligate parasites, their chewing lice (Hafner and Nadler, 1988). This is one of the rather beautiful cases that support so-called Fahrenholz' Rule, which is that parasite phylogeny should mirror host phylogeny (Fahrenholz, 1913).

Another lovely case concerns the relationships between mealybugs and their primary endosymbionts, showing very high congruence over several genera of mealybugs (Downie and Gullan, 2005). In that system the host and symbiont trees share around three-quarters of their internal branches (clades): such a relationship has a vanishingly small probability under a null hypothesis of evolutionary independence, and these common clades correspond to hypothesized episodes of cospeciation, which must have been happening for long periods and dominating the (co)evolution of these species. The close phylogenetic congruence perhaps is not so surprising, given the obligate nature of the endosymbionts with their hosts, but one that is less obvious is the apparent codivergence of populations of Heliconius butterflies with their comimics, through a much more subtle link, Müllerian mimicry (Hoyal Cuthill and Charleston, 2012a).

Evidence of codivergence also can be found in many other interactions, such as the tri-trophic system of flies (Diptera) and their parasitic nematodes, together with their myrtle (Myrtaceae) plant hosts (Nelson *et al.*, 2014); for leaf hoppers and two sets of their own bacterial endosymbionts (Takiya *et al.*, 2006); for ectoparasites of gut flagellates in termites (Desai *et al.*, 2010).

Augustus De Morgan wrote in his Budget of Paradoxes (published in 1872, after his death) (de Morgan, 1872), that

> Great fleas have little fleas upon their backs to bite 'em,
> And little fleas have lesser fleas, and so ad infinitum.
> And the great fleas themselves, in turn, have greater fleas to go on;
> While these again have greater still, and greater still, and so on.

… and this idea has merit in the area of coevolutionary biology as well: it is natural that several levels of codivergence and cospeciation could in principle be uncovered through careful analysis of complex coevolving systems.

How Important Is Finding It?

Useful and interesting information emerges when we know that species or populations are codiverging – if we know such things, then we can say that, for example, two species speciated at approximately the same time. This is not quite as vacuous as it might first seem, because it means that we can lock together events on two phylogenetic trees (or estimates thereof) and then estimate relative evolutionary rates from their matched dates on the evolutionary timescale, for example.

This makes codivergence, where it is well supported by our inference methods, the equivalent in co-phylogenetics of a fossil record.

Once codivergence events are 'locked in' in this sense, then we can place other macroscopic scale coevolutionary events around them: duplication – a generic term that has come to mean a duplication of the *dependent* lineage such as a parasite, independently of the *independent* lineage such as a host.

Identifying cospeciation events has significant consequences for understanding coevolution, because it gives us a very good sense of the degree of dependence between groups of living organisms, such as between lentiviruses (including HIV) and their primate hosts. And it really is important to get those inferences right, because, for example, whether the apparently quite long association between lentiviruses and primates (Compton *et al.*, 2013) is dominated by codivergence or by one in which the viruses happen to match the host phylogeny because of other factors such as *preferential host switching* (PHS) (Charleston and Robertson, 2002; Mindell *et al.*, 1995; Siddall, 1995) has important consequences.

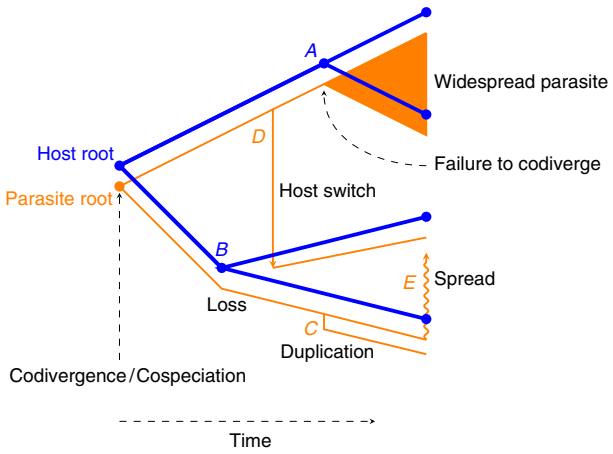

Figure 1 A simple system in which we see some coevolutionary events, including cospeciation, between some parasite species (orange tree) and some host species (blue tree). There is a cospeciation/codivergence event at the root of both trees. At host labelled A, the parasite is failing to codiverge with its host, and has not (yet) undergone speciation, perhaps because there is continued gene flow between the parasites infecting both host species even though the hosts are now genetically isolated. At B we see a loss event, also known as 'missing the boat' *(sensu* Paterson *et al.*, 2003): in this situation the parasite has failed to cospeciate and also failed to diverge, because it survives on only one of the new host lineages. Further down the tree at C we see a *duplication*, where the parasite has speciated independently of the host. The host switch is another case of not being cospeciation: the parasite has established on a new host species that is not the new sibling of its current host, by crossing the 'species barrier.' The last event is the 'spread' event, where a parasite establishes on a new host lineage but does not differentiate from the population on the original host.

Terminology

One of the most well-known kinds of diagram in biology is the *phylogeny*, or 'phylogenetic tree' or 'evolutionary tree.' In a phylogeny, *species*, or *operational taxonomic units* (OTUs) are represented as nodes or vertices, and the links between them are known as lineages. The known species are represented by leaf nodes (or simply 'leaves') in the tree, and hypothetical species are represented by internal nodes (see **Figure 1**). A single link between two nodes is called an edge or arc of the tree, and these represent the periods for which there are no phylogenetic divergences, at least, not marked on the tree.

It is (or was) common to talk about OTUs when creating phylogenies, but it is now more common to use phylogenetic *lineages* instead; this will be the usage here. A lineage in this sense is a group of individuals that is phylogenetically distinct from another such group. Thus a lineage (as with an OTU) can be a species, or a subspecies, or it could be a genetically isolated population, a strain, and so on.

Cospeciation is an idea that has been around for a long time. Clayton, Page, and others call it a process by which speciation in one lineage is accompanied by speciation in an associated, but unrelated, lineage (Johnson and Clayton, 2002; Page, 1991).

With this more generalized usage 'speciation' is a bit too restrictive, so we will speak here about *phylogenetic divergence*, or more simply just *divergence*, which means the splitting into two phylogenetically distinct lineages. The natural continuation of this decision in nomenclature is that we use *codivergence*, even though this article is called 'Cospeciation,' to mean the generalization of cospeciation to any phylogenetic lineages. Still, all the concepts described here apply to speciation and cospeciation as the dominant special cases.

Such a tree is often presented as having a defined *root*, that is, a (generally hypothetical) common ancestor of all the extant species represented by the leaves of the tree (see **Figure 1**). This root gives a sense of direction to the tree, which is necessary to this discussion of cospeciation (codivergence).

For convenience in this article we mainly refer to *hosts* and their *parasites*, because it is in such a context that the ideas of cospeciation (and codivergence) are most easily grasped. The exemplar case of this is the very well-studied gopher–louse system in which there is very strong evidence of coevolution and indeed of cospeciation, but such clean cases are comparatively rare (Hafner and Nadler, 1988). This should be treated as a simplification of the discussion with a view to clarity, but not of the underlying assumptions: it is of course possible that codivergence could occur with an intermediate host, for example, if that intermediate host is an instrumental vector for the parasite, and evidence for codivergence has been found in the Heliconius butterfly co-mimicry complex, driven only by Mullerian mimicry (Hoyal Cuthill and Charleston, 2012a).

Further, cospeciation in host–parasite systems is largely concerned with the *definitive* host of parasites, that is, those that are required for the reproductive phase, because it seems most likely that cospeciation will occur with the host with which parasites have this closest of links, rather than with intermediate hosts or perhaps vectors.

While we might consider genes as 'parasites' of their hosts (Dawkins, 1989), and in some sense species endemic to a given geographical area cospeciating as they undergo vicariant speciation with their range as it is fragmented, we will talk herein about the *dependent* lineages as parasites. The host–pathogen context is often distinguished from the host–parasite one, as there are some significant enough differences between the two situations: pathogens are frequently not as host-specific as parasites are with their definitive hosts, and often are much more motile, even being air-borne; however they share the same overall structure: one set of entities, the independent one, exerts a strong selective pressure on another set when it diverges. Another related set of problems concern the relationships between gene-trees and species trees, and between geographical regions and their endemic species. These scenarios are very similar in general principle to the host–parasite cophylogeny problem, so many of the methods and techniques that apply to hosts and parasites can be carried over to these other contexts.

Correspondingly this discussion will speak of *hosts*, not 'host species' or areas of endemism; this keeps the discussion relatively clean and manageable.

We will further avoid the whole 'Species Concept' question and restrict ourselves only to considering phylogenetic species, that is, those groups of organisms that can be differentiated by their phylogenetic history. Typically two species that are deemed 'phylogenetically distinct' in this sense have accumulated a certain level of divergence in their genetic makeup, for example a few percent difference in nucleotides in the coding genes known for the organisms in question. This avoids the difficulty of establishing whether viable hybrids might form between two lineages, and of gradual differentiation along a geographical range.

Thus we can now talk about *phylogenetic codivergence* or just *codivergence*, which is the process in which two ecologically linked lineages, such as a host species and one of its parasite species as with the now infamous gopher–louse system (Hafner and Nadler, 1988), or a mimicry complex such as the Heliconius butterflies (Hoyal Cuthill and Charleston, 2012a), diverge at the same approximate time, because of uni- or bi-directional influence of one speciation (divergence) event on the other.

How Does Cospeciation Happen?

Suppose we have a population of (for the sake of argument) furry, ground-dwelling creatures that live with plenty of gene flow across the population, over a wide plain, and which are parasitized by a species of fur-dwelling, skin eating ectoparasite that is highly host-specific, preferring only these hosts. Suppose further that the plain becomes broken by a crevasse, perhaps through severe lowering of the water table, and our furry creatures become isolated from each other, forming two sub-populations. Conditions on either side of the crevasse are slightly different from each other: perhaps one area is slightly warmer, favoring shorter, finer hair, and the other area, being cooler, presents an evolutionary advantage to our furry friends if they grow slightly longer, thicker fur. Over time, the slight difference in pressures will mean that the two populations will present different environments to their fur-eating ectoparasites, and will drive their selection also. The different selective pressures on the populations of parasites will lead to their divergence: after all, there is no longer any gene flow between the two so their own smaller populations may easily drift apart from each other. (Note that this is not an argument in favor of climate change driving diversification, as it is also likely that both populations will eventually die out from lack of water.) This toy example links biogeography through vicariant speciation of the furry hosts, to cospeciation of these hosts with their parasites.

In general, if there is population isolation between two nascent host lineages, and gene flow between the parasites of those hosts is severed – which is likely in this situation, then there may be genetic drift of the parasite lineages, and the parasites will then most likely diverge.

A note on timing is pertinent here: after population isolation we expect diversification in the host species, at which point, it then presents variation in the parasites' environment, and therefore there must be an inevitable delay in the initiation of the divergence in the parasite. Thus it is not expected that the host lineages diverge at exactly the same moment as do the hosts, and at any rate it is often the case that hosts, with their generally longer generation times and slower evolutionary rates will diverge from each other more slowly than will their parasites (Page, 1991). But at the scale at which we usually see phylogenetic (evolutionary) trees, the speciation time is close enough to be called contemporary.

Host specificity is of course another major component of cospeciation/codivergence, because without a strong tie to its host, a parasite experiences only weak pressure to co-evolve with it. If the relationship with the host is very tight, such as with the well-studied gopher–louse system described by Hafner and Nadler (Hafner and Nadler, 1988), then cospeciation is to be expected, particularly if the parasite maintains most of its life cycle in the same host species. Certainly this was the view of Fahrenholz (Fahrenholz, 1913), exemplified by the idea that 'parasite phylogeny mirrors host phylogeny.' On the other hand, if the parasite is not highly host specific then it is quite possible that divergence of the host will not have a strong influence on the parasite, and cospeciation will not occur.

Host specificity can be measured in various ways but a standard statistic is S_{TD} of Poulin and Mouillot (Poulin and Mouillot, 2003), which takes into account the mean taxonomic distance across a parasite's hosts. In general, host specificity is said to be high if a parasite shows a strong preference to a given host species, that is, is much more fit on that host species than on any other, and is low in the opposite situation. S_{TD} goes some way toward capturing the idea of 'fitness' for a host, by awarding a higher score to parasites whose hosts are very closely related to each other, than to parasites whose hosts are less related.

It is not likely that there is a simple global trend toward more host specificity or away from it (Poulin *et al.*, 2006), as every system is different, but investigating how it changes over time is still important to understanding how each host–parasite system is behaving, and it is to be hoped and expected that more such studies will shed light on cospeciation in more systems.

One common question next to consider is about dependence: Which species is dependent on which other species? Which one is 'the' host, and which is 'the' parasite? Sometimes it is not clear whether there is a strong dependence–independence relationship between the two groups of species, or whether the relationships is more mutually symbiotic. In the one extreme we expect no effect of speciation in one group on the speciation patterns in the other group, because the independent (host) phylogeny is truly independent of its parasites, which can be considered as merely along for the ride. In this situation there may be some coevolutionary effects by which for example the host's immune system is responsive to changes in its parasites, but this effect is not driving speciation in the host: certainly primates are not speciating in response to the diversification of lentiviruses.

At the other end of this spectrum we might find that the two ecologically linked groups are tightly coupled in a classically symmetric symbiotic relationship, by which divergence in either group commonly leads to divergence in the other. It is even possible, as we increase the sizes of phylogenies being considered for codivergence and other coevolutionary effects, that there could be regions in the phylogenies where both extremes of the spectrum are evident: for one clade the parasites might be completely dependent on the hosts, and for another, the relationship might be balanced.

One way we might find out where on this spectrum a given system lies is to simply attempt to map one phylogeny, X, into the other, Y, according to our chosen method of cophylogenetic analysis, and then to also do the reverse, mapping Y into X. If there is no difference between the measures of fit in the two results, then we cannot differentiate which is the dependent, and which is the independent; thus we can't reject the balanced relationship. However, a survey of over 100 published cophylogeny studies (described in Drinkwater and Charleston (2014b)) in this way shows, perhaps surprisingly, that there is apparently no clear differentiation between the two extremes of this spectrum: despite the explicit or implicit assumptions of dependence in the many studies involved, only a very few show any difference between the quality of the maps when mapped in both directions. One possible reason for this is that the datasets surveyed are simply not big enough to show significant differences in the mapping qualities from X to Y or from Y to X; another reason is that we have simply been unlucky in our choice of the systems to study: we may well have only chosen systems in which there is a strong suggestion of cospeciation already, and the way we recognize these systems is based on simple similarity between phylogenies. Whatever the cause, this is an interesting problem that deserves attention.

Failure to Codiverge

What does it mean to not cospeciate or codiverge?

While we are talking about codivergence we cannot miss out a discussion on what it means for a species or lineage not to codiverge with its host. If a species fails to codiverge when we would have expected it to, then there are a few possibilities (those numbered 1 and 2 as mentioned in Banks and Paterson, 2003 (Paterson *et al.*, 2003)):

1. the parasite species have in fact diverged, but are not distinguishable, in this sense being cryptic – they may be on the verge of divergence though;
2. the host lineage did not actually diverge, despite appearances to the contrary, so there was nothing for the parasite to codiverge with;
3. the host speciated and the parasite (or, recall, pathogen, gene, etc.) did not establish in both new host lineages, populating only one – this is known as 'missing the boat' (Paterson *et al.*, 2003);
4. the parasite populations on two nascent hosts continue to have gene flow between them, therefore maintaining as a single lineage.

This last event is known in the literature as a 'failure to diverge' event, though it should really be 'failure to codiverge' (species 'fail to diverge' almost all the time). 'Failure to codiverge' is one of three events that can lead to widespread associations of parasites with hosts. The second is sometimes called *incomplete host switch* (Clayton *et al.*, 2003) or *spread* (Hoyal Cuthill and Charleston, 2012b) – this makes particular sense in the context of those parasites or pathogens that are not particularly choosy about which host they infect.

Other Coevolutionary Events

It is not possible to talk about codivergence only, when considering the differences between evolutionary histories of linked groups of organisms. If the only event was codivergence, then the trees would be identical, and there would be rather little interest in the topic! Rather, we have several other macro-scale events to deal with: these are usually referred to in the literature as *duplication*, *loss*, and *host switching*.

We will refer to **Figure 1** once more for this part.

Duplication is where the parasite lineage diverges (e.g., speciates) independently of such an event in the host lineage. The terminology comes from the connection of the cophylogeny problem to the gene tree/species tree problem: it is common for genes to duplicate, giving rise to potentially very large gene families, while remaining functional, and not driving speciation in the 'host' organism in which they reside. An example of duplication is shown in **Figure 1** at point 'C.'

Loss is what happens when there is a parasite (pathogen, gene, etc., depending on the context) that is absent from its host, where an ancestor of the host did have (an ancestor of) the parasite (**Figure 1**, at 'B'). It can arise in several ways, and this presents challenges that are very hard to address without targeted coevolutionary studies. One way in which a loss can arise is through one of the cases where codivergence does not occur: number 3 in the list above (section 'Failure to Codiverge'), the host speciates, and the parasite only establishes on one of the two new host lineages.

The second case is where the parasite goes extinct: it did cospeciate with its host, but then after a period of successful infection, died out.

The last is due to sampling error: there may well have been cospeciation, with survival of the parasite to the present, but we simply failed to find it.

The difficulty that these three events present to analyses of coevolution is that they are indistinguishable, when we are only presented with host and parasite trees and their 'known' associations. It is simply not possible to determine which of these processes took place in order for the loss to be observed, and if we are faced with loss then we should spend time looking for other evidence of the loss event being due to extinction or sampling failure; and we should also accommodate the possibility of all these processes when drawing our conclusions, not just assume it is missing the boat (which is the default interpretation in most software).

Host switching has been the main culprit that makes the detection of cospeciation difficult. A host switch (as shown in **Figure 1, 'D'**) is when a parasite infects and establishes on a new host lineage. Another culprit is the 'spread' event, shown at 'E' in the figure, and dealing with it is also a challenge that has only recently seen significant progress (Drinkwater and Charleston, 2014a).

If there is no host switching then it is trivially simple to find where cospeciation could have taken place. However, permitting host switching in the coevolutionary system takes this problem from one that can be solved in an amount of time that is linear in the problem size, to one that is in a class of problems not known to have any polynomial time solution: it is NP-Complete (Ovadia *et al.*, 2011). Dealing with host switching in cophylogenetic studies has been the major challenge in recent years to detecting cospeciation and the other macro-scale coevolutionary events: the computational difficulty means that we cannot guarantee to find optimal solutions (i.e., to find all the cospeciation events), and so recent focus has been on developing faster heuristic methods for this (Conow *et al.*, 2010; Drinkwater and Charleston, 2014a,b).

Detecting Cospeciation/Codivergence

Cospeciation is a consequence of very tight coevolution, and it in turn has consequences. We can use these consequences to detect systems that are consistent with a history of cospeciation, even in situations where the degree of match between say host and parasite species is not very high: still there can be signals left in the parasite tree that can indicate cospeciation has been a significant factor in the coevolution of the system in which we are interested.

One consequence is, as Clay in 1951 noted, that the coevolution of the parasite (in Clays work, Mallophaga, chewing lice) led to "… related groups of birds being parasitized by related groups of lice" (Clay, 1951). Put another way, in general we expect if there is a history of cospeciation that the phylogenetic distance between pairs of parasites should be significantly correlated with the corresponding distances between their hosts. This is straightforward to detect and an approach based on the statistical congruence between distance matrices derived from host and parasite trees is implemented in ParaFit (Legendre *et al.*, 2002), and a simpler test was used by Hoyal Cuthill and Charleston in a study on Heliconius butterflies (Hoyal Cuthill and Charleston, 2012a).

Note that co-speciation implies a direct dependence relation, rather than via a third influence such as through common biogeography: the apparent congruence between phylogenies of Joshua trees (*Yucca brevifola*) and its two (sister) species of obligate pollinators *Tegeticula* spp. moths is unlikely to be cospeciation, as the moths appear to have diversified much more recently than their hosts (Smith *et al.*, 2008).

Apparent congruence can also be explained by PHS, where parasites tend to switch to hosts that are more closely related to their current host than to more distant ones (Charleston and Robertson, 2002; De Vienne *et al.*, 2007).

What confounds the detection of cospeciation? While there are some other systems that show significant congruence between the evolutionary histories of host and parasite (or host and pathogen, and other variants on this independent–dependent theme), other processes can either cover up apparent cospeciation, or mistakenly support it.

PHS was presented as a plausible explanation of how two phylogenies that were *not* undergoing codivergence could still appear to be codiverging, due to their high degree of congruence (Charleston and Robertson, 2002). Under PHS, parasites (in this case, lentiviruses) were observed on hosts that were statistically closer to each other on the primate phylogeny than they would be expected to be, if the host switching was random. It was noted in that paper that PHS could give rise to apparent congruence between the primate and lentivirus trees, even without a long history of codivergence. PHS and the tendency of parasites or pathogens to simply spread, without speciation, to closely related host species, has also been observed and goes part-way to explain the nature of host switch dynamics in general (Hoyal Cuthill and Charleston, 2012b).

Summary

In a sense the community of scientists interested in research in coevolution at the species level has been misled by the remarkable congruence between the gophers and their chewing lice (Hafner and Nadler, 1988). Since that important publication there have been many other studies seeking congruence, with reasonable confidence that there will be some, yet in fact the number of clear cases of cospeciation has been relatively few in comparison with those showing little indication of cospeciation.

Why Is It So Hard to Find Cospeciation?

Partly this comes from a lack of targeted studies of coevolution: reports in the scientific literature commonly have one half of the coevolutionary picture investigated quite well and the other be rather more sketchy, and there are very few studies that seek specifically to find host–parasite associations. Another confounding factor is that even in the presence of some cospeciation there may also be, and commonly is, some host switching, and it does not take very many host switches to

completely mask the congruence that a history of pure codivergence would yield.

There are many complicating factors: host–parasite associations are hard to define, as well as to uncover; parasite life cycles can be cryptic; and cophylogeny mapping is a computationally hard problem. Even small amounts of host switching can mask a history that has many cases of cospeciation.

While it is unlikely that a 'big push' to gain better data is to be expected any time soon though, useful data and better understanding continue to show gains and it is clear that a wealth of understanding of coevolution will come from studying cospeciation.

See also: Parallel Speciation. Phylogeography. Sequential Speciation. Symbiosis, History of. Vicariance Biogeography

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Darwin's Finches, the Galapagos, and Natural Laboratories of Evolution

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Introduction

Darwin's Finches, also known as the Galápagos finches, have achieved iconic status as exemplars of evolution. Darwin famously misidentified several of the finches he collected during his 5 weeks in the Galápagos, but eventually recognized their potential significance to the study of evolution. During the Age of Surveys, many naturalists followed Darwin to the Galápagos and collected numerous finches along with the fauna of the Galápagos. Most confined themselves to the taxonomy of finches, but a few drew conclusions about the ecology or evolution of the group. Darwin's finches emerged as an icon of evolution when David Lack published his study of the finches in 1947. Among others, E.O. Wilson and William Brown cited Lack's work with the finches as a potential model for character displacement. Peter and Rosemary Grant launched a long-term study of the finches on this premise, but over 40 years, their research in this 'natural laboratory' has extended to many aspects of the finches: evolution, genetics, hybridization, ecology, and conservation (Figure 1).

Darwin and the Finches

During the voyage of HMS Beagle, Charles Darwin himself collected specimens of the finches. However, he famously misidentified some of the finches, one as a blackbird and another as a grosbeak. Back in England, the ornithologist John Gould set Darwin straight and described the finches as a highly diverse group of closely related species. In the first edition of *The Voyage of Beagle* (1839), Darwin noted, "a nearly perfect gradation of structure in this one group can be traced in the form of the beak" (Darwin, 1839). Later in his account of the wildlife of the Galápagos, Darwin tentatively suggested that different finches may have been confined to different islands. However, Darwin had labeled his specimens 'Galápagos,' so he could not confirm such suspicions using his own collection. Both the captain and the crew of HMS Beagle collected finches while they conducted hydrogeographic surveys of most of the islands. Darwin and Gould drew on these collections to speculate that different finches occurred on different islands. In addition, Darwin attempted to place the finches he collected to specific islands based on his vague recollections and the other collections. Subsequent research has shown that Darwin's designations constituted educated guesses. However, when he published his suspected localities in *The Zoology of the Voyage of HMS Beagle*, they became inscribed in the ornithological record as later ornithologists at the British Museum relabeled some of the finches to conform to Darwin's conjectural localities (Sulloway, 1982, 1984).

Figure 1 *Geospiza magnirostris*, Plate 37 (Gould, 1839).

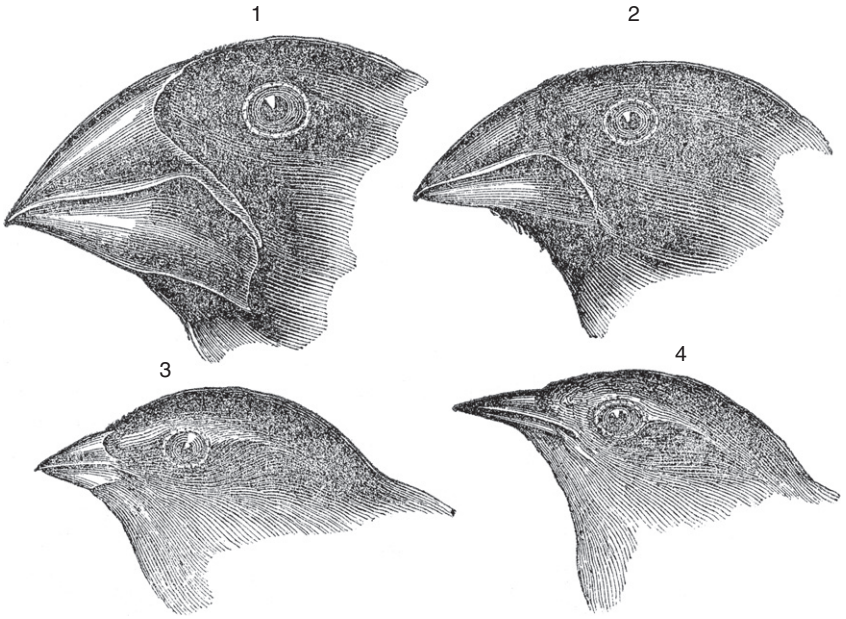

Figure 2 Galápagos Finches as illustrated in Darwin, Voyage of the Beagle, second ed., 1845.

Darwin's thinking about the finches expanded considerably before he published the second edition of the *Voyage of the Beagle*. He elaborated on the 'perfect gradation in the size of beaks' and he included a drawing to illustrate the heads of four of the finches that showed the range of bill size from the largest to the smallest as well as two of the species with intermediate bills (**Figure 2**).

Darwin (1845, p. 380) even speculated on the potential significance of the finches for biology: "Seeing this gradation and diversity of structure in one small, intimately related group of birds, one might really fancy that from an original paucity of birds in this archipelago, one species had been taken and modified for different ends." Such a statement strongly suggests that Darwin's view of evolution or transmutation had developed considerably during the 6 years between the first and second editions of *The Voyage of the Beagle*.

With the exception of the rather provocative statements in the second edition of the *Voyage of the Beagle*, Darwin did not include the finches in his subsequent writings on evolution, or anywhere else for that matter. He mentioned the Galápagos Islands six times in the *Origin of Species* and drew specific attention to the evolutionary significance of islands and animal and plant colonizers, but not the finches (Sulloway, 1982; van Wyhe, 2012). Despite Darwin's reticence on the subject, other naturalists strove to fill the remaining gaps in the natural history of finches beginning with the taxonomy of the closely related group (Donohue, 2011).

The Age of Surveys

Darwin introduced the ornithological community to the Galápagos finches, but his collections included only 31 finches and 64 birds in total from the Galápagos. He sought to obtain representative (or 'type') specimens of each animal (and plant) he encountered. Naturalists on subsequent expeditions to the archipelago sought to represent variations between the finches with larger collections.

In 1868, Habel collected 460 specimens, which Salvin described in 1876. The British Museum of Natural History became the repository of the collections of the 'Beagle' and Habel. In 1897, the ornithologist Robert Ridgway described the collections of the *Albatross* (1888) and George Baur (1891). The 'Albatross' collections remained at the United States National Museum (Smithsonian), Ridgway's home institution, while Baur's collection and that of the Webster-Harris expedition (3075 specimens) became part of the Rothschild collection, which was eventually acquired by the American Museum of Natural History in New York. The Hopkins-Stanford expedition amassed another large collection that Robert E. Snodgrass and Edmund Heller described in 1904. But an expedition by the California Academy of Sciences obtained the largest collection (8691 specimens) in 1905 and 1906. Harry S. Swarth published on this collection in 1929 and 1931 (Swarth, 1929, 1931).

Drawing in part on Baur's collections from the Galápagos, the Smithsonian ornithologist Robert Ridgway (1897) described the many forms of birds from the Galápagos. Unlike Baur, who sought to restrict the number of forms per island, Ridgway represented the other taxonomic extreme in designating numerous forms as full species. He believed that previous taxonomists had been too conservative in designating species. In all, Ridgway added 25 full species to the list of Darwin's finches, including 6 in the genus *Certhidea*, 12 in *Geospiza*, and 7 in *Camarhynchus*. Ridgway generally hesitated to make broader claims regarding the evolutionary significance

of the finches. Baur criticized Ridgway for arranging the different species of finches in a single continuous line to show the gradual connection between the different forms. Baur organized the finches into several parallel series, arguing that species remained true on different islands and never intergraded on the same island, which was consistent with his essential point that natural selection played no role in the development of the finches (Baur, 1897).

Other naturalists did make general claims regarding the evolution of the finches during the Age of Surveys. In one of the first ecological studies of the finches, Robert E. Snodgrass and Edmund Heller preserved the stomachs of 209 specimens of *Geospiza* between December 1898 and June 1899 with a plan to determine whether bill size correlated with food type. Snodgrass hypothesized if the various sizes and shapes of bills within the *Geospizae* were adaptations, then it would be possible to differentiate diets (seed size) by species. The two naturalists separated the seeds by size (they did not attempt to determine the plants whence the seeds came), but found no patterns of consumption. Thus, Snodgrass (1902) concluded: "The evidence, then, seems to be in favor of the general conclusion that *there is no correlation between the food and the size and shape of the bill*. If this is true, then we must look elsewhere for an explanation of the variation of the *Geospiza* bill." After enumerating the remarkable extent of finch collecting efforts, David Lack noted: "As a result of all these visits, Darwin's finches are more adequately represented by museum specimens than almost any other group of birds" (Lack, 1947). Robert Kohler has reframed this period in the history of biology as the 'Age of Survey' (Kohler, 2009).

Percy Lowe (1936) coined the term 'Darwin's Finches' in an address to the British Ornithologist's Union in 1935 to mark the centenary of Darwin's visit to the Galápagos. On the basis of his anatomical studies of finches, Lowe argued that Fringillidae was the appropriate family designation for the finches. To explain the diversity of finches, Lowe proposed that the group represented a hybrid swarm, but he called for actual breeding experiments because Lord Rothschild had already suggested that if the extensive collections in existence could not provide the information, none would.

David Lack

By the 1930s, ornithologists had reached general consensus regarding the systematics of Darwin's finches. From December 1938 to April 1939, the Oxford ornithologist David Lack led an expedition to the Galápagos to study three aspects of the biology of the finches: breeding behavior, ecology, and hybridization. Lack's careful study of the breeding behavior revealed that the finches were very similar to each other. Similarities in breeding behavior corroborated anatomical findings that suggested a close relationship between the species. Ecological studies revealed three aspects of finch natural history: the near absence of food competitors, almost complete absence of predators, and the existence of several islands that provide partial but not complete isolation for island forms. Each of these ecological factors influenced speciation. But Lack (1945, p. 135) concluded: "Differences between closely related species are nonadaptive except that bill characters serve in species recognition. The main genera show adaptive radiation." Lack (1940) also published a brief article on his findings in the journal *Nature*, and he again denied evolutionary agency. Sewall Wright's random drift provided a better explanatory framework.

With the publication of *Darwin's Finches* (1947), Lack revealed that his views on the ecology and evolution of the finches had changed since his earlier writings. He had accepted Geogii F. Gause's (1934) contention that no two species can occupy the same ecological niche in the same place as a consequence of natural selection. If two species did in fact live in the same habitat and ate the same types of food, competition would inevitably result, and one species would eliminate the other. Given that there were three species of ground finches (*Geospiza*) and at least two tree finches (*Camarhynchus*) living together in the same habitat on the Galapagos, Lack theorized that another factor prevented these species from competing.

Lack explained that geographical isolation appeared to be the critical factor in speciation. To bolster his case, Lack surveyed bird species and found that in every species that maintained continental and island populations, the island races differed from each other more strikingly than the continental races. Moreover, the more isolated an island was, the greater the degree of differentiation within the land birds. Thus, Lack (1947, p. 119) concluded: "The primary cause of geographical variation in birds would seem to be not adaptation, but isolation." Darwin's finches provided Lack's case in point. The islands that were the most isolated had the highest proportion of endemic forms, while more central islands had fewer distinct forms. Lack wrote: "Hence in Darwin's finches there is a marked correlation between the degree of isolation and the tendency to produce peculiar forms" (p. 119).

Lack also wondered about forms that were originally geographical races of the same species, which met later in the same region, remained distinct, and formed new species. How might these forms compete? Lack offered four possibilities: one much better adapted form swamps the other and exterminates it; one form has an advantage in the region where it meets another form, but the other has an advantage in an adjacent region; one form may have an advantage in a section of the original habitat and another in the rest; and one form proves better adapted to taking one food, and the other to obtaining other foods. Lack (1947) noted that the differences in food habits were often associated with marked differences in size, including the size of beak. Darwin's finches provided at least two cases of each type of ecological isolation. For example, *Geospiza conirostris* replaced *Geospiza scandens* on three outer (geographically remote) islands. *Geospiza scandens* bred in the same habitat with other ground finches but fed on *Opuntia*. *Geospiza magnirostris*, *Geospiza fortis*, and *Geospiza fuliginosa* occupied the same habitat but their foods were different, at least in part. Lack suspected a similar state of affairs for three species of tree finches, though their dietary preferences remained unknown (**Figure 3**).

Lack's earlier writings on the finches, though transcending the taxonomic studies of the Age of Surveys to make claims regarding evolutionary significance, seem to have been missing a theoretical framework with which he could integrate the data from his ecological studies of the breeding and feeding behavior of the finches with systematic data from the large collection of

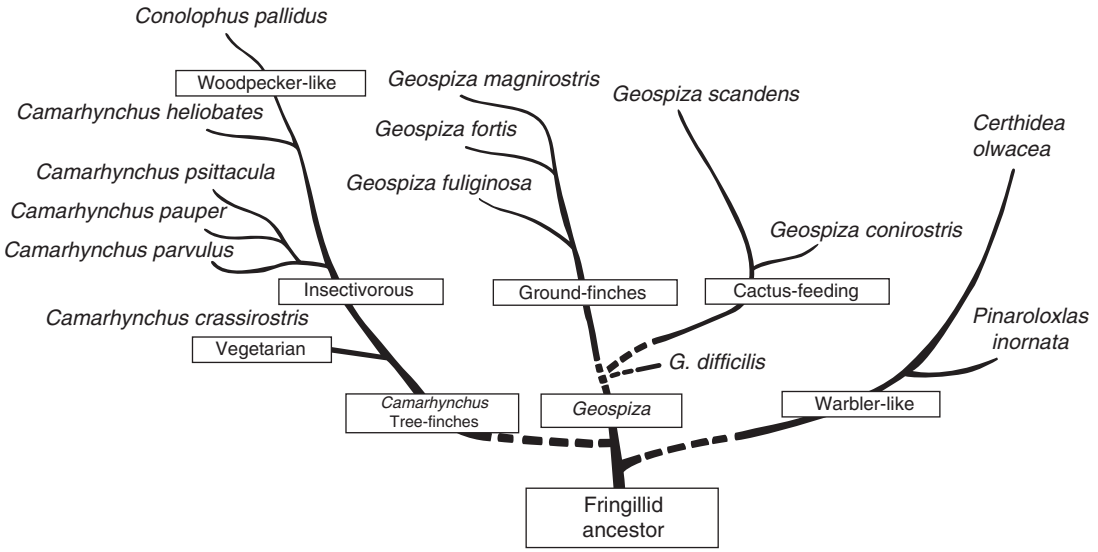

Figure 3 David Lack's tree showing his hypothesis about the evolutionary history of the finches. Reproduced from Lack, D., 1947. Darwin's Finches: An Essay on the General Biological Theory of Evolution. Cambridge: Cambridge University Press, with permission from Peter Lack on behalf of the David Lack Estate.

finches at the California Academy of Sciences. We have seen the influence of Gause's ecological niche concept for Lack's understanding of evolutionary patterns. Lack also cited Julian Huxley's (1942) writing on the modern synthesis and Ernst Mayr's (1942) *Systematics and the Origin of Species*. In this way, the unification of biology provided a new theoretical rigor that enriched Lack's *Darwin's Finches* (and countless biology textbooks).

In 1956, William Brown and E.O. Wilson identified Darwin's finches (*a la* Lack) as a striking case of character displacement. Citing Lack's data regarding *G. fortis* and *G. fuliginosa*, they noted that, on most islands where the two species occur together, the two species could be separated by the measurement of beak depth, and a random sample of this character resulted in two completely distinct distribution curves. However, a comparable sampling on the smaller islands of Daphne and Crossman revealed a single unimodal curve that fell between the curves of *fortis* and *fuliginosa* on the larger islands. Lack's careful analysis of beak to wing proportions revealed that *fortis* occurred on Daphne while the Crossman population was *fuliginosa*. Brown and Wilson (1956) accepted Lack's conclusion that each species had converged toward the other species, thereby filling the ecological vacuum created by its absence.

After two expeditions to the Galapagos, Robert Bowman (1963) argued that the major patterns of differentiation in Darwin's finches were related to adaptations for food getting. Bowman correlated the finches' beaks to various kinds of pliers (**Figure 4**). Although Darwin had confused the finches with similar continental families, Bowman deliberately compared the individual finches with continental families on the basis of the niches field by the different species. This group of songbirds represented no less than seven continental families, which Bowman illustrated with a chart that depicted 'ancestors of the Geospizinae' (**Figure 5**).

The key to the differentiation within the finches was the kind of food they consumed. In this and other respects, Bowman challenged the interpretations of David Lack, who had identified isolation and interspecific competition as the primary engine of adaptation among the finches. On the basis of the field observations and anatomical analyses of the muscular structures supporting the finch beaks, Bowman argued that the evolutionary pattern of the Galapagos finches hinged on differing vegetation between islands and related structural modifications of seeds and behavioral reactions of insects to escape widespread aridity with profound effects on feeding adaptations in the finches. In addition, predation (by Galapagos Hawks and Galapagos snakes) had been selective forces, which led to adaptive differences in plumage between genera, species, and even populations. Finally, Bowman (1961) pointed to the 'genetic constitution of ancestral colonists' as a constraint in the ability of finches to evolve to exploit all ecological niches rather than the presence of 'ecological equivalents.'

Peter and Rosemary Grant: Evolution in a Natural Laboratory

In 1971, Peter R. Grant embarked on a new research project. First, he sought to clarify whether population variation in the size of traits such as beaks was adaptive, as suggested without convincing evidence by Leigh Van Valen. From Lack he knew that Darwin's finches could shed light on this question. Second, following Brown and Wilson, Grant found evidence for character displacement lacking (i.e., the tendency for differences between ecologically similar species to be enhanced where they occur together as a result of natural selection minimizing competition between them). His decision to examine the related process of character release (changes in morphology and ecology of a species resulting from the absence of restraints from a competitor species) led him to the classic case of two species of Darwin's finches as

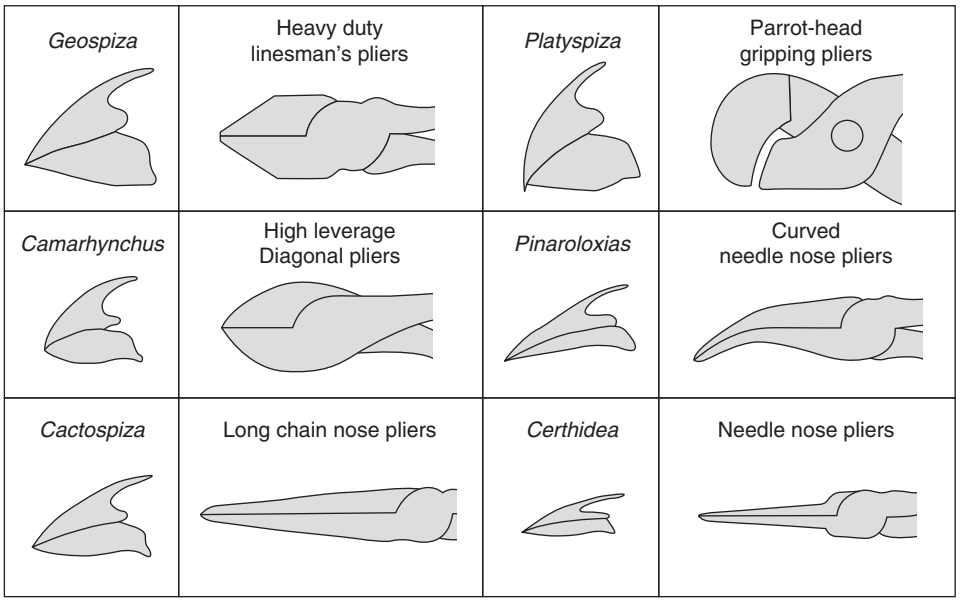

Figure 4 Comparison of shapes of bills with pliers. Reproduced from Bowman, R.I., 1963. Evolutionary patterns in Darwin's finches. Occasional Papers of the California Academy of Sciences 44, 107–140, with permission from California Academy of Sciences.

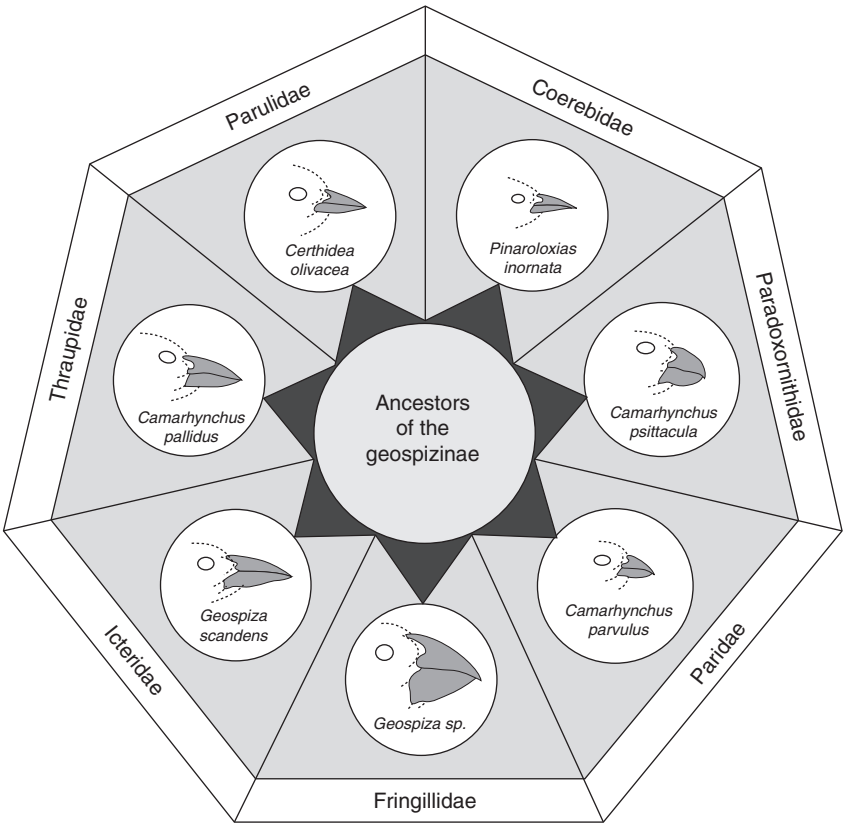

Figure 5 The pattern of adaptive radiation in Darwin's finches. Reproduced from Bowman, R.I., 1963. Evolutionary patterns in Darwin's finches. Occasional Papers of the California Academy of Sciences 44, 107–140, with permission from California Academy of Sciences.

described by Brown and Wilson (1956). Moreover, a graduate student had proposed a study of Darwin's finches on the basis of the conflicting conclusions of Lack and Bowman concerning the same material. Initially, Grant (1985) focused on the Medium Ground Finch (*G. fortis*) population on Daphne Major, the population of which was small enough so that he could uniquely color band every individual, and the birds were variable in both body size and bill shape.

On the basis of the Daphne Major population and through detailed analysis, Grant determined that, though ground finches (*Geospiza*) occurred together in nonrandom combinations, members of all pairs of coexisting species differed by at least 15% in at least one bill dimension with minimal overlap in frequency distributions. By evaluating the nature of the food supply (seed characteristics), Grant revealed a polymodal frequency distribution during the dry season, which in turn determined adaptive peaks. Grant's (1985) long-term study of the Medium Ground Finch (*G. fuliginosa*) and the Small Ground Finch (*G. fortis*) provided evidence of both character displacement (an evolutionary process) and differential colonization (an ecological process).

More generally, Grant asked why there were only 13 species of finches on the Galápagos, rather than twice that many or more. And why did the species range from 8 to 40 g, rather than from 5 to 100? Drawing on the evolutionary history of the finches, Grant suggested that isolation of the islands, ecological differences, and the passage of time all contributed to finch speciation from one to many, but limited ecological opportunity coupled with relatively limited time and the presence of other bird species all placed constraints on the continued diversification of finches.

A few years later, Grant, along with his wife and scientific collaborator, Rosemary, and graduate students, initiated a study of *G. conirostris* on Genovesa, one of the most isolated islands of the entire Galápagos archipelago. Over the course of 11 years, the Grants and their students examined the ecology, behavior, and genetics of the population predominantly through direct observation, because experimentation was limited by National Park regulations and the natural state of the population. Hence, they referred to the islands as a 'natural laboratory.' The Grants found that rainfall was the key environmental factor that influenced the population and its variation. During the 11 years, there were two major droughts and two *El Niño* years. In 1983, the plants, arthropods, and finches all reproduced throughout the prolonged period of rainfall brought about by *El Niño*. In 1985, during a drought, all reproduction ceased. Both events placed selective pressures on the finches and, more specifically, their beaks. Their observations (Grant and Grant, 1989) challenged long-held views that evolution occurs over the course of prolonged periods of time and the corollary that changes are generally imperceptible. In fact, using data from DNA, the Grants have calculated that it could take as little as 200 years for one finch species to evolve into a new species (Grant and Estes, 2009). Widely regarded as one of the most important studies in ecology and evolutionary biology (see Travis, 1990), the Grants' long-term study of Darwin's finches has demonstrated that evolution can be studied in real time. Given the right circumstances, scientists can study evolutionary change as it happens (Figure 6).

Figure 6 Peter and Rosemary Grant on Daphne Minor, Galapagos. Courtesy of Rosemary Grant.

The Future and the Finches

In addition to revealing the ecology and evolution of Darwin's finches, the conservation of these geographically constrained birds has been a recurring theme in the Grants' work. Others have also recognized the need for conservation efforts. The Mangrove Finch (*Cactospiza heliobates*) was the last of finches to be described, and the entire population was restricted to some of the mangrove forests bordering Isabela and Fernandina, but the Grants determined that the latter population may no longer exist and the Isabela birds are under threat from an introduced wasp and habitat destruction (Grant and Grant, 1997). Critically endangered and limited to highland regions of Floreana, the Medium Tree Finch (*Camarhynchus pauper*) also suffers from habitat destruction as well as introduced predators and a parasite (O'Connor *et al.*, 2010). Local extinctions of wider ranging species such as the Warbler Finch (*Certhidea fusca*) on Floreana have also occurred (Grant *et al.*, 2005). Each of these cases offers a cautionary tale for the continued survival of Darwin's finches.

Conclusion

More than 150 years passed between Darwin's first suggestion that the Galápagos finches would serve as an excellent model for evolution in nature and the full realization of that prediction. After Darwin's initial confusion, John Gould described most of the species, but the complexity of speciation vexed biologists during the Age of Survey. Like Darwin, David Lack initially misinterpreted the significance of the finches, but he eventually re-characterized the finches as a model of adaptation by natural selection. The longitudinal studies conducted by Peter and Rosemary Grant (as well as numerous students and collaborators) revealed that in the right circumstances at natural laboratories like Genovesa and Daphne, it is possible to measure evolution as it occurs. Studies of the finches' conservation status suggest that rare and widespread species alike are threatened by anthropogenic change in the Galápagos. The development of Darwin's finches as an evolutionary icon began in a fog of confusion, but a long series of empirical and theoretical studies revealed and continues to provide rich insights into evolution and ecology.

See also: Darwin–Wallace Theory of Evolution. Natural Selection, Introduction to

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Darwin–Wallace Theory of Evolution

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The Darwin–Wallace Theory of Evolution

The Darwin–Wallace theory of evolution is arguably the most important and widely influential in the entire history of science. It has resulted in one of the most revolutionary changes in human thinking in history with profound consequences for research in a wide swathe of areas including geology, paleontology, biology, taxonomy, philosophy, anthropology, psychology, literature, medicine, and theology.

The theory and its origins have therefore been written about, discussed, and debated more than any other. The story of its origins has been retold countless thousands of times over the past 150 years. Like any tale endlessly recounted, the story has considerably evolved in the telling. There are now more myths and legends about this topic and its principal players, Darwin and Wallace, than perhaps any other.

In order to understand the origins of the theory, it is necessary to understand the people and events in their original historical contexts. One of the most persistent myths is that evolution was the answer to an eternal question or mystery which scientists long sought to solve or discover. Nothing could be further from the truth.

The Age of the Earth

In Christian Europe, the world was traditionally held to be about 6000 years old based on interpretations of the Bible, which itself gave no dates or age for the earth. By the early nineteenth century the accepted age of the earth had changed through the work of many scholars studying mining, geology, and fossils. Although most of these scholars were devout Christians, from the continued investigation of the rocks, it came to be universally accepted that the world was extremely ancient.

With the discovery of ever more fossils, another dramatic fact about the history of the world was uncovered and became widely accepted. The history of life on earth had been progressive. The most primitive forms such as shells and ferns had preceded the more complex forms such as crustaceans and conifers. In later rocks reptiles appeared followed in the comparatively recent rocks by the first mammals, although of extinct types. Yet nowhere in the immense history of living things were any human fossils or artifacts found. Humans were clearly a far more recent species. This led some to believe that the creation stories in Genesis only referred to the most recent of many creations, the one that concerned us.

'Catastrophism'

At the beginning of the nineteenth century, the French comparative anatomist Georges Cuvier, through his detailed analyses of fossil bones such as the mammoth and the *Megatherium*, first proved an extremely controversial point. Extinction was a fact. Before this, many scholars held that the suggestion that species could go extinct was irreligious and impossible. Cuvier's excavations in the Paris basin showed that the further back in time one moved, the more different were the kinds of living things. Each era represented in the rocks was characterized by its own flora and fauna. These periods seemed to Cuvier to have been abruptly ended and followed by subsequent environments characterized by a new and unique flora and fauna. For someone who had lived through the French revolution, perhaps it is not surprising that Cuvier characterized these changes from era to era as revolutions. Cuvier's view is usually caricatured in recent accounts as an old-fashioned theory of 'catastrophism.' But it was extremely advanced at the time.

'Lamarckism'

In opposition to Cuvier's theory of extinction, the French naturalist Jean-Baptiste de Lamarck argued that unfamiliar fossil species had not gone extinct but had changed or evolved. For well more than a century now Lamarck's theory has almost always been misrepresented as evolution by the inheritance of acquired characteristics. This is a very inaccurate shorthand. The inheritance of acquired characteristics was not invented by Lamarck, it was not the core of his theory, and it was a very common belief amongst eighteenth- and nineteenth-century naturalists. Lamarck's theory was instead driven by a 'complexifying force' which drove species to become ever more advanced. Along the way Lamarck argued that inheritance of acquired characteristics allowed organisms to adapt to their particular environments. Cuvier so powerfully discredited Lamarck and his theory that for many years any type of evolution was considered unscientific.

More Species

Another great change was the number of species known to exist. This went from a few hundred known in Europe and the Mediterranean areas to countless thousands by the early nineteenth century. As European ships circled the globe, new species became known, from the porpoise and the dodo to the strange marsupial creatures of Australia. Systematists, like the great Swedish botanist Carl Linnaeus, created elaborate systems to arrange and sort them, and it was found that whole classes of organisms were closely related to one another through chains of similarity. Even more fundamentally, it was found that all groups fit as subgroups within larger ones, so that, for example, all species of wolves were classed with foxes, jackals, and dogs as canides. This pattern would later be explained by the Darwin–Wallace theory of branching evolution.

'Uniformitarianism'

Many histories incorrectly attribute the developments outlined so far, such as modern geology with its ancient earth and fossil

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record, to the Scottish geologist Charles Lyell and his influential work *Principles of Geology* (1830–33). But this is another modern shorthand.

Lyell conducted extensive fieldwork. He examined the volcano Mount Etna in Italy and showed that it must have grown slowly over a vast period of time. Lyell argued that events that appeared sudden on a geological scale could be the result of a long sequence of less abrupt changes. This aspect of his theory is the one usually remembered as 'uniformitarianism,' and is held up as the key to Lyell's theory. A supposed debate between 'uniformitarians' and 'catastrophists' is often invoked. Yet this was not the issue at the time, and indeed Lyell was probably the only 'uniformitarian.' Lyell's fellow geologists had no problem with mundane causes explaining past events but they objected to his insistence that causes of the same (or uniform) intensity as those of the present were all that had ever existed.

In addition to his survey of geology, Lyell also discussed what is now called paleontology. He carefully surveyed the evidence for the successive appearance and disappearance of species in the geological record. Here too he tried to show that gradual natural processes were responsible. Because he believed that species could not change, they would eventually become extinct as their environments slowly changed beyond their limited ability to adapt. So, extinction was a gradual and piecemeal process. The question was starting to arise, where did the subsequent species come from? Lyell accepted that species introductions were probably as piecemeal as extinctions and hypothesized that new species somehow appeared in accordance with the new environments. He seemed to allow that supernatural creation was involved with the appearance of new species.

Just as the volumes of Lyell's *Principles of Geology* were coming out, a young English geologist working in South America was able to put some of Lyell's ideas about gradualism to the test. He was also to find Lyell's dodging of the question of where new species came from not entirely satisfactory. His name was Charles Darwin.

Darwin

Charles Robert Darwin (1809–82) was born in the small market town of Shrewsbury, England, to a wealthy upper middle-class family. His mother died when he was eight years old. There is no evidence that he suffered any unusual effects from this as was once believed. Darwin was raised by his elder sisters and maidservants.

He attended the nearby Shrewsbury Free Grammar School as a boarder from 1818 to 1825. In October 1825 he was sent to Edinburgh University with his elder brother Erasmus to study medicine with a view to becoming a physician. While in Edinburgh, Darwin investigated marine invertebrates with the guidance of Robert Grant, a gentleman naturalist. Darwin did not like the study of medicine and could not bear the sight of blood or suffering and so his father proposed the church as a respectable alternative. The advantage to becoming a country parson would be the freedom to pursue a growing interest in natural history. To become ordained in the Church of England it was necessary to first obtain a BA degree from an English university.

On 15 October 1827 Darwin was admitted a member of Christ's College, Cambridge (van Wyhe, 2014a). Darwin was never a model student, but he did mature into a passionate amateur naturalist. He began avidly collecting beetles. His name appeared in print when some of his records of insect captures were published in 1829.

Darwin became the devoted pupil of Professor of botany John Stevens Henslow (1796–1861). Through their close friendship Darwin learned a great deal about the practice of natural science. Darwin passed his BA examination in January 1831. Shortly thereafter he was taught the basics of field geology by Professor Adam Sedgwick during a tour of north Wales.

Voyage of the Beagle

Later in 1831 Henslow recommended Darwin for the post offered by Commander Robert FitzRoy for a naturalist to travel on a Royal Navy survey ship, HMS 'Beagle.' Contrary to recent fashion, Darwin was not on board as the captain's social companion, nor was the ship's surgeon the 'official naturalist' (van Wyhe, 2013b). Darwin was expected to investigate the natural history of the lands visited and this he did to a remarkable extent.

The 'Beagle' surveyed the coasts of the Southern half of South America and the Galapagos islands and returned home via the Pacific. The voyage lasted 5 years. Darwin spent most of these years investigating the geology and zoology of the lands he visited, especially South America, the Galapagos islands, and Pacific oceanic islands. He recorded many of his specimens and observations immediately in field notebooks (Chancellor and van Wyhe, 2009). Throughout the voyage Darwin shipped home specimens which soon earned him a reputation as a collector and observer of the first order. Later he recorded his experiences in a diary which became the basis of his famous book *Journal of Researches* (1839) now known as *Voyage of the Beagle* (Keynes, 2001; Darwin, 1839).

Darwin had the opportunity to witness all of the forces discussed by Lyell, such as erosion, earthquakes, and volcanic eruptions. Darwin made several very important discoveries about the geology of South America, volcanic islands, and the origins of coral reefs by building on Lyell's ideas.

Darwin also unearthed many fossil creatures in South America. He wondered why the fossils resembled the present inhabitants of that continent more than any other species. Where had the new species come from? If species were somehow created to fit their environments, as was then believed, why were tropical species different in Asia, Africa, and South America despite the similarity of climate?

The Galapagos

Only in the middle of the twentieth century did Darwin's visit to the Galapagos come to be seen as a pivotal moment in his life, described as the occasion for a eureka-like discovery of evolution. Darwin did not become an evolutionist on the Galapagos and neither the islands finches nor their now famous beaks prompted a revelation either (Sulloway, 1982;

van Wyhe, 2012). Only much later would the case of the Galapagos animals influence Darwin's thinking.

The Big Three

During the 'Beagle' voyage and after his return, Darwin was particularly struck by three types of puzzling evidence: the succession of allied fossil forms in the same locale, geographical distribution, and the species of the Galapagos (see Darwin, 1958, pp. 118–119). These three factors suggested to Darwin that species must evolve.

The species on the Galapagos, for example, were obviously similar to those of South America, yet their rocky island home bore no connection or resemblance to South America. It seemed to Darwin that stray migrants from South America had come to the Galapagos, after the islands rose from the sea as volcanoes, and then changed over time in isolation on the islands.

Darwin also used the experience and observations of farmers and breeders to reveal that the purported limits or barriers to species mutability was a belief without foundation. Darwin mingled with pigeon breeders to learn how they created extraordinary breeds by careful selective breeding (Secord, 1981).

In September 1838, Darwin read Thomas Malthus's *Essay on the Principle of Population* (1798). For many years it was claimed that Darwin's theory was therefore influenced by the politics or economics of Malthus's book. In fact Darwin noticed none of this but was instead struck by the implications of population growth potential in humans and other species.

Malthus argued that human population growth, unless somehow checked, would necessarily outstrip food production. The focus of this argument inspired Darwin. He realized that an enormous proportion of living things are always destroyed before they can reproduce. This must be true because every species would otherwise breed enough to fill the entire earth in a few hundred generations. Instead, populations remain roughly stable year after year. The only way this can be so is that most offspring (from pollen, to seeds, and eggs) do not survive long enough to reproduce.

Darwin embarked on a vast research program of reading and experimentation that would take many years to complete. In the meantime his primary occupation was the publication of his vast 'Beagle' collections. This would take more than 10 years.

From the 1960s it became widely believed that Darwin kept his theorizing a secret and delayed its publication because he was afraid of the reaction. A large literature emerged to propose reasons or causes for this extraordinary secrecy and 20-year delay. In fact both are modern legends. Darwin told his family, friends, and colleagues about his theory and his plans to publish a large book on it. Like all of his other book projects, the species theory took even longer than he originally imagined (van Wyhe, 2007, 2013a). Nevertheless, the 20 years of work on the theory was far less than the delay in publishing many of Darwin's other works.

Vestiges of Creation

In 1844 an extraordinary anonymous book appeared which became a Victorian sensation: *Vestiges of the natural history of creation* (Secord, 2000). The book was written by the Edinburgh publisher Robert Chambers. *Vestiges* argued that nature operated according to natural laws, and that a fundamental outcome of the way the laws worked was progress. In space, dust evolved to form solar systems, the earth itself evolved to make the planet more suitable for higher life forms and life itself evolved or 'developed.' Organisms produced offspring like themselves. But sometimes, according to an even higher natural law, they would produce an offspring of a higher type. Over time, this led to life progressing ever upwards. Invertebrates, fish, reptiles, mammals, and finally humans had all followed in succession, and *Vestiges* hinted that something higher would follow us. Although widely attacked by the scientific community, *Vestiges* convinced many that life evolved. One of these was a young man named Wallace.

Wallace

Alfred Russel Wallace (1823–1913) came from a rather humble background compared to Darwin's. Wallace's father, a solicitor by training, once had property sufficient to generate a gentleman's income of £500 per annum. But the family's financial circumstances declined so that by the time Wallace was born, they were living in a quiet cottage near Usk, on the Welsh borders. Nevertheless, Wallace was not working class as he is sometimes described by modern commentators.

When Wallace was six years old the family moved to Hertford, north of London, where he lived until he was 14. Here Wallace attended Hertford Free Grammar School which offered a classical education, almost identical to Darwin's at Shrewsbury. Wallace left school aged 14 in March 1837, shortly after Darwin returned from the 'Beagle' voyage. Wallace never attended university. In recent years it has been widely claimed, though erroneously, that Wallace was forced to leave school early for financial reasons.

Over the next decade Wallace pursued a series of jobs from land surveying to an assistant teacher in Leicester. In 1848 he set out with his friend and entomologist Henry Walter Bates to work as a specimen collector in the Amazon basin. In recent years it has become increasingly fashionable to claim that they set out to solve the problem of the origin of species. This is an apocryphal, even if romantic, story (van Wyhe, 2014b). Wallace returned to Britain in 1852 but tragically lost his personal collection in a shipwreck. Undaunted, he set out for another collecting expedition, this time to Southeast Asia, then sometimes called the *Malay Archipelago*.

Malay Archipelago

Over the next 8 years, Wallace and a team of assistants made dozens of expeditions to islands from Singapore to New Guinea and collected 125 000 specimens of insects, birds, mammals, and so forth. Wallace discovered hundreds of new species including the world's largest bee and rarest cat.

In 1855, while living in the province of Sarawak on the great island of Borneo, Wallace wrote his first theoretical paper on species: 'On the law which has regulated the introduction of new species' (Wallace, 1855). Wallace argued that: "Every species has come into existence coincident both in time and

space with a pre-existing closely allied species." Although a lucid analysis of the paleontological and biogeographical evidence of the time, the paper did not explicitly state that species evolve. Instead Wallace left this point to be inferred. Many modern readers, however, mistakenly assume that the essay openly declared evolution.

Wallace continued reading about geology and paleontology and jotting notes about how he believed species changed. As he collected more and more specimens and observed the change of animals from island to island, his ideas about life also evolved. He was convinced that species must be related genealogically – not just somehow created to suit their environments. But he was certainly not, as many modern commentators put it, searching for a 'mechanism' for how evolution works. This is a later manner of thinking.

In February 1858 Wallace was living on the island of Ternate in the Moluccas, the fabled spice islands, west of New Guinea, and then part of the Dutch East Indies. According to his later recollections, Wallace was suffering from a recurring bout of fever when he suddenly conceived of an explanation for the origin of new species. When he recovered, he wrote an essay entitled 'On the tendency of varieties to depart indefinitely from the original type.'

In this extraordinary essay Wallace reminded his readers of the well-known principle of the 'struggle for existence' which kept population numbers in check. Modern readers often misinterpret this initial discussion as if it were about natural selection, but this is a mistake. After establishing the point about how in the normal state of the natural world balance is maintained by the strong living and the weak dying, Wallace then made an analogy: just as there was a struggle for existence amongst individuals of a species, the same was true for varieties or races (essentially modern subspecies). As an environment gradually changed over time a species would become unsuited and die out. Among its daughter varieties, there might be one that happened to suit the new environment. This variety would then become the new species. It could never return back to the parental form as that was now inferior in the new environment. This process, if reiterated over a long time, would lead, as Wallace's title declared, "varieties to depart indefinitely from the original type."

It was a brilliant scientific essay and demonstrates Wallace's independent formulation of what Darwin called 'natural selection.' Nevertheless, historians of science have long commented on the many differences between Wallace's and Darwin's views. The essay is heavily influenced by Wallace's study of his copy of Lyell's *Principles of geology*. Years later Wallace recalled that he had been inspired by Malthus. This is certainly possible but should not be repeated incautiously as if a straightforward fact. Wallace made no mention of Malthus in his essay and only did so after reading Darwin who stressed the work of Malthus.

Darwin versus Wallace?

What happened next has been surrounded by confusion and conspiracy theories in recent decades. Wallace did not send his essay for publication. A few weeks after writing it he received an extremely encouraging letter from Darwin who praised Wallace's (1855) paper and mentioned that Charles Lyell also thought highly of it.

Wallace was inspired. If the Sarawak paper had impressed the great Lyell, perhaps the new Ternate essay would impress him too. Perhaps Wallace could even convince Lyell that his own principles actually supported, rather than contradicted, evolution. So Wallace sent his essay to Darwin, whom he knew to be preparing a large work on evolution, with the request that it be forwarded on to Lyell if sufficiently interesting.

For decades it was believed that Darwin might have lied about when he received Wallace's letter and essay. Based on this uncertainty some even claimed, without evidence, that Darwin might have plagiarized from Wallace. In fact, Darwin received Wallace's essay exactly when he said he did (see van Wyhe and Rookmaaker, 2012; van Wyhe, 2013a).

Darwin was by then 2 years away from completing and publishing his big book on species. Surprised by Wallace's essay, but very much the Victorian gentleman, Darwin immediately forwarded it to Lyell as requested. Darwin even proposed sending Wallace's essay for publication and giving up his own 20 years of priority in natural selection.

Concerned that their friend would lose his priority, Lyell and J.D. Hooker had extracts from Darwin's manuscripts written in 1844 and 1857, together with Wallace's essay, read before the Linnean Society of London on 1 July 1858. These documents were published together as a joint contribution in the Society's proceedings in August 1858 (Darwin and Wallace, 1858). Thus began the long series of events that are usually called the Darwinian revolution.

Since the 1960s there has been an increasing number of suggestions that something about the joint announcement was unfair to Wallace or that he has been otherwise unfairly treated or forgotten. The arrangement was perfectly correct according to the standards of the time. Wallace was both flattered and delighted when he found out about the arrangement. Indeed it was described in this way by all of the participants, their contemporaries and later commentators for over a century. Nevertheless the papers were very brief and easy to read as only a contribution to debates about varieties. Few and perhaps no converts to evolution were made by the joint presentation.

Darwin began to prepare a summary or abstract of his larger work for publication. This project too lengthened far beyond his original plan and eventually became the 500-page *On the origin of species* (1859). The book condensed Darwin's massive research program of 20 years into a single volume and all of the most likely objections were openly acknowledged and answered.

The book immediately became extremely controversial and discussed. Despite the fact that it argued against some of the most fundamental scientific views of the time, and for many was also considered religiously or morally unacceptable, the book almost single-handedly convinced the international scientific community to accept that evolution is a fact within 10–15 years.

As Darwin's book had this dramatic impact, the theory and its success were, from the beginning, attributed to Darwin. *On the origin of species* also included many components not present in Wallace's essay. These included the analogy of man's selective shaping of domesticated plants and animals, laws of

variation, transitional varieties, inherited instincts, family selection, hybridism, embryology, taxonomic classification, no inherent progress, sexual selection, vestigial organs as remnants, and natural dispersals rather than former land bridges. Wallace was amongst Darwin's foremost supporters. The theory was therefore commonly called 'Darwinism.' Wallace (1889) also promoted this view, including his own great book on the theory, second only to the *Origin of species*, called *Darwinism*.

After the work of Darwin, Wallace, and many others, the question of whether or not evolution occurred was never again scientifically in doubt, though natural selection and indeed inheritance, along with a number of other aspects of the theory remained problematic until the first few decades of the twentieth century when the so-called modern synthesis of evolution diminished such concerns. Indeed, it remained for generations of workers to work out the many details and profoundly important and complex implications.

See also: Natural Selection, Introduction to. Synthetic Theory of Evolution, History of

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Developmental Biases on Morphological Evolvability

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Conceptual Definition(s) of Evolvability

Evolvability, the ability of biological systems to evolve, is a relatively new concept in evolutionary biology, which has emerged as the field pivots away from a gene-centric view of evolutionary change and places renewed emphasis on the origin of phenotypic variation. Similar to many other biological concepts, the definition of evolvability has not been completely settled. Depending on the scale of interest, evolvability has overlapping yet different meanings (these are described in detail by Pigliucci, 2008). At the microevolutionary level, evolvability refers to "the ability of a population to respond to selection" (Flatt, 2005), which is largely equivalent to heritability. At the macroevolutionary level, evolvability may refer to the propensity to produce major evolutionary novelties, such as the turtle shell or bat wings. In between these two extremes, evolvability may be defined as any property of a genetic/developmental system that facilitates the emergence of new patterns of phenotypic variation.

Another conceptual difficulty of defining evolvability relates to whether one is referring to the 'rate' or 'direction' of evolutionary changes (Figure 1). When considering the rate of evolutionary change, evolvability depends on patterns of phenotypic covariation. For instance, a population that is characterized by a pattern of phenotypic covariation that is closely aligned with the vector of selection should, in theory, be able to evolve rapidly along this axis (Figure 1(a)). Alternatively, if selective pressures shift such that the vector of selection is perpendicular to the primary axis of phenotypic variation within a population, the evolutionary response should be slower. Thus, evolvability with respect to the rate (i.e., how fast/efficiently a population will respond to selection) depends on the pattern of phenotypic (co)variation within that population and the direction of the vector of selection.

In terms of the direction of evolutionary change, evolvability relates more to the level of phenotypic covariation, often referred to as phenotypic 'integration' (more on this below). Highly integrated trait complexes will show high levels of covariation among its component parts. Under this scenario it should be hard to change one aspect of the phenotype without simultaneously altering other aspects. Thus, evolution will be constrained such that movement in certain directions of phenotypic space should be relatively easy to achieve, whereas movement into other dimensions of shape–space should be more difficult, if not impossible (Figure 1(a)). A very different situation is expected to arise when suites of traits exhibit low levels of integration (Figure 1(b)), where the population is free to move along various phenotypic axes, depending on the direction of the vector of selection. Evolvability with respect to the direction of evolution will therefore be high in this situation.

Given these considerations, it is clear that evolvability is operationally context-dependent, and it is perhaps most appropriate to consider this phenomenon at the nexus between evolutionary constraint and opportunity (Klingenberg, 2008, 2010). A constrained, highly integrated phenotype may have limited opportunity to evolve in multiple dimensions, but

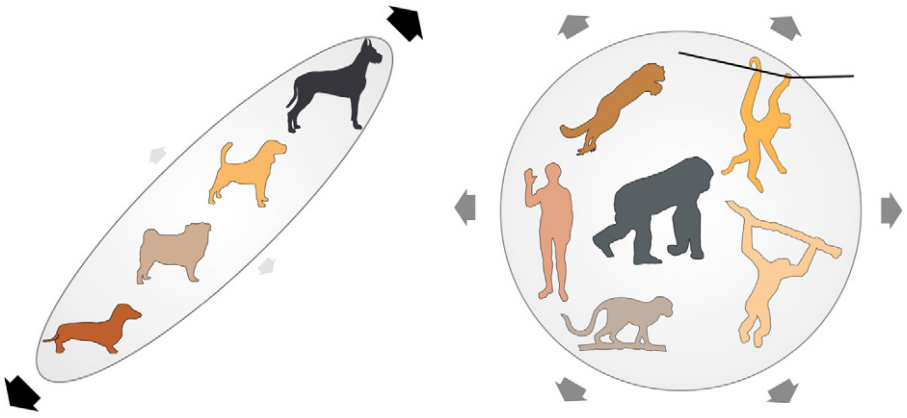

Figure 1 Illustration of how phenotypic covariation (integration) may bias evolution, using limb length as an example (adapted from Klingenberg, C.P., 2010. Evolution and development of shape: Integrating quantitative approaches. Nature Reviews. Genetics 11 (9), 623–635). (a) Among dogs, forelimb and hindlimb lengths covary, resulting in a 2-dimensional ellipsoid distribution of variation in phenotypic space. In this case, the population may respond efficiently to selection along the major axis (dark arrows; i.e., changes in both forelimb and hindlimb length), but it will not be able to respond as well to selection along other directions (small, light arrows; e.g., longer forelimb and shorter hindlimb). (b) Among primates, forelimb and hindlimb lengths are relatively independent to each other, resulting in a circular distribution of variation. In this case, the lineage may respond with equal efficiency to selection on any direction (e.g., longer hindlimb in leaping species, longer forelimbs in arm-swinging species, and equal limbs in quadrupedal species) and thus have maximized opportunity to occupy various regions of phenotypic space.

may respond rapidly to selection along one specific axis. Evolvability of this trait may therefore be considered high with respect to rate, but low with respect to direction. Alternatively, a phenotype with low levels of integration should be relatively less constrained to evolve in multiple directions (i.e., greater opportunity), but will evolve less efficiently along any one axis of variation. Here, evolvability would be considered low in terms of rate, but high in terms of direction. Thus, evolvability can be used as a term to describe the trade-off between the rate and direction of evolutionary response, which operationally is determined by the relative levels of 'constraint' and 'opportunity' that define the system (**Figure 1**).

It is now generally accepted, though still not widely considered, that evolvability in biological systems is strongly influenced by patterns of phenotypic (co)variation. Given this, a renewed effort in evolutionary biology is now underway to understand the mechanisms that determine these patterns, and the field of evolutionary developmental biology (evo-devo) is making significant inroads into this question (Hendrikse *et al.*, 2007).

Development Determines Evolvability

Genetic variation originates from mutation and recombination events in the genome, which are largely considered to be random phenomena. For the better part of the twentieth century, genetic variation has been considered to be the raw material upon which natural selection may act – i.e., the more genetic variation that exists in a population, the greater its potential to evolve. Yet selection does not operate on genes, rather it is the traits translated from the genomic blueprint that ultimately determine the fitness of an organism. The translation from genotype to phenotype is achieved over development, which can act as a 'filter' through which only a subset of the genetic variation will be expressed and thus visible to selection (Jamniczky *et al.*, 2010). For example, many important developmental processes are 'robust' such that little variation is evident in the system. The ultimate cause of robust development is not thought to be reduced genetic variation, but rather the self-regulatory properties of the molecular signaling network, including redundancy, modularity, and negative feedback within the system (Paulsen *et al.*, 2011 and references therein). Thus, the invariant nature of development may belie extensive nucleotide variation in the loci that encode members of the developmental network. It is for this reason that the penetrance of many genetic 'mutation' depends upon the genetic background into which they are introduced (Kitano, 2004).

Another important feature of developmental systems is that they interact with environmental factors, and thus the expression of genetic variation also depends on the external environment. The interaction between genes and the environment is broadly referred to as developmental plasticity, which is predicted to play an important role in promoting phenotypic evolution (West-Eberhard, 2005). Mechanistically, a plastic response may occur when a population encounters a novel environment, revealing cryptic (i.e., hidden) genetic variation that has accumulated over time under normal environmental conditions. This previously hidden genetic variation has the potential to produce new patterns or levels of phenotypic variation upon which natural selection may act (Gibson and Dworkin, 2004). While empirical evidence for such a process has largely been limited to laboratory models (Bateman, 1959; Dilda and Mackay, 2002; Polaczyk *et al.*, 1998), recent work on cavefish evolution provides an excellent example of this phenomenon within a natural system (Rohner *et al.*, 2013). *Astyanax mexicanus* is a species of freshwater fish commonly known as the Mexican tetra that is native to streams and rivers in northeastern parts of Mexico. In certain instances, populations of *A. mexicanus* have become trapped within caves, and have subsequently diverged from their surface-dwelling ancestors. In particular, cavefish have repeatedly lost their eyes and pigment. While much research has focused on understanding the genetic changes that have occurred within cavefish populations that have led to these dramatic shifts in phenotypes (Protas *et al.*, 2006, 2007, 2008; Gross *et al.*, 2009), a recent study by Rohner *et al.* (2013) has demonstrated that the first step in cavefish divergence may involve the release of heritable cryptic genetic variation. They focus on HSP90, which is a chaperone involved in protein folding and is very sensitive to environmental conditions. They show that when surface fish are exposed to a cave environment, a HSP90-related stress response is induced. In particular, they demonstrate that with this environmental shift the function of HSP90 is affected, which leads to a substantial increase in the variation of eye size (notably, other cave-specific phenotypes like pigmentation were unaffected). Thus, changes in the environment are sufficient to produce novel phenotypic variation without genetic mutations, and in the case of blind cavefish, this may have been the first step toward evolved eye loss.

This idea that development filters genetic variation is not new and roots from Waddington's classic concept of canalization (Waddington, 1957), wherein developmental systems exhibit varying degrees of robustness. A robust system is one in which the phenotypic outcome remains the same regardless of variations introduced by genetic mutations, or changes in the environment. Waddington conceptualized development as a marble rolling down a sculpted landscape. In this metaphor, the marble represents a cell or tissue, while the different 'valleys' it encounters along the way represent different developmental pathways. Canalized development would be characterized by a landscape with steep walls and narrow valleys such that there is little variation in the trajectory of development. Importantly, Waddington posited that this landscape could itself evolve by increasing or decreasing the width of the valleys encountered by each marble. In other words, development can evolve to either promote or restrict levels of phenotypic variation. For example, in *Astyanax*, pigmentation is less sensitive to environmental changes (i.e., is more canalized), whereas eye size is relatively more sensitive to the same shift in environment (i.e., is less canalized). The ways in which developmental systems can evolve to effect canalization are likely many, and this represents an active area of research. The common theme however is that these mechanisms act at the intersection between genotype and phenotype, and thus implicitly involve changes in the developmental program (Jamniczky *et al.*, 2010).

Phenotypic Integration and Modularity

Recent efforts to understand how development constrains variation have centered on the related concepts of phenotypic integration and modularity. Phenotypic integration refers to the relative degree to which sets of traits covary. Modularity is a term used to describe trait sets that exhibit a relatively high degree of correlation internally and low degree of correlation with other traits (Pigliucci and Preston, 2004; Klingenberg, 2008). The phenotypic consequence of modularity is that change in one trait will lead to corresponding changes in all other traits in the same module, but little or no change in traits in other modules. Consequently, variation within a module is typically biased/concentrated along certain axes in the phenotypic space that is determined by the pattern of covariation, and would be expected to constrain phenotypic evolution (**Figure 1**). Empirical support for these ideas can be found from a large-scale comparative study of shoulder girdle morphology and integration among mammals by Sears *et al.* (2013). Specifically, investigators found that, compared to placental mammals, marsupials possess a tightly integrated shoulder girdle. These data are consistent with the observation that girdle elements have evolved independently in placentals, but in marsupials they have evolved in a more coordinated manner. Thus, the evolution of the shoulder girdle in marsupials appears to be constrained, and this is coincident with and likely due to their unique reproductive strategy. In particular, marsupials have evolved a highly specialized pectoral girdle to enable a crawl from the reproductive tract to the teat early in life. Moreover, patterns of integration were found to be highly consistent across placental and marsupial mammals. In all this work highlights two attributes of integration: (1) It can impose long-term constraints that bias the production of phenotypic variation within a lineage (i.e., patterns are maintained across marsupial mammals); (2) Integration itself is a trait that can respond to selection (i.e., patterns vary between taxa at a larger scale). The evolvable nature of integration patterns implies that constraint imposed by development is not absolute, and that such processes vary over phylogeny.

Developmental constraints also vary over the course of ontogeny. Evidence supporting this idea can be found in recent studies on the 'phylotypic stage' of vertebrate facial development (e.g., Young *et al.*, 2014). Young *et al.* compared facial shape during embryonic development among multiple species of mammals, reptiles, and birds. They found that after the initial prominence outgrowth, embryonic facial shape converges as the frontonasal maxillary prominences fuse to form the primary palate. After this important process is complete, facial shape then diverges to take on species-specific geometries. Their data are consistent with an hourglass model of development, whereby morphological variation in the face is reduced during the middle/fusion stage compared to the early and late stages. In other words, although adult facial morphology varies dramatically among these lineages, their developmental trajectories are restricted through a bottleneck at the fusion stage. Formation of the primary palate is of critical importance to the survival of the animals, and is the result of the coordinated effects of myriad cellular activities that are under the control of numerous genes and pathways (Wilkie and Morriss-Kay, 2001; Dixon *et al.*, 2011). Variation in this process can have dramatic detrimental effects on the developing embryo (e.g., cleft palate in mammals), thus this stage of craniofacial development appears to be tightly coordinated and highly canalized. The observation that facial development is more variable after this stage implies relaxed constraint within a developmental system that is less susceptible to catastrophic breakdown.

How developmental constraints manifest themselves over ontogeny remains an open question, but integration/modularity are hypothesized to play an important role (Raff, 1996). For example, in the case of amniote facial development, the fusion stage may reflect a period characterized by relatively high levels of integration. Specifically, in order for the various prominences of the embryonic face to properly fuse there must be a high degree of cross talk between these multiple components (e.g., modules). Subsequent divergence in facial shape among amniote lineages may occur as a result of the embryo/face becoming more modularized. With less cross talk between modules, variation could arise within one component of the face without impacting other regions.

'Constraint' in Developmental Systems Is Necessary for Evolution

Note that the word 'constraint' does not necessarily mean that development is an obstacle for evolutionary processes. Rather, theory suggests that constrained variation is actually necessary for the evolution of complex character networks. In their now classic paper, Wagner and Altenberg (1996) used the infinite monkey analogy to illustrate this idea, in which a monkey is given either a pencil or a typewriter to reproduce a verse of Shakespeare. In this scenario, the monkey's behavior is random and analogous to genetic mutations, Shakespeare's text represents an adaptive phenotype/direction of selection, and the pencil and typewriter represent two different developmental systems that translate the random behavior of the monkey. The monkey with a typewriter has a far better probability of reproducing a verse of Shakespeare compared to a monkey with a pencil, since the monkey is constrained by the typewriter to produce letters, and thus has a greater chance to achieve an adaptive phenotype. However, this is only true when the axis of selection is along a Shakespearean axis. If we were to consider selection along other directions, say toward the reproduction of a pencil sketch of Michelangelo, then the pencil would represent a more 'adaptive' developmental system. Thus, it is the environment that influences the direction of selection, and consequently the degree to which a developmental constraint may be considered adaptive. An important outstanding question is how do developmental mechanisms themselves change over time. That is, how easily may a typewriter evolve into a pencil?

Future

How does development bias evolution? How does constraint vary over ontogeny? How do constraints themselves evolve? All of these questions underscore the importance of development when studying the origin of phenotypic variation. Hallgrímsson proposed the palimpsest model as a conceptual

guide/framework for such studies (Hallgrímsson *et al.*, 2009). A palimpsest is an ancient page/script that could be erased and reused. However, with each use the previous text won't be completely washed away, and would leave some trace on the page. Development is a similarly hierarchical process in that each developmental event will add on and reorganize the phenotype established from previous events. The final outcome (e.g., adult phenotype, which is often the topic of evolutionary investigations) is therefore the product of multiple layers of developmental processes superimposed upon each other (Figure 2). Consequently, investigating adult morphology alone cannot inform a comprehensive understanding of developmental constraints. Concerted efforts are therefore needed to dissect the palimpsest, and examine the specific genetic, molecular, and developmental networks that are acting within each layer. We also need to gain a better understanding of the extent to which each layer influences subsequent layers over ontogeny, as well as how these mechanisms interact with the environment. These are lofty goals, but necessary to achieve a holistic understanding of how development translates/filters genetic variation into phenotype.

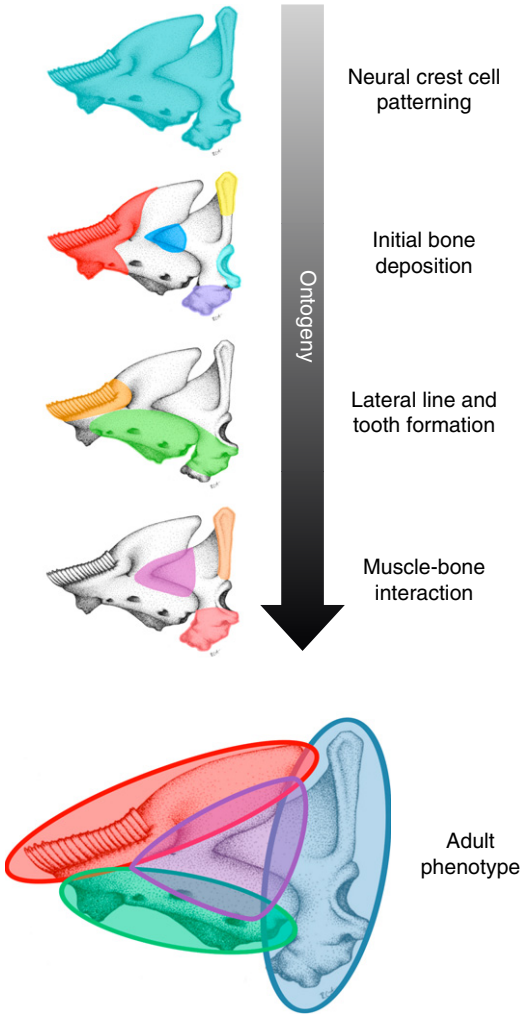

Figure 2 Illustration of a proposed palimpsest model for the cichlid mandible (adapted from Hallgrímsson, B., Jamniczky, H., Young, N. M., *et al.*, 2009. Deciphering the palimpsest: Studying the relationship between morphological integration and phenotypic covariation. Evolutionary Biology 36 (4), 355–376). Various developmental events influence the shape of the jaw at different stages over ontogeny. Each event affects certain regions of the jaw, and has the potential to establish different patterns of integration at different stages. The pattern of covariation observed in the adult is therefore the result of a complex interplay between events over development. A change at any one stage in terms of either the pattern or strength of phenotypic covariation will affect the pattern of integration observed in the adult.

To this end, there are several methods that may prove to be especially helpful. This is by no means an exhaustive list, but rather one that highlights what we feel are promising directions in the field. Classic forward genetics (mapping techniques) would be very useful as an unbiased search for genes and epistatic interactions involved in specific developmental processes (e.g., Albertson *et al.*, 2005; Roberts *et al.*, 2011). Revealing epistatic interactions among loci could be especially informative, because they show how different loci interact, and if the genetic effects are acting at different developmental stages these insights could provide an inroad toward interactions between different layers of the palimpsest. Mapping studies targeting different/multiple stages of ontogeny should also be useful in understanding how different regions of the genome affect phenotype at different developmental stages. Notably, new methods have also recently been established that allow investigators to more explicitly examine the genetic basis for phenotypic integration via forward genetics (e.g., Pavlicev *et al.*, 2011; Parsons *et al.*, 2012; Hu *et al.*, 2014). Induced laboratory mutants (e.g., reverse genetics) are another potentially valuable resource with which to dissect the basis for developmental constraint. Here, instead of characterizing the role for a particular gene in phenotypic development, investigators could use mutant animals to examine how genetic changes translate to shifts in patterns of phenotypic covariation (e.g., Hallgrímsson *et al.*, 2009). Finally, because developmental constraints are unlikely to manifest the same way in divergent lineages, it will be important to explore these ideas in multiple systems and across different phylogenetic levels. We anticipate this to be an especially rich area of future research, which should help to reveal the full range of influence that development has on the production of phenotypic variation, as well as identify any common themes and first principles with respect to the role of development in phenotypic evolution.

See also: Developmental Plasticity and Phenotypic Evolution. Ecological Evolutionary Developmental Biology. Evolvability, Quantitative Genetics of. Genotype-by-Environment Interaction. Modularity and Integration. Modularity and Integration in Evo-Devo. Novel Structures in Animals, Developmental Evolution of. Novel Structures in Plants, Developmental Evolution of. Robustness and Evolvability in Molecular Evolution

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Developmental-Genetic Toolkit for Evolutionary Developmental Biology

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Glossary

Ectopic Occurring in an abnormal position or in an unusual manner or form.
Homeobox A highly conserved 180-base-long sequence present in genes encoding Hox proteins. Importantly, *Hox* genes are not the only genes that encode a homeobox; this important motif exists in many other transcription factor genes that are not found in Hox clusters.
Homeodomain A 60-amino acid DNA-binding domain encoded by the homeobox.
Homeotic Adjective of the term homeosis, which describes a mutation that causes one body part to assume the identity of another.
Homologous Genes or structures that share common ancestry or evolutionary origin.
Tetrapod The superclass comprising the first four-limbed vertebrates and their descendants, including the living and extinct amphibians, reptiles, mammals, and birds.

Introduction

A striking characteristic of our planet is its diversity of animal life. From the most varied landscapes, ranging from the deep ocean to deserts, rainforests and mountaintops, vertebrate and invertebrate life abounds, showcasing a vast array of forms. It has long been the quest of naturalists to describe the patterns within the animal kingdom. Nineteenth-century comparative anatomists realized that behind the manifest variation of forms in living creatures, there was order. For example, creatures as dissimilar as bats and whales share a fundamental anatomical blueprint, or body plan (Valentine, 2004).

Charles Darwin's theory of evolution provided the explanation for the existence of this basic body plan: it was present in, and inherited from, the common ancestor of all animal species, and then subsequently modified to produce the various shapes seen in extant and extinct species. Soon after the mechanisms of heredity were elucidated, evolutionary biologists began working on what would become the modern evolutionary synthesis. In the decades that followed, the primary concern of evolutionary biologists became understanding how genes, allele frequencies at a population level, and natural selection combined to explain the arrival of new species, while neglecting a central question: how do new forms arise? Thus, evolutionists attempted to explain the survival of the fittest, without addressing the mechanisms behind the making of the fittest (Carroll, 2006; Müller, 2008).

To address this quandary, it was necessary for biologists to turn their attention to the process that actually builds animal bodies, namely embryonic development. By performing mutagenesis screens on animal models that were easy to breed in the laboratory, underwent rapid development, and produced abundant offspring, scientists revealed for the first time that genes were required to build bodies. This approach paved the way to the discovery of the developmental-genetic toolkit, namely the genes that controlled development, pattern formation, and identity of body parts. And then came the biggest surprise: these genes were deeply conserved across phyla and were shown to set up the body plans of every animal examined, from flies to humans.

The Discovery of the Homeobox Genes

Mutations in genes controlling development often give rise to defective tissues or structures and developmental arrest. Nevertheless, on occasion an unusual mutant results in a phenotype that does not fall in one of the aforementioned categories. In the *bithorax* mutant, the fruit fly, *Drosophila melanogaster*, develops an additional set of wings (Lewis, 1978), and in the *antennapedia* mutant, legs in place of the antennas (Wakimoto and Kaufman, 1981). These abnormalities were exceptional because the genetic change did not result in a breakdown of embryonic development; it instead transformed the identity of body parts. Additional aberrant flies such as *bithorax* and *antennapedia*, where body parts were made in the wrong places, became collectively known as homeotic mutants (Lewis, 1994).

The search for the genes responsible for homeotic mutants led to many surprising findings. First, the homeotic genes were located on the same chromosomal region in the fruit fly. Second, the genes were clustered in such a manner that genes involved in the formation of anterior structures were on one end and genes directing posterior structures on the other end of the cluster. Third, the order of the genes in the chromosome roughly matched the order of the body structures they influenced. And finally, the homeotic genes (which became known as *Hox* genes) all shared a conserved DNA sequence, the homeobox, that encoded a 60-amino acid 'homeodomain' predicted to function in DNA binding. Altogether, these findings offered for the first time an explanation for how a complex organism is built: a set of genes encoding DNA-binding proteins regulate genetic targets that will organize the formation of body parts in the developing embryo (Slack, 1984). As astonishing and elegant as it was, the discovery of *Hox* genes and its role in fly development represented, of course, an explanation for how an insect builds its body. However, the search for *Hox* genes across metazoa produced the next set of surprising findings that revolutionized developmental biology, and later, evolutionary biology (**Figure 1**).

When researchers set out to find *Hox* genes in other organisms, not only did they discover an abundance of them,

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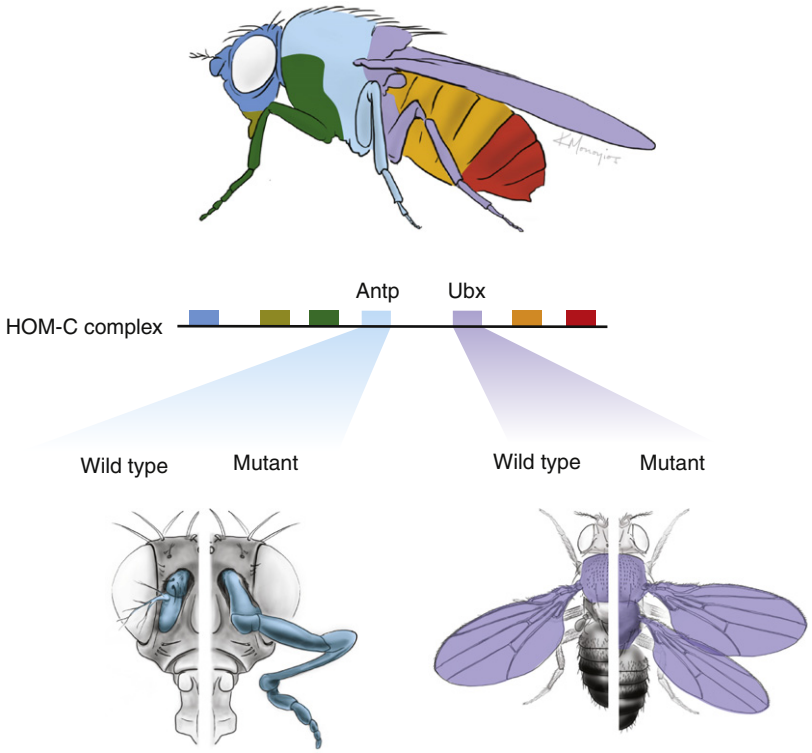

Figure 1 The fruit fly homeobox gene cluster. Top: Genes in the *Drosophila melanogaster* homeobox cluster (HOM-C) are color coded to match the corresponding body segment it controls. Bottom: Alterations to the *Antp* and *Ubx* genes result in changes in corresponding body parts, resulting in an ectopic leg in the place of an antenna or an extra set of wings, respectively. Illustration by K. Monoyios.

there were poignant similarities to the ones previously identified in fruit flies. *Hox* genes in mice were also grouped in the genome, except there were four clusters instead of one, and the position of a *Hox* gene within a cluster and its respective expression pattern along the body axis were relatively compatible (Duboule, 1994; Graham *et al.*, 1989). The outcome of studies on *Hox* genes in flies and mice was a new vision of development and evolution, one in which the bodies of all metazoans were being shaped over 600 million years of evolution. Further research on the developmental-genetic toolkit identified many more genes and showed that their use was not restricted to organizing the main body axis: body structures developed as modules with their own set of toolkit genes.

The Expanded Toolkit: Genes for Body Parts

The eyeless mutation in fruit flies is caused by a defect in the *ey* gene and is characterized by partial or complete loss of eyes. The *ey* gene is, therefore, crucial to the development of the fly's compound eyes. When researchers first isolated the DNA encoding *ey*, two features were particularly exciting: *ey* encodes a DNA-binding protein carrying a homeodomain and it had clear mammalian counterparts. The human version, called *Pax6*, is a gene required for proper development of the iris and the mouse counterpart was called *Small eye* or *Sey*, as mouse mutants for this gene displayed reduction or absence of eyes (Quiring *et al.*, 1994). Therefore, homologous genes in mice and flies were involved in the development of the compound and the camera-type eye, two structures considered at the time to have evolved independently. Researchers suspected that *ey* might have the qualities of a 'master' control gene, and could be capable of guiding the formation of the eye. They hypothesized that, as in homeotic mutants, ectopic *ey* expression might result in ectopic eyes. To test this, *ey* gene expression was induced on the embryonic precursors of wings, legs, and antennas, and the outcome was, as predicted, ectopic eyes. Remarkably, the mouse *Sey* gene, known today as *Pax6*, was also capable of inducing ectopic compound eyes in the fly (Halder *et al.*, 1995). In fact, the association of *Pax* genes and eyes is so profound that in jellyfish, eye development is dependent on *PaxB*, a member of the *Pax2/5/8* subfamily (Kozmik *et al.*, 2003), and the jellyfish *PaxB* gene can also induce ectopic eyes in flies (Suga *et al.*, 2010).

As in the case of the eye, other body parts also develop under control of the developmental-genetic toolkit. In land vertebrates, or tetrapods, the limb originates as four outgrowths on the body wall that will form the fore and hind limbs. Many genes that guide limb growth and patterning also act in similar ways to regulate fin development in fishes. Even *Hox* genes, which specify identity along the head–tail axis, are deployed to specify identity of the different parts of limbs and fins (Schneider and Shubin, 2013). The *Tbox* genes *Tbx4* and *Tbx5* represent a particularly striking example of limb toolkit genes deeply rooted in the metazoan phylogenetic tree. During development, *Tbx5* is expressed in the forelimb and the *Tbx4* gene in the hind limbs, where both are required for limb bud initiation, as mice lacking any one of these genes fail to

develop limbs (Naiche and Papaioannou, 2003; Rallis *et al.*, 2003). Yet, *Tbx4* and *Tbx5* arose by duplication of an ancestral gene named *Tbx4/5*. The lancelet, a cephalochordate also known as amphioxus, is a marine invertebrate that lacks paired appendages and possesses the *Tbx4/5* gene. Remarkably, when the single lancelet *Tbx4/5* gene is expressed in mice that lack the *Tbx4* or *Tbx5* genes, it can promote limb development (Minguillon *et al.*, 2009).

The homeobox gene *tinman* is another outstanding example of a conserved toolkit gene for a body part. Geneticists have discovered that *tinman*, named after the Wizard of Oz character that lacked a heart, is required for heart development in fruit flies. As was the case for many other homeobox genes, *tinman* turned out to have a genetic equivalent in vertebrates, including mammals (Bodmer and Venkatesh, 1998; Harvey *et al.*, 2002). The mammalian version, known as *Nkx2.5*, is also crucial for heart development, as mice lacking this gene display severe heart malformations (Tanaka *et al.*, 1999).

In sum, the findings considered above and many others not addressed here point to the origin of an ancestral developmental-genetic toolkit for body parts. Homologous genes are used to build appendages, eyes, and hearts in species as disparate as mice and flies, whose evolutionary lineages have diverged over half-a-billion years ago. We will next discuss how modifications to such genes may contribute to the diverse morphologies seen in organisms alive and extinct.

Tinkering with the Toolkit Genes and the Origin of New Forms

In our previous sections we examined examples of profoundly conserved toolkit gene function. With all of these deeply conserved genes shared by animals as distantly related as flies and mice, how does diversity arise? Here we discuss studies that highlight how the location, time, and intensity in which a gene is expressed can have a profound effect on development and, consequently, on morphology.

During his visit to the Galapagos Islands, Charles Darwin studied the wide variety of finches inhabiting the archipelago. The finch species varied with respect to beak morphology: some species have long, thin beaks while others, deep and broad beaks. Darwin proposed that these different birds were descendants of a continental species that colonized the archipelago. But what is the mechanism behind the emergence of various forms of beaks among birds of the Galapagos? For some time, biologists speculated that gradual accumulation of mutations in several genes might have been responsible for modifying beak shapes. More than a century after Darwin's trip to Galapagos, researchers found that the thickness of the beaks of Galapagos finches was strongly correlated with the activity of *Bmp4*, a gene encoding a growth factor of the bone morphogenetic protein family. *Bmp4* is expressed in the developing finch beak and the deeper and broader the beak, the greater is the expression of *Bmp4*. To demonstrate that the intensity of *Bmp4* gene expression was responsible for the thickness and width of the beak, researchers artificially increased expression of this gene in chicken embryos. The result was chicks with deep and broad beaks, resembling those seen in Galapago's finches (Abzhanov *et al.*, 2004). This remarkable discovery paved the way for a new overarching hypothesis: could *Bmp4* be involved in craniofacial variation in other animals? When researchers investigated variation in jaw shape in cichlid fishes, they found it was associated with *Bmp4* expression. Furthermore, experimentally modulating *Bmp4* in zebrafish yielded jaw shapes reminiscent of cichlids (Albertson and Kocher, 2006). Collectively, these studies support a fundamental role for BMP4 in vertebrate craniofacial evolution and illustrate how changes in regulation of a toolkit gene can produce phenotypic variation.

Another prime example of nodulation of gene expression associated to morphological variation is pelvis loss in stickleback fish. The reduction or complete loss of hind limbs is a relatively common occurrence among vertebrates. In many aquatic mammals such as manatees, hind limbs are vestigial. In marine stickleback fish, pelvic fins are modified into spike-shaped spines, which protect the fish from predators. By contrast, freshwater sticklebacks have very reduced or absent pelvic fins. The gene associated with the reduction of the pelvic fins turned out to be *Pitx1*, a gene encoding a transcription factor that works in concert with *Tbx4* during hind limb outgrowth (see above). By analyzing its sequence, researchers found no difference between the *Pitx1* gene of freshwater and marine sticklebacks, however, the expression pattern during pelvic fin development was reduced in the freshwater species (Shapiro *et al.*, 2004). It was later shown that the mutation did not alter the gene itself, but rather a region that controls expression of the gene in the developing pelvic fins (Chan *et al.*, 2010) (**Figure 2**).

As previously discussed, homeotic mutations can produce spectacular phenotypes with body parts developing in abnormal positions. Nevertheless, homeotic transformations may occasionally lead to morphological innovation. Crustaceans are subdivided into different groups according to the types of appendages they display in each segment. In some crustaceans, appendages in all segments look similar and are used for locomotion. In others, such as lobsters, modified thoracic appendages called maxillipeds serve as additional feeding appendages, as they are morphologically similar to mouthparts. One *Hox* gene, *Ultrabithorax* (*Ubx*), had long been suspected of playing a role in determining the identity of crustacean appendages, as its anterior expression is invariably absent from the jaw-like maxilliped appendages. Researchers had long known that *Hox* genes are often causally linked to the identity of the body parts in which they are expressed. It was hypothesized that an expansion of *Ubx* expression into the body segments that form maxilliped appendages could result in their transformation into a thoracic-like appendage used for locomotion. This could explain why in some crustaceans, like brine shrimp, no maxillipeds are observed and all appendages look alike. To test this idea, researchers studied *Ubx* gene expression in the *Parhyale hawaiiensis*, a crustacean that has a single pair of maxillipeds. By experimentally reducing *Ubx* expression in *Parhyale*, walking legs were transformed into maxilliped-like legs, as in lobsters (Liubicich *et al.*, 2009). Conversely, expressing *Ubx* ectopically in anterior appendages led to homeotic maxilliped to leg transformations, resembling the morphology seen in brine shrimps (Pavlopoulos *et al.*, 2009).

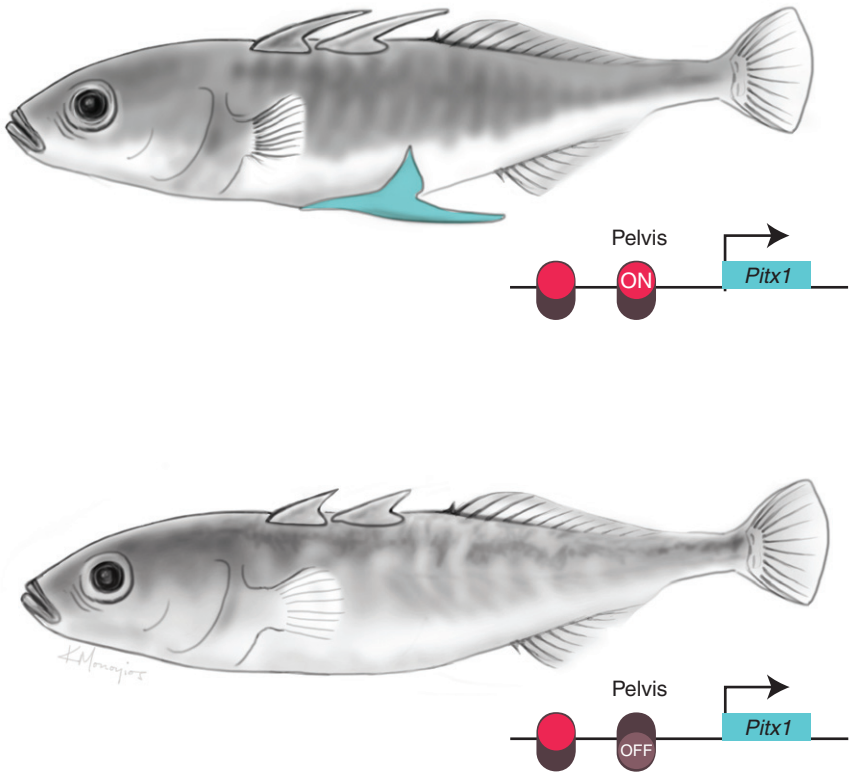

Figure 2 *Pitx1* and pelvic fin loss in freshwater sticklebacks. Top: In marine sticklebacks, *Pitx1* expression in the developing pelvis is controlled by a specific regulatory region. Bottom: A mutation in this regulatory region in freshwater sticklebacks results in the lack of *Pitx1* expression during pelvic development and in reduced or absent pelvic fins. Illustration by K. Monoyios.

The Developmental-Genetic Toolkit and the Hourglass Model

The developmental toolkit genes that set up head–tail segment identity, control eye and appendage development, and regulate craniofacial morphology have many common attributes. Many are DNA-binding proteins that directly modulate downstream genetic targets. Others, like *Bmp4*, are secreted proteins that bind to receptors in the target cells and subsequently bring about changes in gene expression. When the expression pattern or activity of a toolkit gene is altered, profound phenotypic consequences ensue: an entire body part may be produced in the wrong location, or have its serial identity changed, or it may not form altogether. Yet another shared attribute of these and many other toolkit genes is related to when, during development, they become active.

During early development of both vertebrates and invertebrates, there is a high degree of variation in embryonic form and developmental process. Different life histories and adaptive strategies translate into a great deal of divergence, at least until mid-development. Then, embryos reach what various researchers call the 'phylotypic' period, when embryos converge into a stage where they share a basic body plan (Wagner, 2014). After the phylotypic stage, embryos of different species will follow distinct ontogenetic paths that will lead to the adult organism. The observation that disparate embryos converge on a phylotypic period only to diverge as development proceeds became known as the Hourglass Model (Duboule, 1994; Raff, 1996).

The phylotypic period seems to be largely resistant to variation, as pharmacological perturbations during this developmental stage often lead to systemic malformations and developmental arrest (Galis and Metz, 2001). Interestingly, it is during this conserved phylotypic period that many toolkit genes (*Pax6*, *Nkx2.5*) and gene families (*Hox*, *Tbx*, *Bmp*, etc.) are deployed to assemble the basic body plan. In the following section we will survey new tools and approaches that have allowed us to examine, among other things, how regulatory changes in toolkit genes may have contributed to morphological innovation at a macroevolutionary scale.

The Future of Experimental EvoDevo

The recent emergence of high throughput and inexpensive DNA sequencing techniques and computer algorithms to assemble and assimilate the data are revolutionizing many fields in biological sciences, including evolutionary biology. This greatly enhanced capacity for DNA sequencing has created the opportunity for broadening the range of sequenced genomes to include species other than those of medical or economical relevance. For instance, recent genome sequencing efforts have focused on sampling branches of the phylogenetic tree that inform important macroevolutionary events and/or that have not yet been assessed. For example, sequencing of key vertebrate species such as the sea lamprey, chimera, and coelacanth are revealing much information about what it takes to be a

vertebrate as well as the kinds of genetic innovations that have been acquired along the way (Smith *et al.*, 2013; Venkatesh *et al.*, 2014; Amemiya *et al.*, 2014). The pace of genomic sequencing is staggering and efforts are underway to sequence representative genomes of every major animal group (Genome, 2009).

In addition, high throughput sequencing enables the study of the near complete collection of genes expressed in given tissues, so-called transcriptomes. As a result, our ability to compare gene expression profiles between different cells, tissues, or species has expanded profoundly. In fact, recent studies comparing cross-species genome-wide expression of orthologous genes during development have shown that the most conserved expression profile is found at mid-embryonic stages in both invertebrates and vertebrates, supporting the hourglass model in both (Irie and Kuratani, 2014).

In summary, we have described the basic tenets of the developmental-genetic toolkit for evolutionary developmental biology. This toolkit provides a framework for which to carry out empirical investigations into the presumed changes that organisms have undergone throughout their histories. Finally, the recent infusion of high throughput DNA sequencing is helping to drive the field by expanding the developmental-genetic toolkit. This, in turn, is facilitating novel investigations into all manners of evolutionary problems and in all manner of organisms, something not possible just a few years ago.

See also: Evolutionary Biology, History of. Genome Evolution's Role in Developmental Evolution. Model Systems: The Key Roles of Traditional and New Models in Evolutionary Developmental Biology. Novel Structures in Animals, Developmental Evolution of. Regulatory and Coding Changes in Developmental Evolution, Roles of. Synthetic Theory of Evolution, History of

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Developmental Mechanisms Controlling Cell Fate, Evolution of

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Glossary

Epidermal growth factor (EGF) Secreted growth factor that binds to an EGF receptor.
Epithelial to mesenchymal transition (EMT) Cellular behavior where individual cells lose epithelial characteristics such as adhesion and cell–cell polarity and adopt a mesenchymal phenotype.
Equivalence group Set of cells arising from the same lineage that have equal potential to respond to neighboring inductive cues.
Gene regulatory networks (GRNs) Set of genes and the control elements (*cis*-regulatory elements) that control a specific cell biological process.
Metazoa It is another name for the animal kingdom.
Primary mesenchyme cells (PMCs) Embryonic echinoderm cells located in the vegetal plate that will undergo EMT and ingress giving rise to mesoderm.
Vulval precursor cells (VPCs) Set of ectodermally derived cells that can give rise to the adult nematode vulva, or egg-laying organ.

Introduction

The field of evolutionary developmental biology has matured substantially from its earliest days in the pre-genomic era. We have begun to generate answers to many important outstanding evo-devo questions, and today there are few limits on the questions that can be asked, or the organisms in which to ask them. There are many reasons for this. First, decades of intensive study in a few distantly related 'model' organisms such as the fly, roundworm, and mouse have uncovered an astonishing level of detail about the genetic control of cell function, explaining how their cells build disparate body plans. These studies provide an intellectual framework to generate specific hypotheses about how additional body plans (or cell types, tissues, or processes) may have evolved. Second, a number of new tools are available for perturbing gene function and visualizing cellular behavior, making it possible to investigate the mechanistic basis of development in almost any group of animals (Moczek *et al.*, 2015). Third, the fields of systematics and phylogenetics have established the branching order of most of the major and minor nodes of the animal tree (Aguinaldo *et al.*, 1997; Dunn *et al.*, 2008; Halanych *et al.*, 1995; Hejnol *et al.*, 2009; Moroz *et al.*, 2014; Ryan *et al.*, 2013), a requirement for assessing similarities and differences in animal development in an evolutionary framework (**Figure 1(a)**).

Data from many different organisms and developmental processes clearly shows that complex gene regulatory networks (GRNs) control embryonic development (Davidson *et al.*, 2002; Maduro, 2006; Rottinger *et al.*, 2012). These GRNs ultimately specify the identity and behavior of cells (such as proliferation, migration, shape changes) that determine the adult body plan. This article will focus on the evolution of mechanisms for cell fate specification, a field that has its roots in classical embryology (e.g., Conklin, 1905; Driesch, 1892; Roux, 1888). In that era, any cell or organelle that exhibited a phenomenon of interest was worthy of study, so that, as E.B. Wilson wrote "the problems of evolution have been reduced to problems of the cell" (Wilson, 1911). Today, we are able to be more reductionist, thanks to both our ability to rapidly generate transcriptome data and the increasing ease of functional studies. This allows researchers to characterize the underlying GRNs that, in combination with inductive signaling pathways, specify cell fate. These approaches have expanded our ability to generate comparisons from between homologous cells and cell types to more disparate structures and processes based on changes in gene regulatory network architecture.

Here, we review data on three different aspects of metazoan cell specification (**Figure 1(b)**). The first deals with the behavior of an 'equivalence group' in nematodes. We describe powerful techniques developed in the model nematode, *Caenorhabditis elegans*, which have been used to understand how a group of initially equivalent cells are able to acquire distinct fates through induction from other tissues. These data have been leveraged to study related nematodes, uncovering cryptic variation governing fate specification in a homologous group of cells. A second aspect of specification that we will discuss is the emergence of a novel cell type: the sea urchin skeletogenic mesoderm. A detailed GRN has been established for this cell type, making it possible to compare the network across echinoderms to reveal how this unique cell type arose during evolution. Our third section focuses on the decision between cell differentiation and stemness in the context of adult tissue and how the underlying mechanisms vary over the course of animal evolution. The lessons learned from mature developmental systems are combined with data from emerging models in each case study. In this way we gain a better understanding of the evolution of cell fate specification strategies by examining taxa across the metazoan tree, revealing the 'experiments' that have already occurred over the course of animal evolution. To that end, we begin our discussion of cell fate specification using a study group that allows for functional manipulation of homologous cells at single cell resolution, the rhabditid nematode vulva.

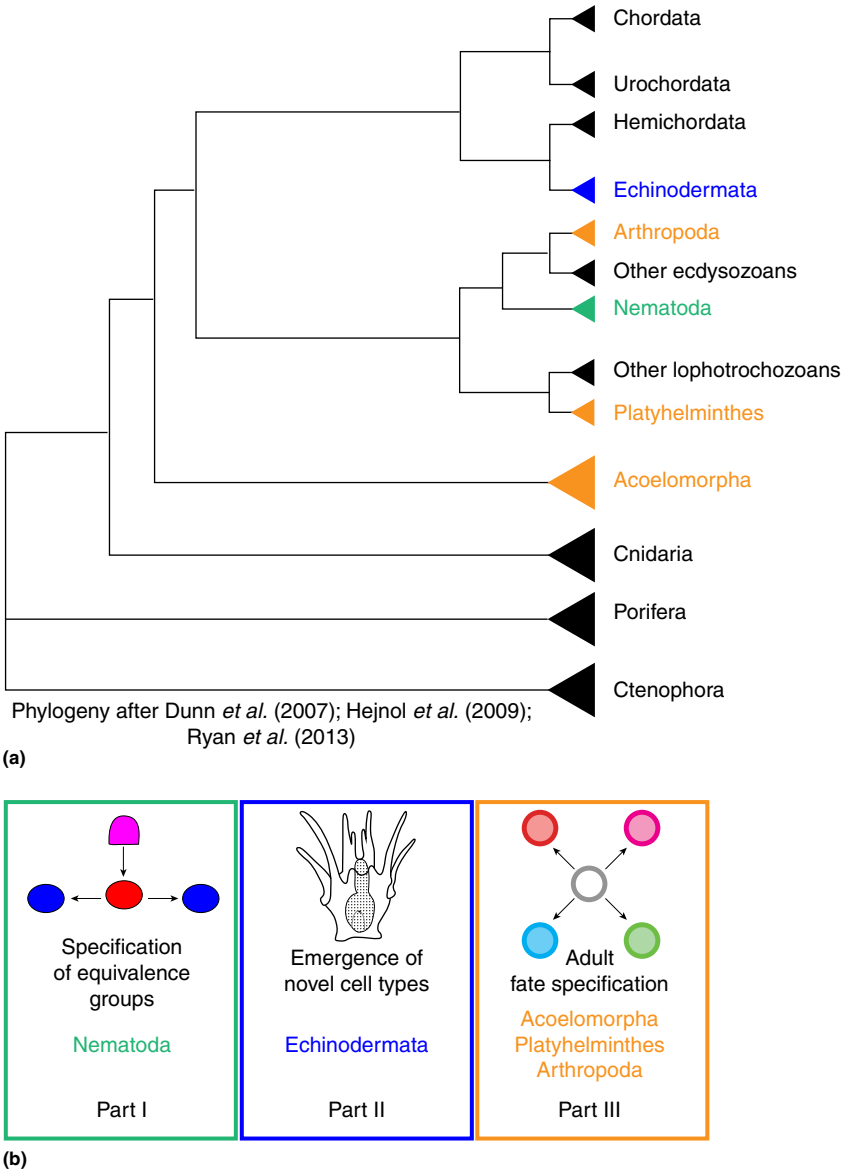

Figure 1 Phylogenetic relationships of animals discussed in this article. (a) Cladogram represents taxonomic relationships. Phylogeny based on recent phylogenomic studies (Dunn *et al.*, 2008; Hejnol *et al.*, 2009; Moroz *et al.*, 2014; Ryan *et al.*, 2013). Colored lineages match taxonomic groups discussed in (b) in each section of this article.

Part I: Competency, Equivalence Groups, and Cryptic Variation

Evolution of Equivalence Groups

A fundamental feature of metazoan cell fate acquisition is the establishment of competency, or the ability of a group of lineage-related cells to respond to inductive signaling. When multiple cells are capable of responding to the same signaling system they are said to form an 'equivalence group' (Kimble, 1981). The discovery of most equivalence groups stems from the ability to map cell lineage patterns during embryogenesis, and thus equivalence groups have been identified in organisms with stereotyped development where individual cells can be both followed over developmental time and destroyed using targeted ablation (e.g., transparent, fast-developing animals, see Table 1). Due to space limitations, in this article, we will focus on the best-studied equivalence group, the rhabditid nematode vulva, or egg-laying apparatus, and refer readers to Table 1 for investigating competence groups in other taxa.

The Rhabditid Nematode Vulva: An Evo-devo Model for Cell Fate Specification

Equivalence groups were first described in nematodes (Kimble, 1981), and due to a wealth of experimental and genetic tools available in *C. elegans*, we have the best molecular understanding of how they function. The most thoroughly characterized equivalence group is the six ectodermal cells (P3.p–P8.p) that

Table 1 Metazoan equivalence groups. Taxonomic examples of specific equivalence groups

Organism	*Phylum*	*Equivalence group*	*Reference(s)*
Mnemiopsis leidyi	Ctenophora	m_1 daughter cells (m_{11}/m_{12})	Henry and Martindale (2004)
Helobdella triserialis	Annelida	O/P ectodermal teloblasts	Weisblat and Blair (1984)
Helobdella robusta			Zackson (1984)
Helobdella stagnalis			Keleher and Stent (1990)
			Huang and Weisblat (1996)
			Kuo and Shankland (2004a,b)
			Kuo and Weisblat (2011)
			Kuo *et al.* (2012)
Drosophila melanogaster	Arthropoda	R7	Greenwald and Rubin (1992)[a]
			Chang *et al.* (1995)
			Dickson *et al.* (1995)
			Crew *et al.* (1997)
			Shi and Noll (2009)
Insects	Arthropoda	Neurogenesis	Doe and Goodman (1985)[a]
			Stollewerk and Simpson (2005)[a]
Caenorhabditis sp.	Nematoda	VPC specification	Sternberg and Horvitz (1986)
Oscheius tipulae			Sulston and White (1980)
Pristionchus pacificus			Kimble (1981)
Other rhabditids			Dichtel-Danjoy and Félix (2004)
			Sternberg (2005)[a]
			Kiontke *et al.* (2007)
			Tian *et al.*, 2008
			Wang and Sommer (2011)
			Penigault and Felix (2011a,b)
			Felix and Barkoulas (2012)[a]
			Kienle and Sommer (2013)
Halocynthia roretzi	Urochordata	Ocellus/Otolith specification	Nishida and Satoh (1989)
			Akanuma *et al.* (2002)
Danio rerio	Chordata	Posterior tailbud progenitors	Martin and Kimelman (2012)
Danio rerio	Chordata	Adaxial cells	Nguyen-Chi *et al.* (2012)

[a]Refers to review article.

will give rise to the adult vulva (Sternberg, 2005). These six vulval precursor cells (VPCs), born in the first larval stage, are all able to generate vulval fates, but under normal (wild-type) conditions only the three inner cells, (P5–7.p) are induced to become vulval cells, adopting either a 1° fate (P6.p) or a 2° fate (P5.p and P7.p). The remaining three cells adopt a default 3° fate and fuse with the epidermis; thus the final pattern is depicted as '3° 3° 2° 1° 2° 3°' (**Figure 2(a)**).

Researchers have spent the past three decades using a variety of experimental and molecular genetic approaches to decode the mechanisms that regulate the fate of these six cells (Sternberg, 2005). Briefly, the VPCs are initially patterned by the expression of a central class Hox5 gene (LIN-39) during the L1 larval stage. Later, in the L3 stage, an EGF signal (LIN-3), secreted by the gonadal anchor cell (AC), which is dorsally situated to the VPCs, induces the 1° fate of P6.p. Upon adopting the 1° fate, P6.p expresses Notch ligands (three delta orthologs, *apx-1*, *lag-2*, and *dsl-1*) (Chen and Greenwald, 2004). Delta ligands activate the Notch receptor (LIN-12) in the neighboring P5.p and P7.p cells which induces the 2° fate. The Wnt pathway functions during VPC specification in a maintenance role to prevent the acquisition of a 3° fate and epidermal fusion (Braendle and Felix, 2008; Eisenmann *et al.*, 1998; Gleason *et al.*, 2002; Myers and Greenwald, 2007).

Comparative work in other nematodes has identified the same signal transduction pathways (Wnt/Notch/EGF) that are used during *C. elegans* VPC specification, although to varying degrees in a taxon-specific fashion (**Figure 2(b)**). For example, data from forward-genetic screens and use of mitogen-activated protein kinase enzyme (MEK) inhibitors in *Oscheius tipulae* has identified a role for EGF/MAPK signaling in VPC induction (Dichtel-Danjoy and Félix, 2004). The initial forward genetic screens to identify vulval development mutants in the diplogastrid nematode *Pristionchus pacificus* led to an unexpected result – rather than a reliance on EGF and Notch/Delta signaling as in *C. elegans*, vulval induction in *P. pacificus* utilizes redundant Wnt signaling from two spatially distinct signaling centers (Tian *et al.*, 2008; Zheng *et al.*, 2005). With many nematode genomes now sequenced and the potential for precise genome engineering offered by new technologies such as CRISPR-Cas9 (Witte *et al.*, 2015) it should now be possible to determine the identity of the signaling pathways that are required to induce VPC fates in different nematode species.

Cryptic Evolution and Developmental System Drift

Not only can we observe striking examples of evolutionary flexibility in signaling pathway usage during nematode VPC induction between related species, but recent work both in *C. elegans* (Barkoulas *et al.*, 2013) and *P. pacificus* (Kienle and

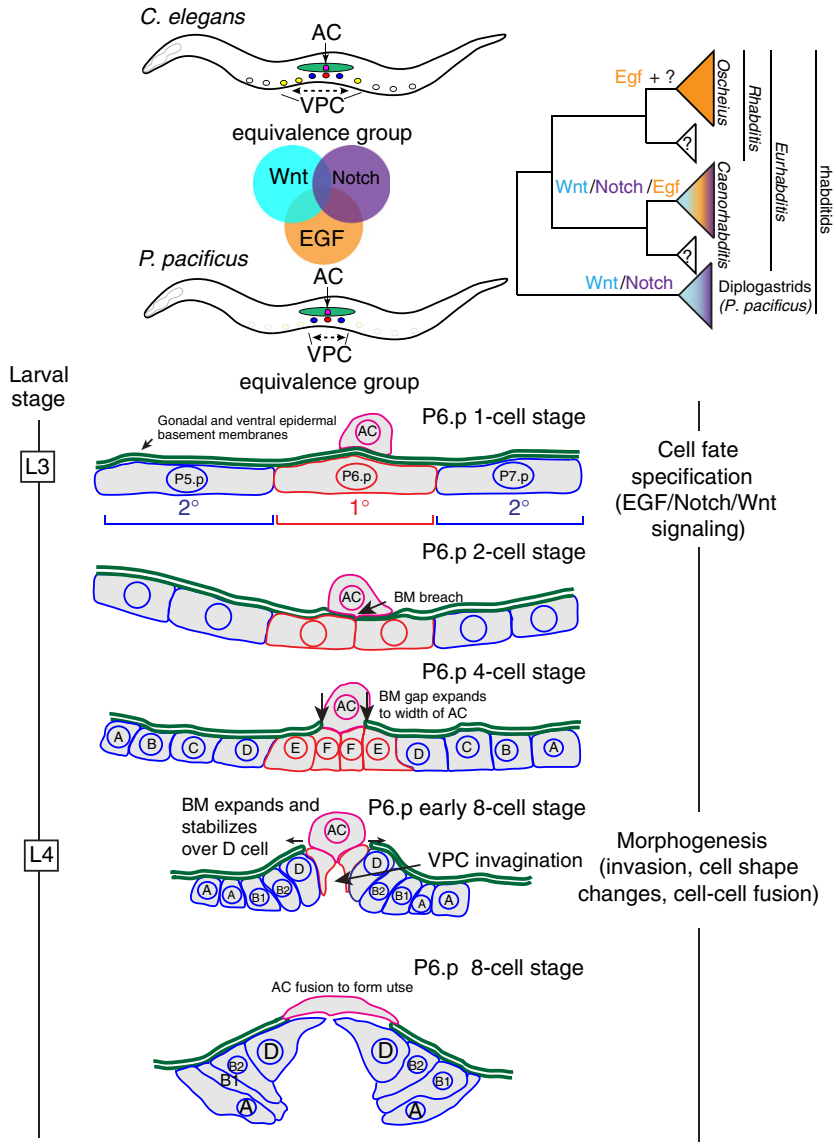

Figure 2 Evolution and development of the rhabditid nematode vulval precursor cell (VPC) equivalence group. (a) Schematics depict VPC fate specification in *C. elegans* (top) and *P. pacificus* (bottom). VPC colors refer to fate (primary, red; secondary, blue; tertiary, yellow (*C. elegans* only)). Venn diagram depicts the contribution of three signaling pathways in VPC specification and corresponds to the colored gradient used in the rhabditid nematode phylogeny shown in (b; phylogeny based on Kiontke *et al.*, 2007). Based on previous research, a role for EGF (orange) signaling (via MEK) has been identified in VPC specification in *Oscheius tipulae*. *P. pacificus* utilizes contributions from both the Wnt (light blue) and Notch (purple) pathway to specify VPC fate, while *C. elegans* receives contributions from all three pathways. It is unknown what signaling pathways specify fate in other rhabditid nematodes (denoted with '?'). (c) Schematic of *C. elegans* uterine–vulval cell specification and morphogenesis (stages defined by division of P6.p and its daughters (e.g., 1-cell stage, 2-cell stage, etc.), with each individual VPC designated by the specific letters, (A)–(F); modified from Matus, D.Q., Chang, E., Makohon-Moore, S.C., *et al.*, 2014. Cell division and targeted cell cycle arrest opens and stabilizes basement membrane gaps. Nature Communications 5, 4184.

Sommer, 2013) highlights cryptic variation that occurs in VPC fate specification within species between natural wild isolates. For example, the Sommer group recently showed that a single *cis*-regulatory change in the conserved Notch ligand, *apx-1*/Delta (one of three Delta ligands redundantly used in 2° fate specification in *C. elegans*) led to gain of a HAIRY binding site in the reference strain of *P. pacificus* (PS312/CA) originally isolated from Pasadena, California. The presence of this HAIRY binding site results in repression of transcription of *apx-1*/Delta in the 1°-fated P6.p cell in PS312/CA. However, most other wild isolates lack this HAIRY binding site and express *apx-1*/Delta in P6.p. Expression of *apx-1* is sufficient to induce 2° fate in the absence of induction from the gonad (Kienle and Sommer, 2013). Their elegant experiments reveal cryptic variation in a core developmental pathway, as a single *cis*-regulatory change can result in the abolition of Notch/Delta signaling as a patterning system in *Pristionchus* VPC specification, and provides a plausible explanation for the diversity in signaling systems that pattern homologous vulval cells between nematode species.

Cell Fate Specification Leads to Differentiation and Morphogenesis

Once the VPCs are properly specified, they execute lineage specific morphogenetic behaviors required to form the adult vulva, including cell division, invagination, and cell fusion (**Figure 1(c)**). *Caenorhabditis elegans* anchor cell (AC) invasion has become a powerful model to understand the genetic control of cell invasive behavior (Matus *et al.*, 2010; Sherwood *et al.*, 2005). Recent work has investigated AC invasion in related nematode species, identifying conserved features: there is only a single AC in all species examined and the AC is required to breach the basement membrane to initiate the uterine–vulval connection (Matus *et al.*, 2014). Following AC invasion, the basement membrane gap expands outward, likely due to forces generated from cell division of the underlying VPCs (Ihara *et al.*, 2011; Matus *et al.*, 2014). The size of this basement membrane gap is tightly regulated, as in all species examined the edges of the gap are stabilized by the same vulval cell, the innermost 2° fated VPC, the D cell. Strikingly, the D cell is the only cell in all nematodes examined to date that never divides (Kiontke *et al.*, 2007). Cell cycle exit of the D cell allows for localization of the extracellular matrix adhesion protein, integrin, to the basal surface of the D cell in response to an increase in the basement membrane component, laminin, at the edges of the basement membrane gap, stabilizing gap expansion (Matus *et al.*, 2014; **Figure 1(c)**). Thus, comparative studies in nematode uterine–vulval development have identified a new mechanism to stabilize basement membrane gaps, a cell biological process that occurs in both developmental contexts and disease pathogenesis (Matus *et al.*, 2014). Connecting cell specification strategies to the cell biology of morphogenetic behaviors across nematode evolution will be informative, especially identifying whether a similar amount of cryptic variation exists between species in executing morphogenetic behaviors as it appears to during cell fate specification.

Part II: Evolution of a Novel Cell Type – Echinoderm Larval Skeleton

The previous section focused on identifying evolutionary changes that alter specification strategies of a homologous group of cells. It is also critical to examine changes in cell fate specification that lead to the origin of new cell types, as they can offer insight into the evolution of novel structures and morphology. To illustrate this point we discuss the echinoderm pluteus larva, which has been a model for developmental, evolutionary, and ecological studies for over a century (Ettensohn, 2009; Lyons *et al.*, 2014; McClay, 2011; Raff and Byrne, 2006; Vaughn and Strathmann, 2008).

The phylum Echinodermata consists of five extant classes: crinoids (sea lilies), asteroids (sea stars), ophiuroids (brittle stars), holothuroids (sea cucumbers), and echinoids (sea urchins, sand dollars). Crinoids are the earliest-branching class, and among the remaining four classes, sea stars and brittle stars are more closely related, forming a clade that is sister to a clade comprised of sea urchins and sea cucumbers (Cannon *et al.*, 2014; Reich *et al.*, 2015; Telford *et al.*, 2014). Members of all five classes develop through an indirect life cycle that includes a planktonic, bilaterally symmetric larva, and a benthic, pentaradial adult. All echinoderms share a homologous calcite endoskeleton at the adult stage, but only ophiuroids and echinoids possess a larval calcite skeleton in the larval stage (**Figure 3(a)**). The skeletonized ophiuroid and echinoid pluteus larva is considered to be a derived form, having evolved from an ancestral auricularia-type larva, shared by the other echinoderm classes (Cannon *et al.*, 2014; Reich *et al.*, 2015; Telford *et al.*, 2014), and their closest out-group, the hemichordates (Rottinger and Lowe, 2012; **Figure 3(a)**). This suggests that the sea urchin and brittle star larval skeleton evolved independently by convergent evolution (or alternatively was lost by crinoids, asteroids, and holothuroids, which is less parsimonious). The development of the sea urchin larval skeleton provides an entry point into understanding how the pluteus skeleton evolved in these two lineages.

The Primary Mesenchyme Cells Build the Pluteus Skeleton in Sea Urchins

The sea urchin skeletogenic lineage arises during cleavage stages as the result of asymmetric cell divisions of vegetal pole blastomeres (Ettensohn, 2009; McClay, 2011). Progeny of these cells undergo an epithelial-to-mesenchymal transition (EMT) and crawl into the blastocoel (the fluid-filled central region of the blastula stage embryo). Once inside the blastocoel, these cells are referred to as primary mesenchyme cells (PMCs). The PMCs then migrate in response to cues coming from the ectoderm/endoderm boundary (Adomako-Ankomah and Ettensohn, 2014; McIntyre *et al.*, 2013, 2014). Although the micromere lineage is autonomously specified at birth (Okazaki, 1975; Oliveri *et al.*, 2008), and will even make skeletal elements *in vitro* (Okazaki, 1975), the pattern of the resulting skeleton is dictated by localized cues emanating from the overlying ectoderm (Armstrong and McClay, 1994; Duloquin *et al.*, 2007; McIntyre *et al.*, 2013; Piacentino *et al.*, 2015; Rottinger *et al.*, 2008). During gastrulation stages, the PMCs fuse to one another and the syncytium forms a ring next to the posterior ectoderm. Soon after ring formation, two aggregates or ventrolateral clusters of PMCs form, where skeletogenesis will begin in response to induction by vascular endothelial growth factor (VEGF) signaling (Adomako-Ankomah and Ettensohn, 2013, 2014; Duloquin *et al.*, 2007; **Figures 3(b)** and **3(c)**). Ventrolateral clusters, expressing the VEGF receptor (**Figure 3(g)**) form directly under signaling centers in the ectoderm expressing the VEGF ligand, where posterior ectoderm and the ciliary band territory intersect (**Figure 3(b)**; McIntyre *et al.*, 2013). When VEGF signaling from the ectoderm is impaired, skeletal patterning within the mesodermal PMCs is perturbed (Adomako-Ankomah and Ettensohn, 2013, 2014; Duloquin *et al.*, 2007). Within the ventrolateral clusters, the PMCs secrete the rudiment of the skeleton, called the triradiate (**Figure 3(d)**). Each prong of the triradiate then grows in a unique and characteristic way to build the mature pluteus skeleton (**Figure 3(e)**; Lyons *et al.*, 2014). The PMC lineage has been used as a model for building GRNs to explain fate specification (Ettensohn, 2013; Oliveri

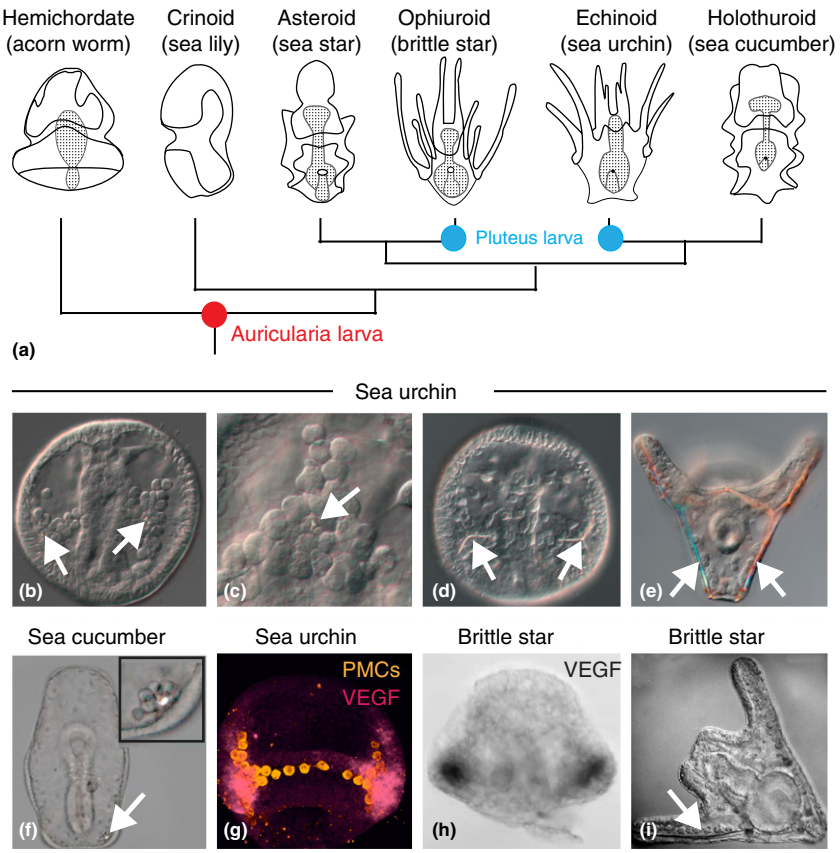

Figure 3 Evolution of larval skeletogenesis in echinoderms. (a) Cladogram showing the evolutionary relationship of echinoderm classes, and their closest outgroup, hemichordates. The auricularia larval form is considered ancestral for echinoderms, making the evolution of the pluteus larva in sea urchins and brittle stars an example of convergence. Cartoons modified from Primus, A.E., 2005. Regional specification in the early embryo of the brittle star *Ophiopholis aculeata*. Developmental Biology 283, 294–309. (b–e) Stages of skeletogenesis in the sea urchin, *Lytechinus*. Arrows point to spicule. (b) Bilateral spicule granules appear within the ventrolateral clusters. (c) Close up of a ventral lateral cluster. (d) The granules grow to form the triradiates during gastrulation stages. (e) Triradiates grow and branch to form the pluteus-stage skeleton. (f) Spicule granule (arrow and inset) in the sea cucumber, *Parastichopus*. Modified from McCauley, B.S., Wright, E.P., Exner, C., Kitazawa, C., Hinman, V.F., 2012. Development of an embryonic skeletogenic mesenchyme lineage in a sea cucumber reveals the trajectory of change for the evolution of novel structures in echinoderms. EvoDevo 3, 17. (g) Double-*in situ* hybridization of *Lytechinus* msp130 transcript (yellow, PMCs) and VEGF transcript (magenta, ectoderm). (h) VEGF transcript is also expressed in the ectoderm overlying the growing arms of the brittle star, *Amphipholis*. Modified from Morino, Y., Koga, H., Tachibana, K., *et al*., 2012. Heterochronic activation of VEGF signaling and the evolution of the skeleton in echinoderm pluteus larvae. Evolution & Development 14, 428–436. (i) Pluteus of the brittle star, *Ophiopholis*; arrow points to one of the skeletal rods supporting the arms. Modified from Primus, A.E., 2005. Regional specification in the early embryo of the brittle star *Ophiopholis aculeata*. Developmental Biology 283, 294–309.

et al., 2008). This detailed knowledge of PMC specification can be used to ask many evolutionary questions, including how the larval skeleton evolved in echinoids, and what role genes required for PMC specification play in clades that lack a larval skeleton (Hinman and Cheatle Jarvela, 2014).

Co-Option of Adult Skeletogenesis for Larval Skeleton Formation

Genes expressed after metamorphosis, during adult skeletogenesis, appear to have been heterochronically shifted into embryonic stages and expressed in the sea urchin PMC skeletogenic lineage (Gao and Davidson, 2008). Using the sea urchin PMC GRN as a starting point, Gao and Davidson (2008) compared the genetic circuitry upstream of the larval and adult skeleton to identify the points at which the programs overlap, and at which they diverge. They found that whereas the transcription factors *ets1*, *alx1*, and *hex* are expressed in both the micromere lineage and the adult rudiment in sea urchins, *tbr* is expressed only in the PMC lineage. *ets1*, *alx*, and *hex* are also expressed in the rudiment of adult sea stars, but *tbr* is not. These data suggest that the *alx/ets1/hex* node of echinoderm adult skeletogenesis is ancient, and was co-opted for larval skeletogenesis in the sea urchin lineage. *tbr*, which is necessary for larval skeletogenesis, but not adult skeletogenesis, was an independent acquisition into the micromere lineage.

What Can Out-Groups Tell Us About the Evolution of the Sea Urchin Pluteus?

Sea cucumbers are the echinoderm class most closely related to sea urchins and sand dollars (Cannon *et al.*, 2014; Reich *et al.*,

2015; Telford *et al.*, 2014), but they lack a larval skeleton. However, a small spine or spicule granule has been observed in the larvae of some sea cucumber species, but it never grows into a triradiate, or makes arms, as occurs during the development of the sea urchin pluteus skeleton. Recent work has investigated the morphological and molecular basis of granule formation in the sea cucumber *Parastichopus* (**Figure 3(f)**; McCauley *et al.*, 2012). In this species, the granule is made by cells that enter the blastocoel early, and these cells go on to form a dorsal cluster underneath the posterior-dorsal ectoderm. *Parastichopus alx1* is expressed in cells at the vegetal pole, and in the cells that enter the blastocoel early and make the dorsal cluster and spicule granule. Knockdown of *Parastichopus alx1* abolishes the dorsal cluster, and the spicule, suggesting that, as in sea urchins, *alx1* is necessary for specifying cells capable of making skeleton material. In the sea cucumber *Holothuria*, *ets1/2* is expressed in mesodermal cells, including a patch of posterior-dorsal cells that might be the mesodermal cells that secrete the larval spicule granule in that species (Koga *et al.*, 2010). These data suggest that sea cucumber mesodermal cells express some of the same genes when making a spicule granule, as the sea urchin PMCs express when making the pluteus skeleton. Koga *et al.* (2010) propose that ancestrally, *ets1/2* had two functions: one specifying larval mesoderm that was non-skeletogenic, and a second specifying adult skeletogenesis. In the echinoid lineage, the *ets1/2* transcription factor gained the ability to upregulate the skeletogenic program in the larval mesoderm.

In fact, many genes associated with the sea urchin PMC lineage are expressed in the larval mesoderm of species that do not make larval skeletons, such as the sea stars (Koga *et al.*, 2010; McCauley *et al.*, 2010; Morino *et al.*, 2012). These studies support the idea that the genes involved in skeletogenic mesoderm in sea urchins were likely expressed in the larval mesoderm of the echinoderm common ancestor and then later became able to promote skeletogenic cell fate by subtle changes in gene regulation, such as downstream gene target switching (Koga *et al.*, 2010).

Convergent Evolution of the Pluteus in Brittle Stars?

How similar is the development of the larval skeleton between sea urchins and brittle stars? Unlike sea urchins, the brittle star skeletogenic lineage does not arise from a noticeable asymmetric cell division at 4th cleavage (Primus, 2005). Instead, the skeletogenic cells, similarly called PMCs, become obvious inside the blastocoel before gastrulation and form bilateral rudiments of the larval skeleton (Yamashita, 1985). The behavior of the brittle star PMCs suggests that they might also migrate in response to signals from the ectoderm. In fact, in the brittle star *Amphipholis* Morino *et al.* (2012) found that homologs of VEGF are expressed in bilateral patches of ectoderm (**Figure 3(h)**), very much like the pattern in urchins; VEGFR is likewise expressed in adjacent ventrolateral PMC clusters. As in sea urchins (Adomako-Ankomah and Ettensohn, 2013; McIntyre *et al.*, 2013), the expression of brittle star VEGF becomes restricted to lateral ectodermal patches.

This remarkable similarity in expression patterns of VEGF and VEGFR between sea urchins and *Amphipholis* demonstrates that there are fundamental similarities in how the two groups make their larval skeleton. More work on brittle stars will be necessary before we fully understand how deep the similarities go, on a cellular or molecular level. For example, *Amphipholis* (Koga *et al.*, 2010) also expresses the *ets1/2* gene in its PMCs, and a transcriptome of *Ophiocoma* gastrula-stage embryos (Vaughn *et al.* 2012) revealed that many homologs of genes involved in sea urchin PMC specification and skeletogenesis are expressed in this brittle star species. Studies of both the transcripts and proteins made by *Ophiocoma* show that the skeletogenic tool kit is similar, but not identical, to that in sea urchins (Seaver and Livingston, 2015; Vaughn *et al.*, 2012).

The fact that in both sea urchins and brittle stars members of the VEGF signaling pathway are expressed in analogous territories (VEGF ligand in the ectoderm, and VEGF receptor in the PMCs) suggests that communication between mesoderm and ectoderm was critical for the evolution of the pluteus in both echinoderm lineages. Whether sea cucumber or sea star skeletogenic cells are responding to cues from the ectoderm during the larval stage remains to be answered. The expression of *vegf* and *vegfr* has been examined in sea stars (Morino *et al.*, 2012), and no transcripts for either gene were detected by *in situ* hybridization or qPCR during early larval stages. Yet later, *vegf* (expressed in the ectoderm), and *vegfr* (expressed in the mesoderm), were associated with the rudiments of the adult skeleton. Thus VEGF signaling might have been heterochronically activated during larval stages, in ectoderm and mesoderm, in sea urchins and brittle stars independently. How this occurred poses a fascinating open question for future investigations. In order to understand the evolution of a novel cell type, we will need to hone in on the conversation between these two tissues.

Part III: Cell Fate Specification in Adult Animals

Specification of cell fate from undifferentiated cells is essential to the maintenance of adult form, as adult tissue can be lost during homeostatic turnover or due to damage. Some animals (e.g., planarians), have pluripotent adult stem cells that can acquire many distinct cell fates, whereas other animals (e.g., vertebrates) possess lineage-restricted stem cells. Very little is understood about how the mechanisms that underlie stem cell pluripotency and subsequent fate specification compare across these diverse species, but recent studies in previously understudied animals have revealed valuable insight.

Differentiation in Lineage-Restricted Adult Stem Cells

Adult mammals have the capacity to continually replace tissues that are lost to homeostatic turnover or to injury. Pools of lineage-restricted stem cells that are maintained throughout adulthood provide new cells. These include hematopoietic stem cells in the bone marrow, which generate myeloid and lymphoid lineages of blood cells; slow-cycling cells of the intestinal crypt that make transit amplifying cells to replace secretory and digestive cells of the gut; and stem cells located in the bulge regenerate hair follicles (Clevers, 2013; Fuchs and

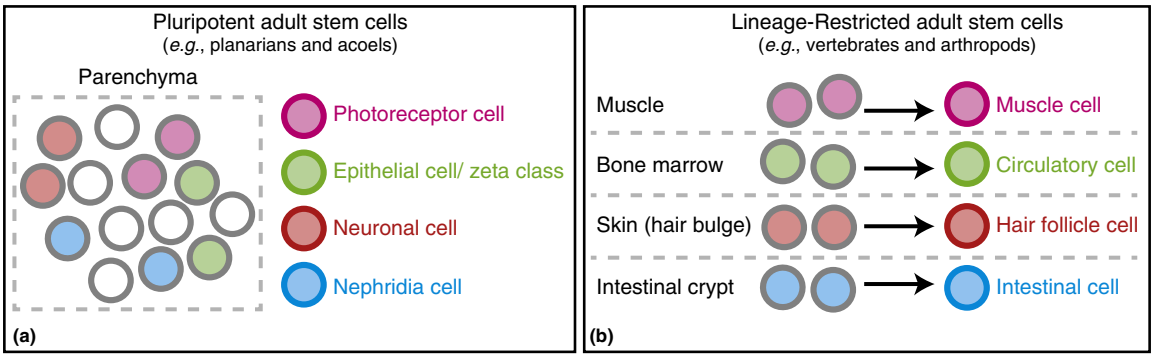

Figure 4 Schematic representation of cell fate specification from planarian adult pluripotent stem cells and lineage-restricted stem cells in vertebrates. (a) Planarian stem cells (neoblasts) are a broadly distributed population of cells that include progenitors that have become committed to specific lineages. The clonogenic-neoblasts (white circles with grey outlines) and progenitors (colored circles with grey outlines) are not separated spatially, but terminally differentiated cell types (colored circles with colored outlines) incorporate into tissues at specific positions in the adult body. Clonogenic-neoblasts are presumed to give rise to the committed progenitors, but this has not been shown directly. (b) Vertebrate organs harbor lineage-restricted stem cells (colored circles with grey outlines) that generate terminally differentiated cells (colored circles with colored outlines) for the specific tissue. These stem cells are spatially restricted to their tissue of origin.

Nowak, 2008; Seita and Weissman, 2010; **Figure 4**). These independent stem cell populations express distinct molecular markers and acquire their restricted cell fates under the control of different factors depending on their varied tissue contexts.

In contrast to blood, intestinal crypts, or hair, which are unique to vertebrates, muscle is present in all bilaterian animals, providing an opportunity to compare specification mechanisms across species. Vertebrates have quiescent stem cells called satellite cells that express *Pax7* protein, may or may not express *Pax3*, and may have sequestered mRNA for the myogenic factor *Myf5* (reviewed in Cerletti *et al.*, 2008; Motohashi and Asakura, 2014). Upon injury, these cells respond to signals from their niche, i.e., the surrounding microenvironment, by dividing asymmetrically to produce a myoblast. This cell downregulates *Pax7* expression, upregulates the expression of *Desmin* and *MyoD*, proliferates, and, with increasing expression of other myogenic factors such as *MRF* and *Myogenin*, differentiates into a muscle fiber.

A recent study on muscle regeneration in a new arthropod model system provides the first opportunity to compare regenerative mechanisms across distantly related bilaterian phyla. Whereas *Drosophila*, the classic model arthropod, cannot regenerate limbs, the crustacean *Parhyale hawaiiensis* is able to regrow its appendages upon amputation. The *Parhyale* ortholog of vertebrate *Pax3* and *Pax7*, *Pax3/7*, labels cells that are present adjacent to muscle fibers and morphologically resemble vertebrate satellite cells (Konstantinides and Averof, 2014). Transgenically-labeled satellite cells were isolated, and upon transplantation into host limbs, contributed to newly formed muscle fibers in regenerating limbs. This finding suggests that arthropod and vertebrate muscles regenerate in a very similar manner, using lineage-restricted stem cells that are labeled by an evolutionarily conserved marker, *Pax3/7* (**Figures 1** and **4**). Moving forward, it will be fruitful to investigate more broadly in cell types besides muscle, commonalities between lineage-restricted stem cells, which are present in many animal phyla, including the early branching non-bilaterians (e.g., ctenophores, cnidarians) (Alie *et al.*, 2011; Plickert *et al.*, 2012).

Specification in Pluripotent Adult Stem Cells

Adult planarians show amazing regenerative abilities. They can regenerate virtually any missing tissue through the activity of a large population of parenchymal cells called neoblasts, which are required for regeneration (Reddien *et al.*, 2005; **Figure 4**). Based on their shared expression of homologs of *piwi*, neoblasts were considered to be a homogeneous population. Single neoblasts transplanted into irradiated animals expand clonally (thus referred to as clonogenic- or c-neoblasts), differentiate into all tissue types of the adult animal, and restore the regenerative capacity of their hosts (Wagner *et al.*, 2011). It is unknown what proportion of the total neoblast population is clonogenic.

Studies of regeneration in planarians of specific organs, such as nephridia and eyes, revealed that transcription factors that are expressed in and required for the regrowth of these structures, also label a small number of *piwi*+ cells (**Figure 4**). These cells are thought to be committed progenitors that begin to express a tissue-specific marker (*Sp6-9*, *Dlx*, *Six1/2*, and *Eya* for pigment cups of the eye; *Six1/2-2*, *Eya*, *Osr*, *POU2/3*, and *Sall* for nephridia), lose *piwi* expression, and become terminally differentiated (Lapan and Reddien, 2011; Scimone *et al.*, 2011). Several progenitor classes for different neuronal lineages have also been recently identified (Cowles *et al.*, 2013; Scimone *et al.*, 2014). Clustering of neoblasts, based on the expression of 96 genes in single cells, revealed progenitor lineages that differentiate into the gut and epidermis (van Wolfswinkel *et al.*, 2014). Thus, neoblasts represent a dynamic population of stem cells that differentiate into varied cell types, presenting a great opportunity to understand the mechanisms of fate specification in adults.

It is unknown whether the mode of adult cell fate specification uncovered in planarians is broadly conserved among animals. Acoel worms diverged from planarians 550 mya and likely represent the earliest-diverging lineage of animals with bilateral symmetry (Hejnol *et al.*, 2009; Philippe *et al.*, 2011; Srivastava *et al.*, 2014). Acoels also have a population of proliferative cells that resemble planarian neoblasts based on

morphology, expression of *piwi*, and sensitivity to radiation (De Mulder *et al.*, 2009). A recent study in *Hofstenia miamia*, a new model acoel species, revealed that expression of *piwi* in neoblasts is required for regeneration (Srivastava *et al.*, 2014). Additionally, Wnt and Bmp signaling pathways are required for correctly regenerating tissues along the anterior–posterior and dorsal–ventral axes respectively in *Hofstenia*, suggesting that the decisions upstream of fate specification are also shared between acoels and planarians. *Hofstenia* is amenable to mechanistic studies of differentiation within the neoblast population which, combined with similar studies in planarians, could inform us on whether fate specification in adult pluripotent stem cells is evolutionarily conserved or independently-evolved.

Many other regenerative animal species have putative pluripotent adult stem cells that express piwi (e.g., sponges, cnidarians, annelids, and ascidians) (Alie *et al.*, 2011; Brown *et al.*, 2009; Funayama *et al.*, 2010; Giani *et al.*, 2011; Juliano *et al.*, 2014; Plickert *et al.*, 2012; Rinkevich *et al.*, 2013), but fate specification mechanisms in these species are currently unknown.

The Evolution of Stem Cell Fate Specification

Given that both lineage-restricted and pluripotent modes of adult stem cells are broadly distributed across animal phylogeny, it is unclear which mode represents the ancestral condition (**Figure 1**). For example, ctenophores and sponges, the two earliest-diverging animal lineages, feature lineage-restricted and pluripotent stem cells respectively (Alie *et al.*, 2011; Funayama *et al.*, 2010).

Fate specification mechanisms may be conserved, regardless of the source of undifferentiated cells. Small populations of lineage-committed progenitors within the total neoblast population in planarians could be analogous to the lineage-restricted pools of stem cells in vertebrates and crustaceans. If the satellite-like cells in *Parhyale* and vertebrates maintain stemness and differentiate into muscle fibers via the same molecular mechanisms, then one would infer that these mechanisms appeared in the bilaterian ancestor. Planarians also evolved from this same ancestor, and one might hypothesize that a subset of neoblasts that are committed to differentiate into muscle would resemble satellite cells, for example requiring the expression of a *Pax3/7* homolog. One *Pax3/7* homolog reported thus far from planarians is required for the formation of progenitors for *dopa-beta-hydroxylase*+ cells of the nervous system (Scimone *et al.*, 2014). Recently reported MyoD-expressing *piwi*+ cells may represent planarian muscle progenitors (Cowles *et al.*, 2013; Scimone *et al.*, 2014). Thus, a detailed investigation of muscle progenitors within the neoblast population is needed to illuminate this hypothesis.

An alternative explanation for shared mechanisms underlying lineage-committed progenitors could be independent co-option of developmental pathways in the adult. For example, *Pax3* and *Pax7* expressing cells form muscle during mouse embryonic development (Relaix *et al.*, 2005), and if these transcription factors are conserved regulators of muscle cell fate in other animals as well (e.g., in arthropods and flatworms), then their role in adult muscle progenitors would reflect a hard-wired control over downstream genes that mediate muscle cell function. Investigating the details of how *Pax3/Pax7* are regulated to hold progenitor cells in a paused state, and subsequently to release them to acquire muscle fate in distantly related species, will be crucial to understanding the overlap between developmental versus adult muscle specification.

Studies of fate specification in adults not only inform how animal body plans are maintained, but how adult stem cells are regulated. Emerging model systems such as planarians, acoels, and crustaceans offer opportunities for mechanistic investigations of fate specification in a variety of cell types in the context of pluripotent and lineage-restricted adult stem cells. In addition to cell-intrinsic control of fate specification, it will be important to compare niche signals, which are essential regulators of vertebrate adult stem cells.

Conclusions and Future Directions

This is an exciting time to be investigating the molecular basis of cell fate specification in an evolutionary context. We are fortunate to have a wealth of data generated by traditional model systems that provides a framework for empirical testing. The ease of adapting CRISPR/Cas9 to both induce mutations in genes of interest as well as introduce GFP and other fluorescent markers into endogenous loci is enabling researchers to move beyond the descriptive approaches that dominated the field of evo-devo for the past two decades. These new genome engineering techniques will allow us to directly test gene function and visualize cellular behaviors in nearly any taxon of choice. These molecular approaches are critical if we are to understand how alterations in cell fate specification strategies result in evolutionary change. Whether they are represented by cryptic variation in the formation of homologous cell types and tissues, changes in GRNs that result in the formation of novel cell types, or changes in the molecular cascades that maintain the balance between stemness and differentiation in response to injury or environmental stress in adults, the next decade should see huge leaps forward in our understanding of the evolution of cell fate specification in multicellular organisms.

See also: Cellular Behaviors Underlying Pattern Formation and Evolution. Novel Structures in Plants, Developmental Evolution of

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Developmental Paleontology and Paleo-Evo-Devo

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Glossary

Ambulacral field Part of the plate system of an echinoderm bearing the tube feet.
Cambrian Earliest geological age during which animal life is known with certainty.
Cycloneuralia Group of worm-shaped animals which are characterized by an anterior nerve ring; it includes Nematoda, Nematomorpha, Priapulida, Kinorrhyncha, and Loricifera.
Disparity Morphological diversity.
Doushantuo Geological formation in China, dating shortly before the Cambrian.
Eryoneicus Larva of polychelid lobster, adapted to long-term pelagic life.
Heterochrony Evolutionary shift in developmental timing.
Metamorphosis Short phase during post-embryonic ontogeny with rapid and drastic change in morphology.
Metanauplius Early larva of (eu-)crustaceans, subsequently adding segments and appendages.
Monophyletic group Group including all species descending from the same stem species, including the latter.
Ontogeny Individual development of an organism.
Orsten Special fossil preservation type, in the strict sense limited to early Paleozoic deposits; three-dimensional uncompressed preservation, including finest details down to the sub-micrometer range.
Otolith Small stones in the static organ, for example, of fishes, with growth lines often used to determine the age of the organism.
Phyllosoma Larva of achelate lobsters with a very thin, leaf-shaped body.
Phylogram Diagram of relationship, following traditional phylogenetic systematics, additionally giving a relative timing of the species splitting events.
Plesiomorphic Ancestral trait inherited from the stem species.
Precambrian Geological age before the Cambrian.
Proximal endite Median sclerotization in the body-appendage membrane in crustaceans, important for further evolution within crustaceans.
Spinicaudata Group within branchiopod crustaceans, with two large shield valves showing growth lines.
Taxonomy Discipline of naming and classifying species.
Viviparous Giving birth to living offspring (in contrast to, e.g., laying eggs).

Developmental paleontology is a scientific discipline that focuses on the ontogeny, i.e., the individual development of extinct organisms. A primary goal of paleo-evo-devo, which is an expansion of developmental paleontology, is to set developmental information from extinct organisms into an evolutionary context. To achieve this goal, paleo-evo-devo scientists investigate two specific questions:

1. How did ontogenetic patterns change through time?
2. How did changes of ontogenetic patterns contribute to evolutionary patterns in general?

Paleo-evo-devo research differs in its approach from traditional evo-devo research on extant organisms in certain aspects. For example, traditional evo-devo studies on extant taxa often rely heavily on modern genetic tools, while paleo-evo-devo studies tend to rely more on morphological comparisons of structures of various developmental stages and ontogenetic sequences.

In the following, we will first explain why a paleo-evo-devo approach is not only an interesting amendment of 'normal' paleontological or evo-devo studies, but also is absolutely crucial for the understanding of evolution. In the second part, we present how paleo-evo-devo studies are practically performed, especially in the light of an often scarce-appearing dataset. As we are zoologists and work on animals, we restrict our examples to these. However, paleo-evo-devo studies can also be performed on other groups of organisms, for example, on plants or unicellular organisms such as foraminiferans (e.g., Brummer *et al.*, 1986; Boyce, 2010; Gerrienne and Gonez, 2011). Therefore, several aspects of what is discussed below can also be transferred to nonanimal groups.

Why Is a Developmental Paleontological and Paleo-Evo-Devo Approach Necessary?

We here discuss five reasons why a developmental paleontological and paleo-evo-devo perspective is an important component of modern synthetic research.

Taxonomy

A comparably underestimated value of developmental paleontology, and not straightforward to recognize, is its taxonomic significance. If two fossil specimens differ morphologically, it is important to differentiate if these two morphs represent different species or simply different developmental stages of the same species (or other possible intraspecific morphs such as sexes, seasonal morphs, and castes). This issue also played an important role in the initial identification of some modern species, for example, many aberrant larval stages of crustaceans were originally described as separate species (and even higher categories; Harvey *et al.*, 2002).

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The most prominent examples of such ontogeny-confounded taxonomic issues can be found in dinosaurs. Horner and co-workers have proposed that supposed separate species among Marginocephalia (= Pachycephalosauria or dome-headed dinosaurs + Ceratopsia or horned dinosaurs) indeed represent different growth stages of a single species (Horner and Goodwin, 2009; Scannella and Horner, 2010; see also below). Although these interpretations remain controversial (see e.g., Longrich and Field, 2012; Maiorino *et al.*, 2013), they raise a significant question: how should one place 'morphological differences' among fossils in a biological context? This issue has also been raised in arthropod groups (e.g., Haug *et al.*, 2012).

Furthermore, it should be noted that it is also possible that supposed larval stages in the fossil record may well represent separate dwarf species (example in extant animals: Worsaae *et al.*, 2012).

These taxonomic issues have several potentially significant implications:

a. An important aspect raised by Horner and co-workers is that of diversity. If several supposed species are in fact representatives of a single species, the species richness and diversity of a fossil community have been lower than originally anticipated. What is however not lowered through such a shift in the interpretation is the disparity of a community. If supposed conspecific morphs in fact represent different species, the diversity would in fact be higher.

b. Certain species may not differ recognizably as adults, but have quite differing larval stages or ontogenetic sequences (examples in De Beer, 1958). Hence, these developmental stages can contribute characters for recognizing two species as separate ones (see also discussion in Haug *et al.*, 2012). Generally it is thought that early stages are more similar between different species and later stages more distinct morphologically (e.g., Haeckel, 1866; Høeg and Møller, 2006). Yet, quite the opposite might play a major role here.

c. Flawed taxonomy also has the potential to confound phylogenetic reconstructions. If developmental stages of a single species are included into a phylogenetic analysis as separate terminals this will lead to significant artifacts. Conspecific but morphologically differing stages will not be resolved as a monophyletic group in the tree (see Wolfe and Hegna, 2014). Hence, a single species will occupy numerous different positions in a single tree. This will not only make the whole topology meaningless, but also obscure character evolution entirely. The same problem also applies to the opposite: if separate species are treated as conspecific morphs they are automatically forced to represent a monophyletic unit, although some of these may in fact be closer related to other species. Hence only a careful a priori discussion before the phylogenetic analysis will lead to meaningful results.

d. Biogeographical insights can also suffer heavily from taxonomic and phylogenetic artifacts if different developmental stages are misidentified. Such a scenario might be the case within certain representatives of Pachycephalosauria. According to the phylogeny reconstructed by Snively and Cox (2008), closely related species occur on different continents, i.e., in Asia and North America. This interpretation leads to a scenario which requires several migrations of the populations. However, when the skull morphology of the co-occurring species is considered, these assumed separate species could also represent different developmental stages of the same species (Horner and Goodwin, 2009; **Figure 1**). Hence, significantly less migration events are necessary to explain this phylogenetic pattern.

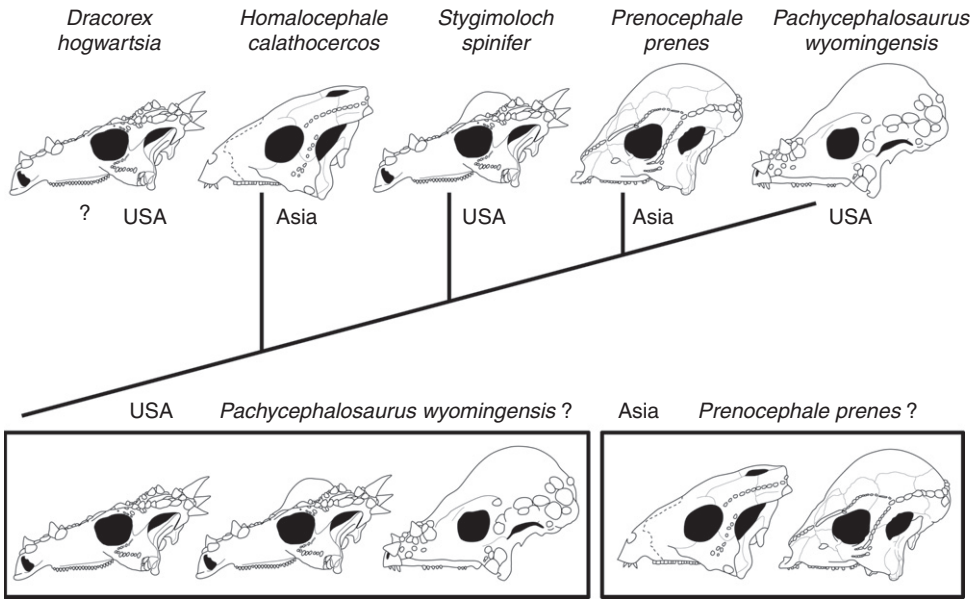

Figure 1 Dome-headed dinosaurs (Pachycephalosauria) as an example for taxonomic, phylogenetic, and biogeographic challenges. According to the simplified phylogeny (top; after Snively and Cox, 2008), several migration events between Asia and North America need to be assumed for these species. However, if some species represent ontogenetic stages of another one, the distribution of the fossils is much easier to explain.

Minimum Ages

A very important aspect of developmental paleontology is that it provides minimum ages for modern developmental patterns. In most cases, minimum ages for developmental patterns are based on phylogenetic inference. While the latter can be comparably reliable, minimum age calculations rely on the assumption that development has remained comparable in the early and modern representatives of a lineage. Fossil data can be used to test such an assumption or also to reject it.

The fossil record of crustaceans, in particular, highlights the insights that can be obtained from the fossil record concerning the earliest appearance of modern larval morphotypes. The oldest known crustacean larva, *Wujicaris muelleri* from the lower Cambrian of China (520 million years old), already represents a modern morphotype, similar to larvae of barnacles (**Figure 2(a)**; Zhang *et al.*, 2010). Therefore, fossil evidence suggests that this larval type, called metanauplius, has evolved more than half a billion years ago.

The fossil record also provides evidence for modern-type larvae in some highly specialized crustacean ingroups. For example, the lithographic limestones of southern Germany (150 million years old) have produced the oldest example of a modern-type larva of mantis shrimps (Stomatopoda; but also now extinct morphotypes, see below), which already possesses all characteristics seen in living stomatopod larvae (**Figure 2 (b)**; Haug *et al.*, 2008).

Additionally, thousands of larval specimens of achelatan lobsters (slipper and spiny lobsters) have been found in the Solnhofen limestones (**Figure 2(c)**; e.g., Polz 1972, 1973, 1984; Haug *et al.*, 2011), the so-called phyllosoma larvae. These larvae are among the most aberrant-appearing larval forms of all

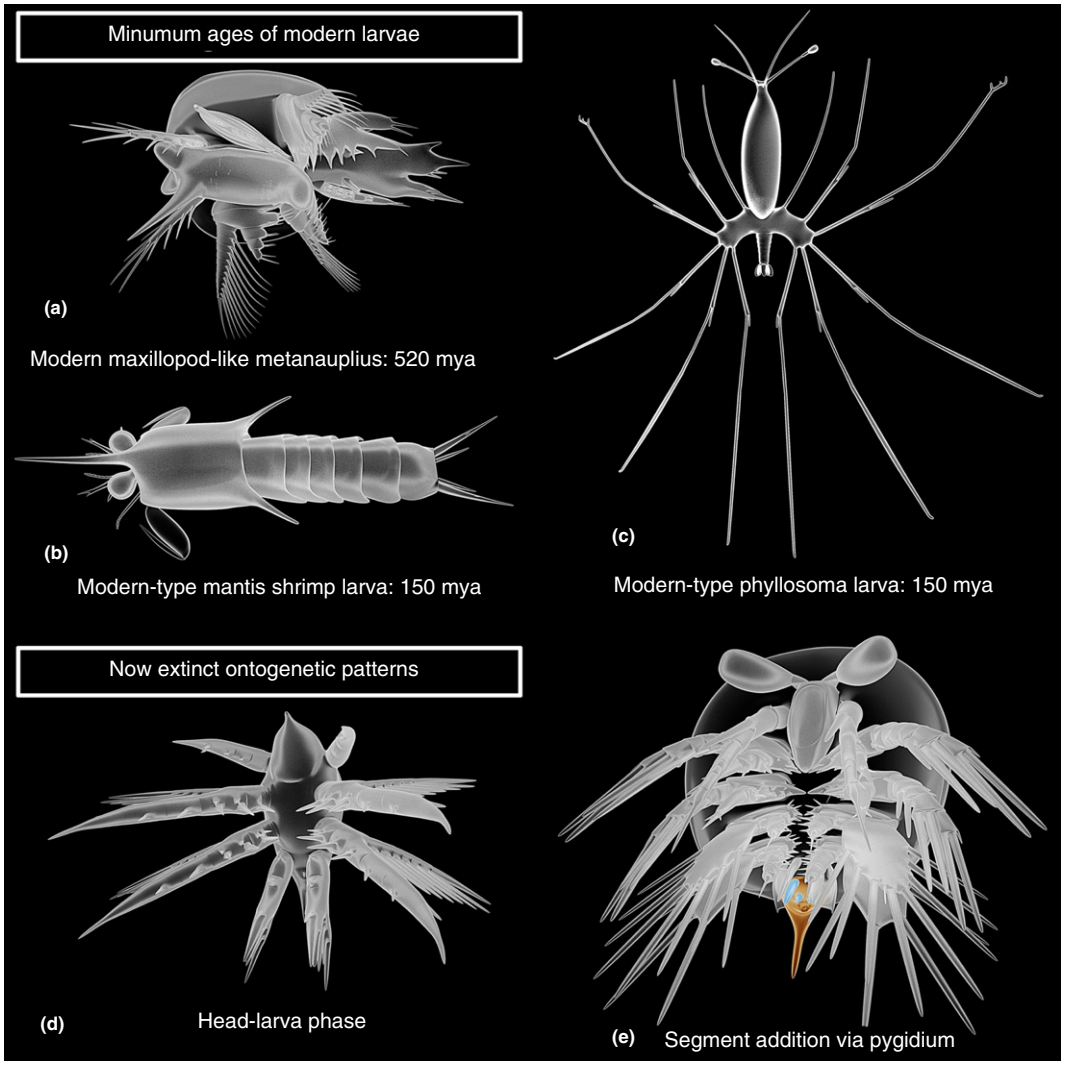

Figure 2 Minimum ages of modern larval morphotypes (a–c) and developmental patterns no longer existing today (d, e). (a) Oldest known crustacean larva, *Wujicaris muelleri* from the lower Cambrian of China (520 million years old), already closely resembling modern larval morphology. (b) Modern-type mantis shrimp larva from the Upper Jurassic Solnhofen limestones (150 million years old). (c) Modern-type phyllosoma larva from the Solnhofen limestones. (d) Head larva of early crustacean, *Martinssonia elongata* from the middle Cambrian Orsten of Sweden (505 million years old). (e) Larva of early crustacean, *Henningsmoenicaris scutula* from Swedish Orsten, with segment addition via pygidium (orange, with limb buds in blue).

crustaceans. The body is leaf-like (name!), flat and translucent; the appendages are very elongate and slender; distally they possess a sub-chelate claw for grasping jellyfish or comb-jellies. Phyllosoma larvae can reach astonishing sizes of up to 150 mm. As in the previous example, fossil phyllosoma larvae look very much like the modern ones. Therefore, the highly specialized larvae of this group had already evolved 150 million years ago.

Ancestral, but Now Extinct Patterns

Fossil developmental data can also reveal ancestral developmental patterns that are now extinct. These extinct patterns may bridge very differing developmental patterns in modern groups that otherwise would remain seemingly unrelated.

As above, several examples of these now extinct developmental patterns can be found among crustacean fossils, some of them in the middle Cambrian Orsten from Sweden (505 million years old; e.g., Maas *et al.*, 2006). One example is the head larva. Early crustaceans such as *Martinssonia elongata* hatched as head larva with four pairs of legs (**Figure 2(d)**), while modern crustaceans hatch with only three pairs (see above). Additionally, the segment number at hatching was retained in early crustaceans for several molts while modern crustaceans add segments subsequently at each molt (e.g., Müller and Walossek, 1986; Walossek, 1993; Haug *et al.*, 2009a; Haug *et al.*, 2010a,b).

Another now extinct developmental pattern present in early crustaceans, trilobites, agnostines, and possibly a number of other early euarthropods is the mode of segment addition via a pygidium (e.g., Haug *et al.*, 2010a). While in modern crustaceans segments bud off an undifferentiated posterior budding zone, the pygidium (e.g., in *Henningsmoenicaris scutula*; **Figure 2(e)**) is a posterior body area with a dorsal shield and well identifiable segmental markings. From this area the segments bud off anteriorly.

Further examples of now extinct developmental patterns can be inferred from crustacean larvae found in the Solnhofen and other Mesozoic limestones. As mentioned above, achelatan lobsters and mantis shrimps are both represented in these deposits with modern as well as ancestral-appearing larvae. In achelatan lobsters, a number of larval forms in these limestones combine phyllosoma and post-phyllosoma characters in very different combinations (**Figure 3(a–c)**; Haug *et al.*, 2009b, 2013; Haug and Haug, 2013). Such character combinations are unknown from any modern achelatan lobster.

For the evolution of developmental patterns in achelatan lobsters, three scenarios were offered by Haug and co-authors, which are probably all represented in the material:

1. Some of the fossil larvae could represent intermediate stages between modern-type phyllosoma and post-phyllosoma stages, but which were evolutionary skipped in modern forms (condensation).

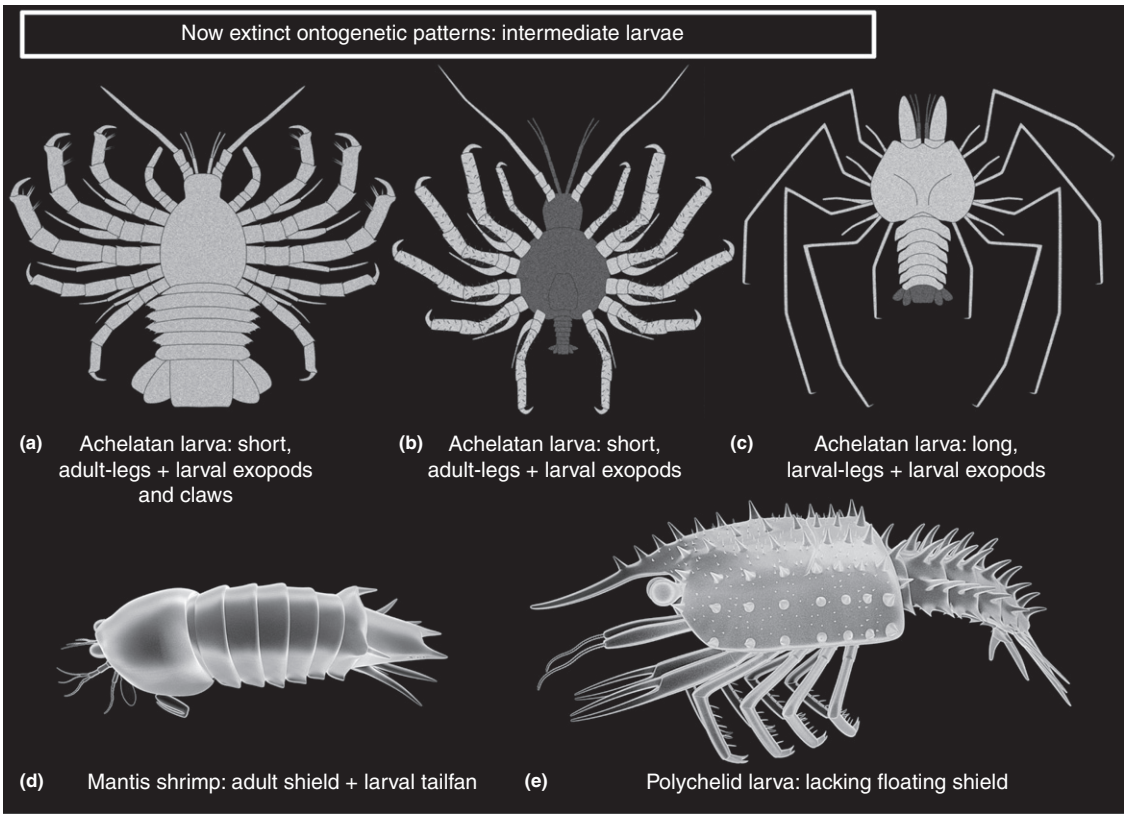

Figure 3 Developmental patterns no longer existing today, continued. (a–c) Different types of achelatan larvae with a mixture of typical phyllosoma and post-phyllosoma characters from the Upper Jurassic Solnhofen limestones (150 million years old). (d) Late larva of the mantis shrimp, *Spinosculda ehrlichi* from the Solnhofen limestones. (e) Larva of a polychelid lobster from Upper Cretaceous Lebanese limestones (90 million years old).

2. Some of the fossil larvae could be evolutionary early 'proto-phyllosomes' which did not yet possess all specifications modern-type phyllosoma larvae have.
3. Some of the fossil larvae could represent paedomorphic species, so they would have retained larval characters into the 'post-larval' phase.

The ancestral-appearing mantis shrimps larval type found in the Solnhofen limestones differs from the co-occurring modern type in its relatively advanced morphology at a still rather small body size (**Figure 3(d)**). While still retaining the larval tail fan, it already possesses the short adult head shield. Other features characteristic for modern-type stomatopod larvae are lacking, such as the long spines on the shield. As also other developmental stages of the stomatopod species with this ancestral larval type have been discovered (Haug *et al.*, 2009), it appears that the ontogeny of this species did not comprise a drastic metamorphosis as in modern species, but rather happened more gradually.

Another crustacean group from Mesozoic limestones in which an ancestral developmental pattern occurred, which is no longer present today, is polychelidan lobsters. Today, polychelidan adults and larvae both live in the deep sea. In the Mesozoic, however, representatives of this group lived in much shallower areas. This change of the habitat resulted in morphological changes in the larvae: while modern polychelidan larvae, also called eryoneicus larvae, are large, blind, and possess an extremely blown-up spinose shield, fossil larvae from Cretaceous limestones of Lebanon (ca. 95 mya) have eyes and relatively normal-sized, though spinose shield, but are in total also quite large (**Figure 3(e)**). In comparison with the supposedly more ancestral developmental pattern of certain polychelidan species from the Solnhofen limestones, these fossil data suggest that modern polychelidan larvae likely evolved through the stepwise acquisition of specialized morphological characters (Haug *et al.*, 2015).

Evolution of Body Organization

In paleo-evo-devo, as well as in an evo-devo approach with extant organisms, developmental patterns are used to explain the evolution of new features of body organization. The advantage of incorporating a paleo-evo-devo approach is that extinct developmental patterns can also be included into analyses. Such ancient datasets are especially valuable for groups in which large radiation events occurred many millions of years ago.

One example is the evolution of echinoderms (sea urchins, sea stars, sea cucumbers, etc.). Certain fossil representatives exhibit a very specific developmental pattern, which can be deduced from the plate arrangement in adult specimens (Sumrall and Wray, 2007; Sumrall, 2008). At first, a pair of lateral ambulacral fields is developed, followed by one anterior ambulacral field. Then, the two lateral fields divide into two fields each, resulting in five fields in total. With this pattern as starting point, the evolution of the very diverse forms of body organization in the different fossil echinoderm groups can be deduced. If the development of the anterior field is inhibited, only four ambulacral fields develop. The inhibition of the split of the lateral fields leads to three ambulacral fields. If anterior field and the lateral split are inhibited, only two fields develop. Finally, if the development of the lateral fields is inhibited, the result is only one ambulacral field. With this, a complete range of adult forms of body organization can be deduced from one ancient developmental pattern.

Evolutionary Scenarios

When evolutionary scenarios are reconstructed based exclusively on adult specimens, evolutionary 'novelties' often appear to have evolved abruptly. However, developmental information from fossils often makes it clear that these 'novelties' could have arisen through slight changes in developmental timing. As a result, fossil data often make it possible to reconstruct a much finer graded evolutionary scenario.

An example of this can be found in the early evolution of crustaceans, where fossil data have demonstrated that changes in developmental timing have driven morphological evolution. A very important morphological structure in crustaceans is the proximal endite, an exclusively median sclerotization in the body-appendage joint membrane, which contributes to the feeding apparatus. In certain appendages of certain crustaceans, the proximal endite is transformed into a coxa, a large and prominent structure, which forms a closed ring and often bears a median shovel-like extension. Data from the fossil record suggest that the evolutionary transformation of this structure occurred through small changes in the timing of its appearance during ontogeny. In the earliest crustaceans, the proximal endite is not present in early ontogenetic stages (**Figure 4(a)**) and remains small in later stages (**Figure 4(b)**). However, the proximal endite appears earlier in ontogeny later in crustacean evolution (**Figure 4(c)**). As a result, the proximal endite has more time to grow larger in later ontogenetic stages than in earlier crustaceans (**Figure 4(d)**). The repetitive occurrence of such a heterochronic pattern finally leads to the development of a coxa (**Figure 4(e)**; for more details, see Müller and Walossek, 1985; Haug *et al.*, 2010a,b).

Another example of a finely graded evolutionary scenario, in which the necessity to include fossil data becomes evident, occurs in achelate lobsters. The antenna of modern achelate lobsters show two distinct morphologies: a very long and slim antenna in spiny lobsters (**Figure 4(f)**), and a shovel-like, stout antenna in slipper lobsters. Fossil representatives, however, appear to exhibit intermediate antenna morphologies, especially when developmental stages are examined. For example, *Cancrinos claviger* from the Solnhofen limestones (**Figure 4(g)**) begins its development with a spiny-lobster-like antenna (**Figure 4(h)**) which then develops a broader proximal, set-off component (**Figure 4(i)**) and finally loses the thin distal component (**Figure 4(j)**; Haug *et al.*, 2009b). In this case, antenna development in a fossil achelate lobster recapitulates the evolution of antennal form within the group.

How Is a Study in Developmental Paleontology and Paleo-Evo-Devo Performed?

Largely Post-Embryonic Ontogeny

In general, developmental paleontological studies are based on post-embryonic stages, or stages occurring after the animal

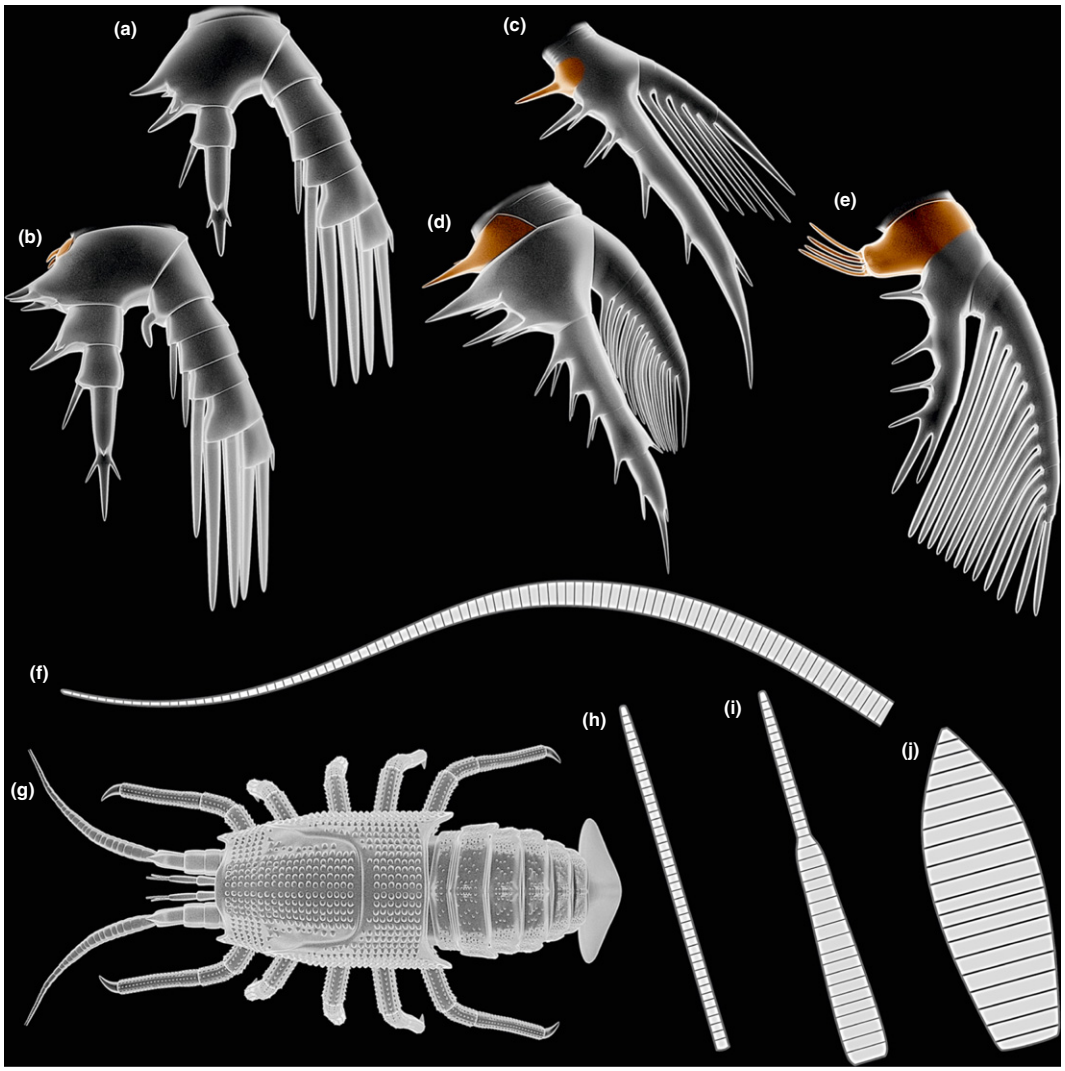

Figure 4 Evolutionary scenarios. (a–e) Evolution of the crustacean coxa as a specialization of the proximal endite, exemplified on the (second) antenna of half a billion years old Orsten crustaceans. (a) Young ontogenetic stage in the early crustacean *Henningsmoenicaris scutula*, still without proximal endite. (b) Later stage of *H. scutula*; proximal endite present (orange), but remains small also in this later stage. (c) Young ontogenetic stage of *Martinssonia elongata*, which is closer related to modern crustaceans (Eucrustacea) than *H. scutula*; the proximal endite (orange) appears here earlier in the ontogeny than in the earliest crustaceans. (d) Later stage of *M. elongata*; the proximal endite (orange) has grown larger than in similar stage of the earliest crustaceans. (e) Putative adult of *Skara anulata*, a eucrustacean; the repetitive occurrence of heterochronic events finally led to the development of a coxa (orange). (f–j) Evolution of the antenna within achelate lobsters. (f) Very long and slim antenna in spiny lobsters. (g) Reconstruction of a late juvenile of *Cancrinos claviger*, Solnhofen limestones. (h) Spiny-lobster-like antenna in early developmental stage of *C. claviger*. (i) Antenna in later ontogenetic stage of *C. claviger* with a broader proximal, set-off part. (j) Antenna in still later ontogenetic stage of *C. claviger* with thin distal part lost.

is born or has hatched from the egg. The reason is simply that embryonic stages are usually relatively small and very soft, which lowers their preservation potential and makes them difficult to find.

However, there are numerous exceptions to this rule. Viviparous species have the potential to preserve embryos within the mother when the latter becomes fossilized. Well-known examples come from vertebrate fossils, such as adult marine reptiles with late embryos preserved within the body, or nests with dinosaur and pterosaur eggs (e.g., Carpenter *et al.*, 1994; Carpenter, 1999; Caldwell and Lee, 2001; Norell *et al.*, 2001; Cheng *et al.*, 2004; Chiappe *et al.*, 2004).

Additionally, isolated invertebrate fossil embryos have also been preserved in late Precambrian and Cambrian strata as a result of Doushantuo and Orsten-type preservation (e.g., Chen and Chi, 2005; Donoghue and Dong, 2005; Donoghue *et al.*, 2006a,b, and references therein). The systematic position of these specimens is often difficult to assign; a notable exception is the embryo of a cycloneuralian worm, assigned to different species of *Markuelia* (e.g., Donoghue *et al.*, 2006a,b; Haug *et al.*, 2009c; Zhang *et al.*, 2011; Duan *et al.*, 2012). Such fossils appear to be highly restricted to a specific preservational window. Nevertheless, the preservation can be so intricate that even the finest details such as pores or tiny hairs are still

present. Hence, such fossils are immediately comparable to their modern counterparts.

However, even if only an older animal is preserved, information can sometimes be gleaned about earlier developmental stages. Certain animals such as molluscs or brachiopods may, for example, preserve aspects of the embryonic shell (see below) at older stages of development. Yet, one has to admit that such a late embryonic shell is presumably more or less identical to the early larval shell and will therefore mainly provide post-embryonic information. Thus, in most cases the study of ontogeny in fossil organisms is restricted to the post-embryonic and in most cases post-larval ontogeny (ambiguous term, see Anger, 2006) or juvenile development.

How to Obtain Such Data

Data for developmental paleontological studies are commonly obtained using two approaches, which can be combined:

a) Development at a single stage can be investigated using isolated, preserved specimens.
b) Ontogenetic sequences can be reconstructed from numerous specimens representing different developmental stages.

Single specimens with ontogenetic information

The adult forms of certain groups of animals, such as shell-bearing organisms (molluscs), preserve information about their ontogeny. In these animals, information about earlier ontogenetic stages can be preserved in the most proximal area of the shell. Interestingly, these late embryonic or larval shells can possess a completely different morphology than the adult shell, for example, a twist in the other direction in snails (for a recent summary, see Nützel, 2014 and references therein).

The adult shell can also provide ontogenetic information in ammonites, an exclusively fossil mollusc group. To access this information, the shell is sectioned and changes of the shape of the sutures inside the shell or of the whorl expansion rate can be detected (e.g., Landman *et al.*, 1996; Ubukata *et al.*, 2008; De Baets *et al.*, 2012).

Arthropods usually shed their exoskeleton while growing. Due to this process of molting, information concerning earlier developmental stages is lost as growth continues. However, there are exceptions to this rule. For example, spinicaudatan crustaceans retain their post-larval shield valves instead of shedding them during each molt. Hence, they show growth lines that can be used to investigate developmental evolution (e.g., Wang, 1989; Olempska, 2004).

Adult legs can also provide developmental information in certain arthropods. Numerous arthropods grow by posterior addition of segments, which subsequently develop their equipment such as legs to achieve the final adult morphology. Therefore, anterior segments are further developed than posterior ones. As a consequence, a single older specimen gives insight into its earlier development. Such well preserved specimens are also present in the fossil record, for example, in crustaceans of the Cambrian Orsten (e.g., Walossek, 1993; Zhang *et al.*, 2007).

Ontogenetic information can also be deduced from single adult specimens in echinoderms. Each single plate in the adult has growth lines that can be used to generate developmental data. Additionally, the mode of plate addition can to a certain degree be deduced from the pattern of plate arrangement in the adult (Sumrall, 2008).

In vertebrates, structures such as bones and teeth can store developmental data from earlier stages, and these structures are commonly preserved in the fossil record. For example, the internal structure of the bones and teeth can give insight into the speed of growth (e.g., Starck and Chinsamy, 2002; Chinsamy-Turan, 2005; Woodward *et al.*, 2011). In addition, otoliths are often an important source for ontogenetic data in fish. Otoliths are small stones inside the fish balance organ, which are used to sense gravity. They grow together with the fish (though there may be a more direct correlation with the metabolic rate instead of growth itself; e.g., Woydack and Morales-Nin, 2001), assembling prominent growth lines which can be mined for developmental information.

Ontogenetic sequences from several specimens

Scientists incorporating paleo-evo-devo approaches commonly use several conspecific specimens of different ontogenetic stages to construct ontogenetic sequences for fossil taxa. In the best case, these developmental sequences are relatively complete and ontogenetic changes can be clearly detected. There are indeed some rather complete ontogenetic sequences preserved in the fossil record, for example, among crustaceans in Orsten-type preservation (e.g., Müller and Walossek, 1988; Walossek, 1993; Maas *et al.*, 2003; Haug *et al.*, 2010a) or in lithographic limestones (e.g., Polz, 1972, 1973, 1999; Bravi *et al.*, 2014), echinoderms (e.g., Sumrall *et al.*, 2006, 2013), amphibians (e.g., Schoch, 1992; Schoch and Fröbisch, 2006), or dinosaurs (e.g., Horner and Goodwin, 2006, 2009).

However, in many cases huge gaps in the developmental sequences make it difficult to determine if specimens represent different developmental stages of the same species or if they belong to different species. There are a number of cases in which representatives of previously separately described species are now assumed to be different ontogenetic stages of the same species (e.g., Horner and Goodwin, 2009; Scannella and Horner, 2010; Haug *et al.*, 2012). The most prominent example is the still ongoing discussion about the horned dinosaurs *Triceratops horridus* and *Torosaurus latus*. Some paleontologists provided evidence that the first species is a juvenile form of the latter (Scannella and Horner, 2010), while others see these presumed developmental characters as clear species differences (Longrich and Field, 2012). However, these uncertainties need to be solved before an evolutionary scenario can be reconstructed.

Evolutionary Scenarios

We have explained how fossil developmental data can be collected, but have not yet addressed how to reconstruct an evolutionary scenario using paleo-evo-devo approaches. To reconstruct how the transformation from one developmental pattern to another took place, ontogenetic data from both

extant and fossil representatives need to be included. This is not a trivial undertaking, and most analyses to date have included ontogenetic data from either extant or fossil taxa, but not both.

The basis for the reconstruction of an evolutionary scenario is a relatively well-resolved phylogram ('diagram of relationships'; see discussion in Haug *et al.*, 2012). Developmental information can either be directly included into the phylogenetic analysis that constructs the phylogram, or the developmental patterns for each monophyletic group (species or larger unit) can be mapped onto an already existing phylogram. The latter may result in a reevaluation of the phylogeny, which means that the mapping has to be repeated based on the new phylogeny hypothesis.

By plotting developmental data onto the phylogram, researchers can identify developmental patterns that are ancestral and derived, and thereby hypothesize the direction of evolution. The inclusion of fossil developmental data is especially crucial to this process, as these may provide ancestral conditions no longer present in the modern fauna (although fossils also often possess modern character conditions). Here we discuss an example of this in achelate lobsters (slipper and spiny lobsters). In modern representatives of this group, animals hatch as flat, swimming, so-called phyllosoma larva with very tiny tails. At the end of the phyllosoma phase, the larva changes drastically within a very short time (metamorphosis) into an almost adult-appearing, ground-living lobster with a very pronounced tail. As this developmental pattern characterizes both slipper lobsters and spiny lobsters, the general assumption was that this pattern of development was present in the last common ancestor of these two groups. However, recent investigations on fossil ontogenies of achelate lobsters made clear that the condition in modern representatives must have evolved convergently as different fossil in-group representatives show a much less metamorphic pattern (Haug and Haug, 2013; Haug *et al.*, 2013). This is a striking example of the type of errors that can occur when fossil developmental data are not incorporated into phylogenetic and evolutionary analyses.

Interactions of Paleo-Evo-Devo and 'Neo'-Evo-Devo

One central question remains: How can we effectively integrate paleo-evo-devo and modern evo-devo approaches into one combined, more holistic approach? A few such enterprises have been successfully undertaken already (e.g., Ortega-Hernández and Brena, 2012; Garwood *et al.*, 2014). Yet, additional steps will be necessary before an even closer integration of the two fields will become possible.

As we pointed out above, paleo-evo-devo is often restricted to studies of post-embryonic development. In contrast, modern evo-devo is almost entirely restricted to studies of embryonic development. This is especially apparent in arthropods, where all common model organisms have a highly derived ontogenetic pattern, lacking dispersal larval stages. Hence, future studies on extant organisms need to be extended more into post-embryonic development to provide data for comparisons between fossil and extant organisms. Using this approach, the external and internal morphology, for example, of the muscles (e.g., Briggs *et al.*, 2005; Haug *et al.*, 2014), nervous system (e.g., Ma *et al.*, 2012; Tanaka *et al.*, 2013), or circulatory system (Ma *et al.*, 2014) of these organisms should be investigated. We expect that the resulting studies will provide exciting new insights into the evolution of developmental patterns.

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See also: Cambrian Explosion: A Molecular Paleobiological Overview. Insects and Ecdysozoa, Diversification of. Land Vertebrates, the Origin and Evolution of

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Developmental Plasticity and Phenotypic Evolution

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Glossary

Baldwin effect The effect that occurs when a population encounters a novel environment and preexisting plasticity allows for the appearance of novel phenotypes. Selection then acts on these novel variants, influencing, over generational time, the direction of evolutionary change.

Cryptic genetic variation Genetic variation that is unexpressed due to genetic canalization, but which can be exposed to selection in the case of particular changes in genetic background or environmental conditions.

Developmental instability/developmental noise Stochastic perturbations of development independent of specific environmental or genetic variation, typically measured as a deviation from bilateral symmetry.

Developmental plasticity A form of phenotypic plasticity in which the differences in phenotype arise during ontogeny. This type of plasticity is often, though not always, irreversible.

Environmental canalization The developmental process by which a phenotypic trait is buffered against environmental variation, manifesting as a 'fixed' trait or flattened reaction norm.

Epigenetic A mitotically or meiotically heritable change in phenotype or gene function that does not involve a change in DNA sequence. This contemporary usage is distinct from the term as coined by C. H. Waddington and also differs from contemporary uses that focus on functionally significant changes in chromatin structure regardless of heritability. See Bird (2007) for discussion.

Genetic accommodation Changes in allele frequency due to selection on the regulation, form, or side effects of a novel trait. The phenomenon includes both selection on environmentally induced traits (in this case subsuming the second half of the Baldwin effect) or traits induced by novel mutations.

Genetic assimilation A form of genetic accommodation in which selection results in environmental canalization or the loss of plasticity.

Genetic canalization The developmental process by which a phenotypic trait is buffered against genetic variation, manifesting as overlapping reaction norms for different genotypes.

GxE A genotype-by-environment interaction, or genetic variation that responds differently to some aspect of the environment, resulting in reaction norms with different slopes.

Phenotypic plasticity The ability of genetically identical organisms to adopt different phenotypes in response to different environmental conditions. Such responses may or may not be adaptive.

Polyphenism A form of developmental plasticity in which alternative phenotypes, known as 'morphs,' are discrete and typically exhibit a suite of phenotypic differences.

Reaction norm A graphic representation of the phenotypic response (i.e., the value of a particular trait) of a genotype to a range of values for a particular environmental variable.

What is Developmental Plasticity?

The term 'developmental plasticity' refers to the ability of genetically identical organisms to develop different phenotypes in response to different environmental conditions. In practice, developmental plasticity is inferred when one observes different phenotypes developing from genetically similar if not identical organisms, and the observed phenotypic variation is associated with some aspect of the environment rather than with existing genetic variation. Such phenotypic variation can manifest as differences in almost any aspect of the phenotype, including those of morphology, physiology, behavior, or life history, as long as the differences result from a developmental or ontogenetic response to a change in the environment. Such responses tend to be irreversible for an individual organism, but not always. Developmental plasticity is thus a type of 'phenotypic plasticity,' a broader term that refers to the environmentally induced production of alternative phenotypes at any point in the life cycle of an organism. The predator-cued defensive 'helmets' of some cladocerans or the courtship song sung by a male sparrow that possesses the requisite neuroanatomy – both develop or are learned during ontogeny and are thus examples of developmental plasticity (**Figures 1(a)** and **1(b)**; Marler, 1999; Nottebohm, 2005; Tollrian and Dodson, 1999). In contrast, the predator-cued apparent death of an American opossum or the background-inspired camouflage of an octopus, both of which can be reversed and invoked at any time (Gabrielsen and Smith, 1985; Hanlon, 2007), are examples of nondevelopmental phenotypic plasticity (**Figures 1(e)** and **1(f)**); sometimes referred to as 'labile plasticity' (Gomulkiewicz and Kirkpatrick, 1992) or 'activational plasticity' (Snell-Rood, 2013). Although useful conceptually, in practice the developmental versus nondevelopmental distinction can be problematic, as it is often difficult to decide when 'development' ends and 'physiology' begins (Fusco and Minelli, 2010).

Under the right historical circumstances, developmental plasticity can afford populations and individuals the ability to adapt to changing environmental circumstances on short, nonevolutionary timescales. Such 'adaptive developmental plasticity' evolves when (1) a population possesses heritable variation in a developmental response to an environmental

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cue and (2) some responses in the population confer greater reproductive success than other responses. Implicit here is the notion that not all developmental plasticity is adaptive. Indeed, developmental plasticity can be selectively neutral or even maladaptive, especially when it first appears in a population as a result of *de novo* mutations or exposure to a novel environment. Furthermore, developmental plasticity, whether adaptive, neutral, or maladaptive, is often considered to be distinct from 'developmental instability' or 'developmental noise,' which results from stochastic perturbations of development (Waddington, 1957, p. 40; also see Bradshaw, 1965). This latter type of phenotypic variation, observed for a single genotype in a single environment, can be induced by environmental stressors and is typically measured as a deviation from bilateral symmetry, as both sides of an individual develop with the same genotype and in the same macroenvironment (Klingenberg, 2003; Nijhout and Davidowitz, 2003; Palmer, 1994). Unlike developmental plasticity, however, environmentally induced developmental instability results when the environment's influence on development is directionless and effectively random with regard to effects on the resulting phenotype.

A helpful way to visualize developmental plasticity is the 'reaction norm.' First drawn by German zoologist Richard Woltereck (1909), a reaction norm or 'phenotypic curve' was later interpreted as the response of a genotype to a range of values for a particular environmental variable, such as temperature or nutrition, producing a range of values for a particular phenotypic trait (**Figure 2**). In other words, reaction norms illustrate how the environment modulates the phenotypic expression of the genotype (see Sarkar, 2004 for a history of reaction norms). In the case where the environment has little influence on the phenotype – that is, there is limited plasticity – the reaction norm is relatively flat (**Figure 2(a)**). The proximate cause of this flattening is developmental buffering against environmental variation, a process referred to as 'canalization' by Waddington (1942) or 'autoregulation' by Schmalhausen (1949), which itself is the result of a history of stabilizing selection (but see de Visser *et al.*, 2003; Flatt, 2005). For Waddington, however, it is worth noting that canalization included the stable production of the phenotype in the face of not only environmental, but also genetic, variation (i.e., mutations). Thus, strictly speaking, a single flat reaction norm, as the function of a single genotype, reflects only 'environmental canalization' as opposed to 'genetic canalization' (*sensu* Stearns *et al.*, 1995). In contrast, any reaction norm with a significant slope indicates plasticity, even if the reaction norm exhibits curvature and the slope changes as the environment changes. Plasticity can thus take many forms depending on the magnitude and shape of the reaction norm, including responses that are continuous and discrete (**Figure 2**, compare (b) and (c)). When a discrete response is adaptive, developmental, and irreversible, it is often referred to as a 'polyphenism,' with the alternative phenotypes, referred to as 'morphs,' typically exhibiting a correlated suite of phenotypic traits (Mayr, 1963; Michener, 1961; Moran, 1992; see **Figures 1(a)** and **1(d)**).

For a single genotype to produce a reliable, coherent reaction norm, regardless of whether it is environmentally canalized or plastic, requires that the phenotypic trait in question exhibit sufficient developmental stability (i.e., minimal variation at a particular value along the environmental axis). Note that this requirement for developmental stability is not the same as a requirement for genetic canalization; without genetic canalization, reaction norms exhibit greater inter-norm variation among genotypes, whereas reaction norms subject to genetic canalization exhibit limited inter-norm variation with greater overlap (**Figure 2**, compare (d) and (e); Stearns and Kawecki, 1994).

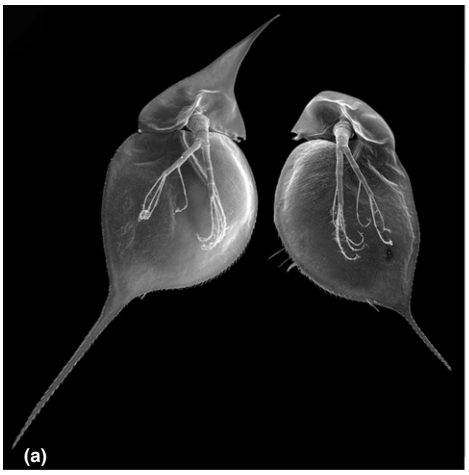
Although developmental plasticity had been discussed in adaptative terms as early as 1914 (Nilsson-Ehle, 1914), Synthesis biologists of the 1930s and 1940s – with some exceptions, notably Waddington (1942) and Schmalhausen (1949) – tended to treat the role of plasticity in evolution only cursorily, as they preferentially focused on phenotypic variation that was due to additive genetic variation and therefore subject to natural selection. Similarly, early geneticists interested in development focused on the role of genes in this process and thus tended to disregard environmentally influenced variation as noise (for a review of the history of views on plasticity, see Sarkar, 2004; Schlichting and Pigliucci, 1998, pp. 29–50; West-Eberhard, 2003, pp. 1–20). Both of these viewpoints changed slowly but steadily in the second half of the twentieth century, with the result that we are now witnessing a surge of interest in multiple aspects of developmental plasticity, including its evolution, its consequences for evolution more generally, the proximate mechanisms that generate it and how these can be altered by natural selection.

The Evolution of Plasticity

Dynamic responsiveness is a hallmark of life itself; indeed, adaptive conditional responses of behavior, physiology, and even morphology are common. This observation, along with the results of early experiments in which plasticity was modified by artificial selection, led Bradshaw (1965) to argue that plasticity is under genetic control and as such is subject to natural selection. Bradshaw went on to discuss the types of conditions under which plasticity would evolve, identifying scenarios in which plants would experience persistent variation in their environments. This work inspired the development of theoretical models for the evolution of plasticity, most dealing with life history traits, beginning in the 1970s and proliferating in the 1980s. Subsequent empirical explorations attempted to test the resulting predictions as to how and under what conditions adaptive phenotypic plasticity evolves.

How Does Plasticity Evolve?

Although few doubted that adaptive plasticity was the product of evolution by natural selection, from 1985 to 1995 there was considerable debate regarding whether phenotypic plasticity is the direct target of selection, or if, instead, plasticity is simply an emergent byproduct of selection for distinct, alternative traits in different environments (Gavrilets and Scheiner, 1993; Scheiner, 1993a,b; Via, 1993; Via *et al.*, 1995). The answer to this question has implications both for predicting evolutionary dynamics as well as for understanding the genetic underpinnings of plasticity. In particular, if plasticity

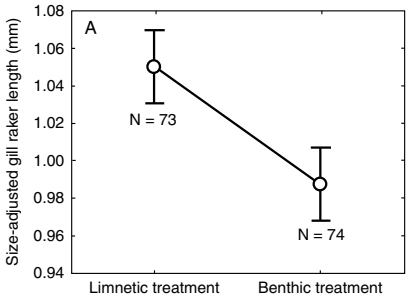


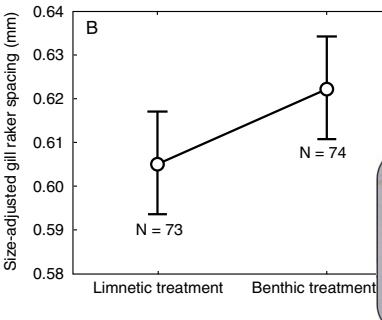
Normal songs

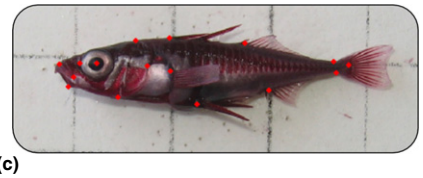
Isolate songs

Swamp sparrow
melospiza georgiana

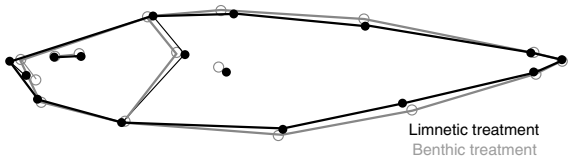
(b)







(c)



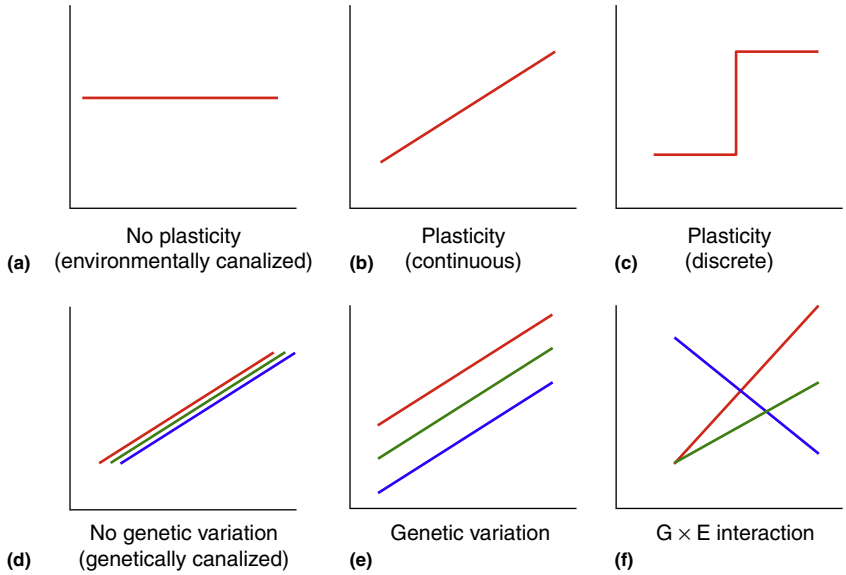


Figure 2 Some examples of reaction norms. In all cases, the *y*-axis represents a phenotype or trait value while the *x*-axis represents the value of some environmental variable. (a–c) Individual single-genotype reaction norms showing no plasticity (*monophenic*) (a), continuous plasticity (b), and discrete plasticity (*polyphenic* response) (c). Note that the term 'reaction norm' is often reserved for a continuous response (e.g., Stearns, 1989) and in this sense would not apply to (c). (d–f) Multiple single-genotype plastic reaction norms showing no genetic variation (reaction norms overlapping) (d), genetic variation, but no variation in the shape or magnitude of the response (e), significant genotype-by-environment interaction, representing heritable variation in the response (g). Although idealized single-genotype reaction norms are shown here, in practice reaction norms are estimated by plotting trait means for closely related families.

is indeed the direct target of selection, then there must exist specific genes that regulate the magnitude or shape of the plastic response that are distinct from the genes that control the development of the trait in alternative environments (Via *et al.*, 1995).

Another way to make the point would be to say that, in order for plasticity to be a target of selection, there must first be heritable variation in the plastic response itself, independent of the heritable variation in mean response across environments. In classical quantitative genetics, the proximate sources of phenotypic variation are partitioned as $V_P = V_G + V_E + V_{GxE}$ where the population's phenotypic variance (V_P) results from contributing genetic variance (V_G), environmental variance (V_E), and variance due to genotype-by-environment interaction (V_{GxE}). For plasticity to be subject to selection, it is not enough that $V_G > 0$ (**Figure 2(e)**), since this does not represent variation in the plastic response (i.e., the slopes of the reaction norms are the same). Instead, for plasticity to be subject to selection requires a significant genotype-by-environment interaction ($V_{GxE} > 0$) (Scheiner, 1993a; Scheiner and Lyman, 1989; Via

Figure 1 Some examples of phenotypic plasticity. (a) Two individuals of a single clone of the Asian and African water flea, *Daphnia lumholtzi*, exposed (left) and not exposed (right) to chemical cues from predaceous fish. The sharp helmet and extended tail spine of the induced morph protect the water flea from fish predators. (b) Sonograms sung by male swamp sparrows after exposure to the stereotyped song as juveniles (normal) and after no exposure (isolate). (c) For a given body size, stickleback fish raised in a limnetic environment have longer and more closely spaced gill rakers than those raised in the benthic environment (gill raker shown in inset). The average body shapes of limnetic-raised fish and benthic-raised fish also differ significantly, as depicted by wireframe graphs (deformations are exaggerated 4x to illustrate differences). (d) Genetically identical wingless and winged female aphids that developed under uncrowded or crowded conditions, respectively. (e and f) Two examples of nondevelopmental and reversible phenotypic plasticity: an opossum playing dead, an involuntary physiological response to a potential predator (e) and *Octopus vulgaris* sequentially showing a deimatic threat display and camouflage (left and right, respectively), dynamic changes that can take place in less than 1 s (f). Other well-known examples of developmental plasticity include seasonal polyphenism in butterflies (Brakefield and Frankino, 2009), horn plasticity in beetles (Emlen *et al.*, 2007; Moczek *et al.*, 2014), caste polyphenism in social insects (Miura, 2005), phase polyphenism in locusts (Simpson and Sword, 2009), cannibalistic larval polyphenism in both tiger salamanders (Collins and Cheek, 1983) and spadefoot toads (Pfennig, 1990), and environmental sex determination in reptiles (Janzen and Phillips, 2006). *Daphnia lumholtzi* images reproduced from Agrawal, A.A., 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294, 321–326. Courtesy of Christian Laforsch and Ralph Tollrian; Swamp sparrow sonogram data adapted with permission from Marler, P., 1999. Nature, nurture and the instinct to learn. In: Adams, N.J., Slotow, R.H. (Eds.), Proceedings of the 22nd International Ornithological Congress Durban, South Africa, pp. 2379–2393. Johannesburg: BirdLife South Africa. Available at: http://www.int-ornith-union.org/files/proceedings/durban/Symposium/S40/S40.3.htm (accessed 31.07.15); painting of swamp sparrow © David Sibley, reproduced with permission. Stickleback data reproduced with permission from Wund, M.A., Valena, S., Wood, S., Baker, J.A., 2012. Ancestral plasticity and allometry in threespine stickleback fish reveal phenotypes associated with derived, freshwater ecotypes. Biological Journal of the Linnean Society 105, 573–583. Aphid images reproduced from Brisson, J., 2010. Aphid wing dimorphisms: Linking environmental and genetic control of trait variation. Philosophical Transactions of the Royal Society B 365, 605–616 and provided courtesy of Jennifer Brisson. Opossum photo by Tony Alter (license) and *O. vulgaris* images courtesy of Roger Hanlon.

and Lande, 1985), which has the effect of producing reaction norms that are not parallel and may even have opposing responses to the environment (**Figure 2(f)**). Although required for selection to shape plasticity, a $V_{GxE} > 0$ on its own does not suggest that plasticity itself is a direct target of selection.

Quantitative genetics experiments have demonstrated widespread heritable variation in plastic responses (i.e., significant 'GxE'; e.g., Danielson-François *et al.*, 2006; Gutteling *et al.*, 2007; and papers reviewed by Windig *et al.*, 2004). Furthermore, artificial selection experiments clearly demonstrate that direct selection on the magnitude of plasticity can lead to evolutionary responses, independently of changes in trait means (e.g., Czesak *et al.*, 2006; Garland and Kelly, 2006; Scheiner, 2002). As such, it is now generally accepted that phenotypic plasticity can indeed be a direct target of natural selection, and that the degree of flexibility of a trait is not necessarily genetically correlated to its environment-specific development, although selection on trait values in one environment may lead to changes in the slopes of reaction norms across environments (Scheiner, 2002; Suzuki and Nijhout, 2006). Numerous examples of reaction norm evolution in natural populations have also emerged, complementing work done on model systems in the laboratory (e.g., Baythavong and Stanton, 2010; Lee *et al.*, 2011; Scoville and Pfrender, 2010). Furthermore, the emergence of evolutionary developmental biology ('evo-devo') and advances in molecular biology have led to plausible models for the nature of so-called 'plasticity genes' and how they might work (Pigliucci, 2005). Applying this integrative biological approach, we now understand plasticity as resulting from any one of several mechanisms, including differential gene expression along an environmental gradient (Aubin-Horth and Renn, 2009), changes in enzyme activity under varying conditions, or the destabilization of posttranslational buffering mechanisms such as the activity of heat shock proteins and other chaperones (Rohner *et al.*, 2013; Rutherford and Lindquist, 1998; Queitsch *et al.*, 2002). Current work aims to further our understanding of how proximate mechanisms first detect environmental cues and then subsequently translate them into adaptive plastic responses, as well as how such complex developmental genetic systems evolved in the first place.

When Does Plasticity Evolve?

Environmental heterogeneity is common, and plasticity seems like an excellent strategy to handle it. So why do organisms sometimes confront a changing environment with a fixed (i.e., environmentally canalized) phenotype? In other words, under what circumstances do we expect a plastic versus fixed trait to evolve? There are at least two important answers to this question. The first is that the benefits of a plastic response might be outweighed by its cost, a price ultimately paid in reproductive success (DeWitt *et al.*, 1998; Relyea, 2002; Callahan *et al.*, 2008). The second is that plasticity is only favored by selection when environments vary predictably and frequently enough to make the ability to produce alternative phenotypes advantageous (reviewed in Schlichting and Pigliucci, 1998).

While it is conceptually intuitive that a plastic response might incur more cost than a fixed, jack-of-all-trades phenotype, efforts to empirically demonstrate the costs of plasticity have met with mixed results (DeWitt *et al.*, 1998; Murren *et al.*, 2015). Such equivocal evidence for the theoretical prediction of plasticity costs may be due, in part, to a tendency among investigators to conflate what is meant by the 'costs,' as opposed to the 'limits,' of plasticity. 'Costs' typically refer to the energetic and functional demands of producing alternative traits, including the physiological and developmental machinery required to detect an environmental cue and mount an appropriate response, which detrimentally affect reproductive success across the entire environmental range. 'Limits,' on the other hand, refer to constraints that prevent a plastic response from generating an optimal trait value for a given environment, such as developmental lag times, unreliable environmental cues, and reduced phenotypic integration that might make it difficult to simultaneously alter more than one or a few aspects of the phenotype (Auld *et al.*, 2010; DeWitt *et al.*, 1998). Such limits also come with costs in terms of reproductive success, but these costs are distinct from the 'costs of plasticity' described above and instead are costs that result from producing a suboptimal trait in a given environment, referred to as the 'costs of phenotype' (*sensu* Callahan *et al.*, 2008; Murren *et al.*, 2015). After reviewing the literature through this lens, Murren *et al.* (2015) conclude that, in most cases, it is in fact the limits of plasticity and their resulting costs of phenotype, as opposed to the costs of plasticity itself, that act as the predominant constraints on plasticity evolution.

One important class of limits of plasticity concerns environmental cues. In the case of adaptive plasticity, the environmental cue that triggers a plastic response may not be the same as the environmental challenge for which the response is an adaptation. This can be advantageous, as in cases where there is a developmental time lag associated with mounting an adaptive response. Here, a time delay between the environmental cue (e.g., a shortened photoperiod) and the environmental challenge (e.g., the cold temperatures of winter) can provide a sufficiently early warning for the adaptive plastic response to manifest on time (Nijhout, 2003). On the other hand, the distinction between environmental cue versus challenge also means that a plastic response can only be consistently adaptive if the cue that triggers the response reliably predicts the appearance of particular environmental conditions (Schlichting and Smith, 2002; David *et al.*, 2004). Thus, in the case of a novel plastic response (either mutationally or environmentally induced), plastic genetic variation will be consistently adaptive and hence favored by selection only if the environmental cue is reliably associated with the appropriate environmental challenge. Also predictive of whether or not selection will favor plasticity over a fixed response is the timescale at which the environment varies relative to generation time, a metric known as 'environmental grain' (Levins, 1968). In fine-grained environments in which the environment varies on timescales shorter than a typical lifespan, a fixed strategy might be favored if the lag time required for trait production is too long to be useful, particularly if environmental cues are also unreliable. In especially coarse-grained environments in which multiple generations pass between changes in the environment, we might also expect fixed

phenotypes because environmental change may be too rare for selection to favor plasticity (reviewed in Schlichting and Smith, 2002). Of course, predicting whether and how plasticity will evolve depends upon the nature of the trait in question. Activational responses (*sensu* Snell-Rood, 2013), such as rapid and reversible changes in behavior or physiology, might be expected to evolve in fine-grained environments, because they can immediately respond to new conditions. In contrast, developmentally plastic responses, whether irreversible or slowly reversible, may only evolve in somewhat less fine- or even course-grained environments due to a typically longer time lag between the detection of an environmental cue and the production of the altered phenotype.

Evolutionary Consequences of Plasticity

In addition to questions concerning the proximate mechanisms and evolutionary origins of developmental plasticity, we can also ask how preexisting developmental plasticity (and phenotypic plasticity more generally) might affect the course of evolution. Research on this question has suggested at least three major ways that plasticity might impact the evolutionary process (reviewed in Wund, 2012). In general, for populations that possess it, plasticity will potentially (1) improve viability in novel environments, (2) alter the rate at which a population responds to selective pressures, and (3) constrain the form of those responses (Ghalambor *et al.*, 2007; Price *et al.*, 2003; Schlichting, 2004; West-Eberhard, 2003).

While potentially counterintuitive, the idea that environmentally induced phenotypic variation might impact the evolution of inherited traits can be traced back to at least the late nineteenth century, when Morgan (1896), Osborn (1896), and Baldwin (1896, 1902) independently conceived of what has come to be known as the 'Baldwin effect' (coined by Simpson, 1953; see Crispo, 2007). When a population encounters a novel environment, either through migration or *in situ* change, plasticity may allow for the appearance of novel phenotypes. If at least some of these novel phenotypes are sufficiently adaptive, the population may remain viable until mutation and recombination can supply natural selection with beneficial genetic variants. It is possible, for example, to imagine that when facing a new environment, preexisting plasticity could place at least some members of a population on the slope of a new fitness peak. Assuming the appropriate genetic variation is or becomes available, natural selection could subsequently push the population toward the fitness maximum, either by changing mean trait values (e.g., the elevation, but not the slope, of the reaction norm) or the regulation of the plastic response (e.g., the slope of the reaction norm) (**Figures 1(a)** and **1(b)** in Crispo, 2007; Ghalambor *et al.*, 2007; Price *et al.*, 2003). It is in this basic sense that plasticity can both 'buy time' for adaptive evolution to take place while also affecting the rate of response to natural selection by providing an immediate (within generation) shift in phenotypic variation.

By revealing specific patterns of variation to selection or even influencing how individuals interact with their environment – as is the case, for example, with behavioral plasticity – the form of the plasticity (i.e., the magnitude and shape of the reaction norm) also constrains the types of adaptations that can evolve. Mary Jane West-Eberhard (2003) coined the term 'genetic accommodation' to describe evolutionary changes in the form, regulation, and integration of traits that adaptively 'accommodate' novel developmental variants, whether they result from novel mutations or a plastic response to a novel environment. The second step of the Baldwin effect, wherein environmentally induced adaptive variants are improved by natural selection, is an example of the latter and thus a type of genetic accommodation (Crispo, 2007; West-Eberhard, 2003). In terms of lasting effects on the course of evolution, the important point here is that the resulting adaptation initially arose as a plastic variant.

Another form of genetic accommodation that also depends on plasticity but instead results in the loss of plasticity was described and referred to as 'genetic assimilation' by Waddington (1957, 1961) and 'stabilizing selection' by Schmalhausen (1949). Like the Baldwin effect, genetic assimilation begins with an exposure to a novel environment that induces an adaptive response in virtue of preexisting but unexpressed plasticity, followed by selection. In the case of genetic assimilation, however, selection for this alternative phenotype necessarily results in a change in the regulation of the plasticity and this change is specifically in the direction of canalization, so that the alternative trait is expressed even if the environmental cue is no longer present (i.e., the reaction norm is flattened). In other words, plasticity has been lost (Pigliucci *et al.*, 2006).

Sometimes the phenotypic variation released by a novel environment is bolstered due to a history of stabilizing (or canalizing) selection. Indeed, canalized development – specifically, genetically canalized development – can lead to the buildup of unexpressed genetic variation because it is invisible to selection. A change in the environment might then release previously 'cryptic genetic variation,' exposing it as selectable phenotypic variation (Gibson and Dworkin, 2004; Le Rouzic and Carlborg, 2008; Paaby and Rockman, 2014; Palmer, 2012; Schlichting, 2008). This 'de-canalization' can occur when novel environmental conditions overwhelm or otherwise disrupt canalizing mechanisms during development – for example, through altered gene expression or changes in the activity of chaperone proteins (Hayden *et al.*, 2011; Iwasaki *et al.*, 2013; Queitsch *et al.*, 2002; Rohner *et al.*, 2013; Rutherford and Lindquist, 1998; Sangster *et al.*, 2008). This storage and subsequent release of cryptic genetic variation could potentially lead to rapid responses to selection where previously little additive genetic variation was observed (Le Rouzic and Carlborg, 2008; Paaby and Rockman, 2014). On the other hand, plasticity can also retard evolutionary change; if multiple genotypes can produce equally and highly adaptive phenotypes, then selection will be unable to distinguish among them and little or no evolutionary change will occur (Ghalambor *et al.*, 2007; Price *et al.*, 2003).

In addition to the microevolutionary effects already described, plasticity may also have macroevolutionary consequences (Pfennig *et al.*, 2010; Schlichting, 2004; West-Eberhard, 2003). By allowing populations to persist in different forms in alternative environments, plasticity may promote reproductive isolation and hence speciation. Plasticity may even foster adaptive radiation due the combined effects of population

persistence, genetic accommodation, and its role in speciation (West-Eberhard, 2005; Pfennig *et al.*, 2010). Whether we consider the micro- or macroevolutionary effects of plasticity, common to all of these processes is the fact that the environment has two interrelated roles: on the one hand it affects the expression of phenotypic variation, on the other it acts as the selective agent upon that variation. This interplay can lead to complex evolutionary dynamics (Wund, 2012).

Although hypotheses concerning the role played by plasticity in evolution remain controversial, a number of important books and reviews (e.g., Jablonka and Lamb, 2005; Pigliucci, 2001; Price *et al.*, 2003; Schlichting and Pigliucci, 1998; West-Eberhard, 2003) have spawned a renewed interest and motivation to evaluate them. A number of models demonstrate the capacity for plasticity to promote responses to selection (Behera and Nanjundiah, 2004; Chevin and Lande, 2011; Lande, 2009; Thibert-Plante and Hendry, 2011) and empirical evidence for this claim has been mounting quickly (reviewed in Schlichting and Wund, 2014). A role for genetic accommodation, for example, has been demonstrated in a number of instances: adaptation to novel predators (Scoville and Pfrender, 2010), the repeated colonization of freshwater by marine copepods (Lee *et al.*, 2011), and the evolution of eye reduction in cave fish (Rohner *et al.*, 2013). A recent study has even implicated genetic accommodation in the initial colonization of land by the ancestors of tetrapod vertebrates by examining the effects of terrestrialization on an extant analogue of stem tetrapods (Standen *et al.*, 2014). The release of cryptic genetic variation has also been demonstrated in several natural systems (Ledón-Rettig *et al.*, 2010; McGuigan *et al.*, 2011; Purchase and Moreau, 2012; Rohner *et al.*, 2013). With respect to impacts on macroevolutionary processes, plasticity has been implicated in originating novel traits (Ledón-Rettig *et al.*, 2008; Moczek *et al.*, 2011), promoting adaptive radiation (Pfennig and McGee, 2010; Wund *et al.*, 2008; Wund *et al.*, 2012) and facilitating speciation (Savolainen *et al.*, 2006).

Mechanisms of Plasticity

Our understanding of how adaptive developmental plasticity both evolves and affects the process of evolution will undoubtedly be informed by a deeper understanding of its genetic, epigenetic, and developmental basis. Although recent technological advances promise continued progress in this area, important insights can still be gained by applying classical techniques such as hormone manipulation and artificial selection (though see Zera, 2007). At least two species of the ant genus *Pheidole*, for example, possess a 'supersoldier' subcaste, which is induced by nutrition mediated by juvenile hormone. Application of a juvenile hormone analog, however, can induce supersoldiers in *Pheidole* species that do not normally produce supersoldiers, suggesting that an ancestral developmental potential for this plastic response was retained and this latent plasticity in turn facilitated parallel evolution of the subcaste (Rajakumar *et al.*, 2012).

Developmental investigations that follow artificial selection can also shed light on the mechanisms that underlie phenomena such the Baldwin effect and genetic assimilation. Suzuki and Nijhout (2006), for example, performed selection in two different lines, each starting with the same mutant stock of tobacco hornworms, which are normally black but become partially green as 5th instar larvae when heat shocked as 4th instars. In the green line, they selected for a greener color when heat shocked, while in the black line they selected for retention of the black color when heat shocked. In a control lineage they did not select after heat shock. After 13 generations, heat shocked larvae of the green line were completely green and heat shocked larvae of the black line were completely black. More interesting, however, were the resulting reaction norms. Rather than assimilating, the green line showed a stronger plastic response to increasing temperature in the form of a steeper, more polyphenic reaction norm relative to the unselected line. This constitutes evidence for the Baldwin effect and genetic accommodation in that phenotypes closer to the optimum were initially produced by a preexisting plastic response to a new environment (heat shock) and those variants (greener larvae) were then selected, with the result that even greener larvae were produced by modifying the plastic response through changes in the frequency of modifying alleles (Braendle and Flatt, 2006; Crispo, 2007; Suzuki and Nijhout, 2006). Here it is important to note that Suzuki and Nijhout did not select for increased plasticity per se; instead, increased plasticity resulted from selection in a single environment (i.e., 4th instar heat shock). In contrast, the black line showed a reaction norm that was both lower in elevation and environmentally canalized (i.e., flat), consistent with genetic assimilation if one takes the 'novel' environment to be the absence of 4th instar heat shock, assimilation having occurred after larvae remain completely black even in the presence of heat shock, where previously being black required the absence of heat shock (Crispo, 2007; Suzuki and Nijhout, 2006).

From a developmental perspective, the most interesting results came from follow-up experiments aimed at elucidating the mechanism of the genetic accommodation and assimilation. In the case of the green line, the more discrete response resulted from higher titers of juvenile hormone in response to heat, either through increased production or reduced degradation. In the case of the black line, the loss of plasticity appears to be due to reduced sensitivity to juvenile hormone under heat shock (Suzuki and Nijhout, 2006). It is important to emphasize that this loss of sensitivity occurs in 'response' to heat shock. Underlying this evolutionary 'loss' of outward phenotypic plasticity is thus an 'increase' in plasticity – in this case changes in hormone sensitivity in response to temperature – a theoretical possibility raised previously in a cautionary note about assuming too much about the nature of canalization (Frankino and Raff, 2004).

More recent advances in our ability to describe transcriptomes and the establishment of model systems for studying plasticity have fueled rapid progress toward understanding the details of alternate developmental trajectories and the nature of environmentally controlled developmental switches (*sensu* Stearns, 1989). In a number of polyphenisms, for example, transcriptomic approaches have identified differences in gene expression between morphs (e.g., Gallot *et al.*, 2012; Grozinger *et al.*, 2007; Wang *et al.*, 2014; Warren *et al.*, 2014), in some cases providing candidate genes that respond specifically to inducing cues (Colbourne *et al.*, 2011; Ishikawa

et al., 2012; Kijimoto *et al.*, 2014; Le Trionnaire *et al.*, 2012) or allowing comparisons with analogous genetic polymorphisms (Brisson *et al.*, 2007). Emerging model systems, particularly among insects (Simpson *et al.*, 2011; Whitman and Ananthakrishnan, 2009), have additionally allowed us to go beyond correlation and test the potential roles played by particular genes, pathways or hormones in generating plasticity. Examples include phenomena as diverse as the specification of caste in honeybees (Kucharski *et al.*, 2008; Li-Byarlay *et al.*, 2013; Patel *et al.*, 2007), horn polyphenism in beetles (Emlen *et al.*, 2012; Kijimoto *et al.*, 2012; Snell-Rood and Moczek, 2012), the wing polyphenism of planthoppers (Xu *et al.*, 2015), the wing and reproductive polyphenisms of the pea aphid (Brisson, 2010; Le Trionnaire *et al.*, 2008), and the phase polyphenism of locusts (Ernst *et al.*, 2015), to name just a few.

Increasingly, 'epigenetic' modifications of chromatin appear to play a key role in the mechanism of plasticity through effects on gene function. Cytosine methylation at CpG dinucleotides in particular is common yet variable among social bees, wasps and ants (Kronforst *et al.*, 2008), and the honeybee, pea aphid, and two locusts have been shown to possess functional DNA methylation systems (Walsh *et al.*, 2010; Wang *et al.*, 2006, 2014). The latter four species also possess an intriguing bimodal distribution of genes showing high versus low levels of CpG methylation (either inferred by the ratio of observed to expected CpG sites or examined directly) in contrast to the unimodal distribution found in other insects that do not exhibit polyphenism (Elango *et al.*, 2009; Falckenhayn *et al.*, 2013; Lyko *et al.*, 2010; Walsh *et al.*, 2010; Wang *et al.*, 2014). Some studies suggest that genes exhibiting caste- or morph-biased expression are hypomethylated, which may make sense if these CpG sites need to be available for *de novo* methylation, which in turn allows for the developmental specification or differentiation of castes or morphs (Elango *et al.*, 2009; Hunt *et al.*, 2010; Srinivasan and Brisson, 2012; though see Lyko *et al.*, 2010). This idea is consistent with the fact that interfering with *de novo* DNA methylation in the honeybee causes would-be workers instead to adopt a queen-like fate (Kucharski *et al.*, 2008).

Direct examination of the methylation status of cytosines by methylation-sensitive restriction enzymes or bisulfite sequencing additionally allows one to detect genes that are methylated differently in tissue from different morphs, as adults or during development (Bonasio *et al.*, 2012; Foret *et al.*, 2012; Lyko *et al.*, 2010; Wang *et al.*, 2014; Weiner *et al.*, 2013). In two species of ants with different degrees of caste polyphenism, the extent of differential methylation correlates with both the degree of caste differentiation in the two species, as well as the onset of caste differentiation during development (Bonasio *et al.*, 2012). Interestingly, this study, as well as another study of the brains of honeybee workers and queens, detected a correlation between CpG methylation and RNA splicing sites, raising the possibility that methylation could foster plasticity by regulating alternative splicing (Lyko *et al.*, 2010; Bonasio *et al.*, 2012). In support of this, interfering with *de novo* DNA methylation has been shown to alter patterns of exon skipping and intron retention in the honeybee (Li-Byarlay *et al.*, 2013) and phase-biased alternative splicing of 45 transcripts has been demonstrated in the migratory locust (Wang *et al.*, 2014). This work will undoubtedly continue to improve our understanding of how morph-biased epigenetic modifications, of both DNA and histones (e.g., Simola *et al.*, 2013), mediate the ability of the environment to shape the ontogeny of alternate phenotypes.

Future Directions and Implications

The role of developmental plasticity in phenotypic evolution continues to be an active area of investigation. Ongoing problems include establishing the relative importance of the various processes described above, asking in particular how common and important the release of cryptic genetic variation is in natural populations and whether adaptive evolution by genetic accommodation is the exception or the rule (Pfennig *et al.*, 2010; Schlichting and Wund, 2014). Answers to these questions in turn have important implications for human health and well-being as we increasingly expose ourselves to conditions well outside the range of our evolutionary history, opening the doors for a mismatch between historically adaptive environmentally cued phenotypes and actual environmental conditions (Bateson *et al.*, 2004; Gluckman *et al.*, 2011a, 2011b). Outdated 'nature versus nurture' debates are also being replaced by more nuanced understandings of the complex interactions among genetics, development, and the environment, and a recognition of how the environmental conditions experienced by one generation may affect disease susceptibility in the next (Gluckman *et al.*, 2007; Whitelaw and Whitelaw, 2008). As we learn more about these processes, as well as nongenetic systems of inheritance such as epigenetic inheritance and cultural transmission (Day and Bonduriansky, 2011; Jablonka and Lamb, 2005; Jablonka and Raz, 2009), some have argued that the Modern Evolutionary Synthesis requires an extension, one that moves us away from a strictly mutation-selection-response view of evolutionary change, if we are ever to grasp fully the complexity of phenotypic evolution (Jablonka and Lamb, 2005; Laland *et al.*, 2014; Moczek, 2012; Pigliucci, 2007; Pigliucci and Müller, 2010).

See also: Developmental Biases on Morphological Evolvability. Epigenetic Inheritance. Evolutionary Medicine III. Mismatch. Evolvability, Quantitative Genetics of. Genetic Architecture. Genotype to Phenotype: Insights from Evo-Devo. Genotype-by-Environment Interaction. Sex Determination. Waddington's Epigenetic Landscape, History of

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Directed Evolution, History of

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Directed Evolution

Is evolution the consequence of environmental circumstances alone? Or do factors internal to the organism cause evolution to proceed in certain directions? For the past two centuries, biologists have sought answers to these related questions. Charles Darwin's theory of evolution by undirected variation and natural selection suggested little or no directionality to the evolutionary process. Given Darwin's popularity, many people might assume that theories of directed evolution are outdated, false theories of a bygone era. While some biologists over the past 150 years have argued that evolution is predominantly nondirectional, many evolutionists have articulated theories and research projects that describe evolution as directed of limited. These modern theories of directed evolution began in the work of Jean Baptiste Lamarck in the early nineteenth century, gained prominence as theories of 'orthogenesis' at the turn of the twentieth century, and continue today as a part of the field of evolutionary developmental biology. Throughout this long history, theorists postulated a wide range of innate tendencies, internal factors, evolutionary laws, and developmental mechanisms that could account for evolutionary directionality. And, while theories about the specific factors causing directed evolution have changed over time, broad trends and long-term outcomes of morphological evolution have dominated the explanatory goals and empirical studies of people explaining apparent trajectories in evolution.

The term 'directed evolution' arose in the late nineteenth century, though its core ideas existed earlier. Put simply, 'directed evolution' denotes evolution taking place along certain trajectories (that is, in particular directions) due to constraints on the production of new morphological features. Thus, theories of directed evolution describe the developing organism as limiting or constraining the outcomes of evolution in certain respects. During earlier periods of history, evolutionists focused on the directionality of or trajectories in evolution quite broadly. In the past few decades, however, they have begun to utilize developmental and genetic analysis to accurately explain evolutionary constraints.

Four points clarify the scientific meaning of the term 'directed evolution.' First, something or some condition in the natural world must cause evolutionary directionality. Put differently, theories of directed evolution do not explain directionality as an accidental, random occurrence. Second, evolution proceeding in a limited direction does not mean that it has an end-goal or ultimate purpose. Therefore, directed evolution is not necessarily teleological; directionality can stem merely from conditions or factors that constrain the course of evolution. Third, theories of theistic evolution – evolution guided by a supreme being – were predominantly directional in nature, yet the scientific community rejected them because theistic evolutionary ideas failed to generate testable hypotheses. Fourth, cultural notions of 'progress' have infused the language of directed evolution throughout history, especially in the nineteenth century. Even today, many people either state directly or imply that both human history and evolution are inherently progressive. Even still, the question of evolutionary directionality is a problem that has been, and continues to be, examined using rigorous scientific methods. Because of these various cultural influences on the idea of directed evolution, understanding the history of scientific theories of directed evolution requires investigating only theories based on scientific naturalism and rigorous empirical evidence.

Variation and Teleology

In *Philosophie Zoologique*, the French biologist Lamarck (1809) proposed an evolutionary explanation for all life on Earth, a central component of which was inherent directionality. While thinkers before him had argued that some species could change over time, Lamarck provided a clear theoretical foundation for how that process occurred. He based this theory on two fundamental principles. First, he argued that organisms become more complex or perfect over time, meaning they become better suited to their conditions of existence. Following in the footsteps of physicists like Isaac Newton, who sought fundamental laws of nature to explain natural phenomena, Lamarck contended that evolution toward perfection was one such law. He argued that new, simple organisms constantly arise spontaneously from inorganic matter, a process that supplies the Earth with the raw material of evolution; the oldest organisms (humans) are the most complex and perfectly adapted to the world. The second principle held that characters an organism acquired during its life would be passed on to future generations; this was known as the 'inheritance of acquired characters.' While the inheritance of acquired characters was not a new concept, Lamarck used the idea to explain evolutionary change over time at the broadest scale – the entire history of life on Earth – which was an unprecedented and ambitious application of the theory. Both of these fundamental features of Lamarck's theory – the principle of perfection and the inheritance of acquired characters – had a lasting and major influence on subsequent theories of evolution. Inherent in the first, however, was the fundamental notion that evolution was inherently directional and progressive.

Fifty years after Lamarck's book, Darwin (1859) published *On the Origin of Species*, which changed the landscape of evolutionary theory dramatically and called into question the inherent directionality of evolution. Importantly, Darwin's theory differed from its predecessors because he described variation as multifarious, or taking place in a wide number of directions. Even though Darwin did not view variation as completely random, his argument that variation was nondirected was an important conceptual move. Based on his understanding of variation, Darwin argued that evolutionary

change was primarily driven by differential survival and reproduction caused by the conditions of existence. In a sense, Darwin recast teleology in evolution as adaptation caused by selection, as opposed to evolution toward a predetermined goal or perfection. Even still, the language of progressive evolution – for example, 'advanced,' 'higher,' 'developed' – persisted throughout Darwin's work. Despite this, Darwin's theory of evolution by natural selection and multifarious variation became the theory against which proponents of directed evolution argued.

Orthogenesis and Nomogenesis

Even though support for the idea of evolution toward perfection waned in the mid to late nineteenth century, theories of evolutionary directionality gained considerable support. These ideas took shape under the name 'orthogenesis,' meaning evolution in specific directions due to limitations on the production of variation. The German biologist Wilhelm Haacke proposed the word 'orthogenesis' in 1893 based on the Greek term 'ortho' meaning 'in a line,' and 'genesis' meaning 'origin.' Like 'directed evolution,' the term 'orthogenesis' came to refer to both the phenomenon itself, of evolution in a particular direction, and the wide range of theories proposed by prominent biologists to account for this phenomenon. Haacke, for example, argued that the material of heredity was crystalline. New variation in nature would follow the crystalline pattern of the hereditary material, and only proceed in certain limited directions. The Swiss-German zoologist Theodor Eimer supported orthogenesis vocally, usually in opposition to the neo-Darwinian biologist August Weismann. Eimer (1890) argued that the developmental process – in which an organism transforms from an embryo into an adult – structures and limits the types of new features that can arise. Based on this idea that development limits variation, Eimer claimed that evolution is 'definitely directed,' because variation produces the raw material required for evolution. According to Eimer, because development produces variation only along limited trajectories, evolution can only proceed along the trajectories created in development. Like other supporters of directed evolution, such as Edward Drinker Cope, Henry Fairfield Osborn, and Othenio Abel, Eimer argued that orthogenesis could account for a wide range of empirical observations that followers of Darwin and Lamarck struggled to account for, such as patterns of directionality in the fossil record, overspecialization of parts, and patterns of variation.

The Soviet biogeographer and taxonomist Leo Berg developed a theory of evolution in the 1910s (see e.g., Berg, 1969) called 'nomogenesis,' or evolution according to law. Although not the most influential theory in the history of biology, Berg's nomogenesis nevertheless exemplified theories of directed evolution around this time in important ways. Much like Lamarck and Eimer before him, Berg argued that evolution proceeds in defined directions because evolution operates according to natural laws. He claimed that the origin and change of evolutionary variation were strongly constrained by morphological forms along certain trajectories. Berg argued against what he claimed to be the Darwinian ideas that variation was random and that evolution proceeded simply according to environmental circumstances. Strongly anti-Darwinian, Berg also criticized the idea of descent with modification, emphasizing evidence from the fossil record and studies of extant animals that showed evolution as fundamentally convergent, not divergent. Like Berg, many orthogenesists directly opposed the neo-Darwinian conception of multifarious variation and adaptation to local conditions as the primary mode of evolutionary change. While most did not deny outright the operation of natural selection as a cause of evolution, they focused instead on ideas and evidence that showed morphological evolution to be a highly ordered and constrained process, proceeding almost always in definite directions.

The Evolutionary Synthesis and Beyond

In the mid-twentieth century in Britain and the United States, biologists from a wide range of disciplines (zoology, paleontology, cytology, botany, etc.) rallied around the confluence of Mendelian genetics and Darwin's theory of natural selection. This modern evolutionary synthesis brought an intense focus on gradual, genetic changes or adaptations to local conditions, often at the level of populations of organisms; and, thus, support for orthogenesis waned. In Germany and elsewhere, however, directed evolution remained a vibrant part of evolutionary theorizing. The German paleontologist Otto Schindewolf used extensive evidence from paleontology to showcase directionality in the evolutionary history of life on earth. His theory to explain directed evolution, called 'typostrophism,' described evolution as a cyclical, three-part process. In each of these phases of evolution, Schindewolf discussed the evolution of entire taxonomic groups, related by a common ancestor (see e.g., Schindewolf and Reif, 1993). In the first phase, a group of species evolves rapidly into multiple different morphological forms, all based around a common architecture called a 'body plan.' In the second phase, the morphologically distinct organisms in the group evolve along similar lines – that is, directions – for a long period of time. Schindewolf argued that these trajectories result from internal factors or developmental processes that constrain organismal evolution. In the final phase of evolution, the entire group of species went into decline, with many or most going extinct due to maladaptive features. His theory nicely explained gigantism, overspecialization, evolutionary directionality, and mass extinction, highlighting sets of evidence that were difficult to account for in the modern evolutionary synthesis framework.

Evo Devo and Devo Evo

In the 1970s and 1980s, developmental, molecular, and evolutionary biologists, alongside paleontologists, focused on the question of how developmental and evolutionary phenomena are causally related. Modern theories of directed evolution emerged as one component of the complex and related interdisciplinary research programs today known as evolutionary developmental biology (evo devo) and developmental evolution (devo evo). These research areas built off of work like Gould's (1977) *Ontogeny and Phylogeny*, and that

of others who, like Gould, were dissatisfied with the explanatory sufficiency of the approach from the modern synthesis. An important workshop in Berlin in 1981, chaired by developmental biologist John Tyler Bonner, clarified two important considerations that have become central to modern attempts to understand the apparent directionality in evolution (see Alberch and Bonner, 1982). First, conference attendees argued that the ways in which genes regulate development is a major contributor to the trajectories and directionality in evolution. Second, the developmental process constrains the conditions under which selection can operate. These conceptual advancements resulted in a view of evolution as deeply constrained and contingent on development. Soon thereafter, in 1985, an important paper further articulated several foundational ideas concerning directed evolution, and defined 'developmental constraints' as 'a bias in the production of variant phenotypes or a limitation on phenotypic variability caused by the structure, character, composition, or dynamics of the developing system' (Maynard Smith *et al.*, 1985, p. 266). This definition specified features of the developmental process that could constrain or direct evolution, thereby identifying concrete causes of directionality in evolution. Furthermore, this article distinguished developmental constraints from constraints of history, physics, or selection. Set out in this way, the idea that the genetics and mechanisms of development guide and limit evolution became an important and coherent research project for future generations.

Throughout the 1980s and 1990s, investigators from various disciplines further studied the relationship between evolution and development. Evo devo tended to focus on the role of evolution in development, and devo evo emphasized ways that development influenced or constrained evolution. Both areas of research – with their associated theories and study of directed evolution – became important parts of the emerging trend toward interdisciplinarity in the biological sciences. Devo evo especially emphasized ways in which the regulation of gene activity in the developing organism structures and limits the production of new variation, and constrains evolution. The discovery of homeobox genes, which regulate the patterns of anatomical development, and studies showing the similarity of regulatory genes across all metazoans have dramatically shaped our understanding of the limits to morphological evolution. Through careful analysis of these deep genetic homologies, developmental geneticists and evolutionists have come to understand the networks of regulatory genes shared across all organisms with a common body plan.

Evolutionary Directionality

Theories explaining evolutionary directionality have persisted throughout the past two centuries of evolutionary thinking, beginning in the work of Lamarck and continuing today as a part of evo devo and devo evo. While the proposed cause of directionality has changed over time – from progress toward perfection and laws of evolution in the nineteenth century to gene regulatory networks and deep homology in the twentieth and twenty-first centuries – the theories of directed evolution represent an important part of the history of evolutionary theory. While early hypothesized causes of directed evolution were vague, or inconsistent with our modern understanding of developmental constraints, orthogenesists nevertheless asked a set of questions that biologists are still trying to answer today. Orthogenesists identified directionality as a real phenomenon and something worth investigating carefully. Throughout this history, the question of the explanatory relationship between directed evolution and natural selection based on environmental circumstances has arisen time and time again. While most orthogenesists viewed natural selection as operating in a limited capacity, they also positioned their ideas as explicitly against the neo-Darwinian view. Supporters of evo devo and devo evo argue for a much larger role for natural selection in evolutionary causation generally, though directionality caused by developmental constraints also features importantly in these subfields. Consequently, supporters of directed evolution today uphold developmental constraints and inherent limitations to phenotypic variation as one part of the complex set of factors causing evolutionary phenomena.

See also: Adaptive Mutation Controversy. Darwin–Wallace Theory of Evolution. Developmental Biases on Morphological Evolvability. Epigenetic Inheritance. Epigenetics and Genome Evolution. Evolutionary Biology, History of. Synthetic Theory of Evolution, History of. Waddington's Epigenetic Landscape, History of

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Directional Selection and Adaptation

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Glossary

Ancient DNA DNA obtained from fossils or museum specimens. It is usually very fragmented and contains chemical modifications that tend to accrue after the expiration of the individual.

Diffusion approximation A continuous approximation to a process that undergoes discrete changes in state over discrete periods of time. It is based on the assumption that only very small changes happen in any particular time step. In the case of the Wright–Fisher process, neutral drift may only cause very small allele frequency changes at a specific generation, so long as the population size is large. The diffusion approximation maps the discrete time and allele frequencies onto a continuous scale, which simplifies the mathematics of the system.

Identity-by-descent (IBD) Continuous stretches of DNA sequence that contain sites with the same allele, because they were all inherited from a common ancestor without being broken up by recombination. Long segments of IBD are a hallmark of recent selective sweeps.

Linkage disequilibrium Nonrandom association between alleles at different loci. It can be caused by low-local rates of recombination along a chromosome, specific forms of population structure and/or selection.

Introduction

Natural selection is the process by which particular traits become more or less abundant in a population, as a consequence of their effects on survival and reproduction. A major branch of population genetics involves the study of the genetic variants that code for beneficial or deleterious traits, and how those variants rise or fall in frequency throughout time.

A new variant that enters a population (e.g., by mutation) can be harmful, beneficial, or neutral. If it is harmful, then negative selection will act to remove it from the population because it causes a fitness disadvantage. If the variant is beneficial, then positive selection will act to increase its frequency in the population because it confers a fitness advantage. This increase in fitness may manifest itself, for example, through a larger number of viable offspring in those individuals that carry the mutation. A neutral variant (neither harmful nor beneficial) may also remain in the population for some time, and even reach fixation (become present in all individuals in the population), merely due to chance.

In this article, we will study natural selection and its effects on genetic variation. We will focus on the process of positive natural selection, because this can result in the appearance of new adaptive phenotypes. Two key questions in population genetics are how long it takes for a beneficial variant to fix in a population and what the probability of fixation is. In order to study these questions, biologists have historically modeled the frequency trajectory of neutral and beneficial alleles using the 'Wright–Fisher' model, which we will briefly explain below (see Ewens, 2004 for an extensive treatment of this model). Then, we will survey different types of selection in the context of linkage and recombination, including 'hard sweeps,' 'soft sweeps,' and 'adaptive introgression.' Finally, we will explain how particular signatures left by selection on the genome can help us detect selected genes, both in humans and other organisms. Whenever possible, we will attempt to illustrate theoretical ideas with concrete examples from the biological literature.

The Dynamics of Selection

To understand the dynamics of natural selection in a population, we will first begin by assuming this population is infinitely large. This abstraction allows us to ignore the stochastic sampling of alleles that occurs in each generation (see 'Genetic drift and the Wright–Fisher Model' below) and concentrate only on the effects of fitness differences on the composition of a population. Here, we will focus on viability selection, meaning selection operating on the ability of individuals to survive until they can reproduce rather than on their fertility. We will also restrict ourselves to the case of an additive locus. If A is the advantageous allele, that means that the relative fitnesses of the three possible genotypes, aa, Aa, and AA, are $w_{aa}=1$, $w_{Aa}=1+s$, and $w_{AA}=1+2s$, respectively. These relative fitnesses express the viabilities of each genotype, relative to the other two. Here, s is the selection coefficient, which determines how advantageous a particular genotype is, relative to the aa genotype.

Let us see what happens in one generation of selection favoring the A allele. Suppose the allele frequencies in the parents are f_A and f_a, that the size of the population remains constant across generations, and that parents mate at random. In that case, the frequency of offspring containing each of the three genotypes in the next generation will be in Hardy–Weinberg equilibrium: $f_{aa}=f_a^2$, $f_{Aa}=2f_Af_a$, and $f_{AA}=f_A^2$. However, not all of these offspring survive to adulthood, as selection will remove some individuals before they get to successfully reproduce. In fact, the three types of zygotes will eventually reproduce in the ratio $w_{aa}:w_{Aa}:w_{AA}$. Therefore, the

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ratio of reproducing adults will be $f_a^2 w_{aa}$: $2f_A f_a w_{Aa}$: $f_A^2 w_{AA}$. We can transform each of these terms into relative frequencies by dividing by their sum, which yields that:

$$f'_{aa} = f_a^2 w_{aa}/w_{\text{sum}}$$
$$f'_{Aa} = 2f_A f_a w_{Aa}/w_{\text{sum}}$$
$$f'_{AA} = f_A^2 w_{AA}/w_{\text{sum}}$$

where

$$w_{\text{sum}} = f_a^2 w_{aa} + 2f_A f_a w_{Aa} + f_A^2 w_A$$

To obtain the allele frequencies in this new generation, we need to account for the number of copies of each allele in each genotype:

$$f'_a = f'_{aa} + (1/2)f'_{Aa} = (f_a^2 w_{aa} + f_A f_a w_{Aa})/w_{\text{sum}}$$
$$f'_A = f'_{AA} + (1/2)f'_{Aa} = (f_A^2 w_{AA} + f_A f_a w_{Aa})/w_{\text{sum}}$$

Finally, we can quantify the net change in allele frequencies in a generation by taking the difference between the allele frequencies in the new and the old generations:

$$\Delta f_A = f'_A - f_A = f_A f_a [f_A(w_{AA} - w_{Aa}) + f_a(w_{Aa} - w_{aa})]/w_{\text{sum}}$$

We can also express this difference in terms of the selection coefficient:

$$\Delta f_A = f_A f_a s/(1 + 2f_A s)$$

Thus, we see that if the selection coefficient is positive, the frequency differential will also be positive and the allele frequency of the A allele will increase. Furthermore, the most rapid change will occur at intermediate frequencies, when $f_A f_a$ is largest.

If a new advantageous allele appears in an infinite population, it is guaranteed to rise to high frequencies, but it will only asymptotically reach fixation, as the population is infinite. The speed at which it reaches asymptotic fixation will not only depend on the selection strength, but also on the dominance relationship between the two alleles (Figure 1). While we have focused above on an additive case, it is also possible that the advantageous allele could be dominant or recessive

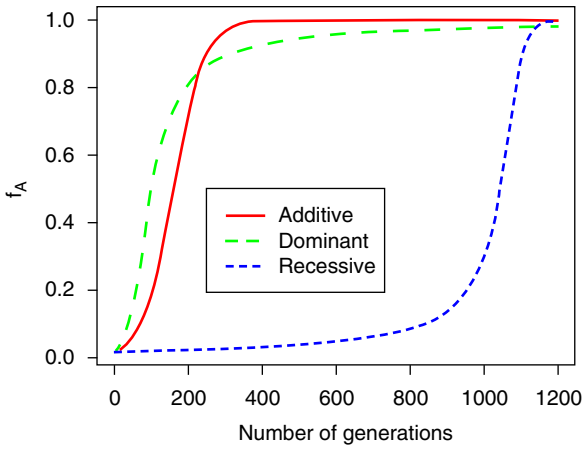

Figure 1 The change in the frequency of an advantageous allele when it is additive, dominant or recessive. Here the selection difference between *aa* and *AA* is 5%.

(Figure 1). For further details about the dynamics of selection, we refer the reader to more extensive treatments of this topic in Crow and Kimura (1970), Hartl and Clark (1997), and Nielsen and Slatkin (2013).

The Interplay between Selection and Drift

Evolution in finite populations is stochastic. At each generation, individuals undergo meiosis, and only a proportion of the gametes make it to the next generation. Additionally, not all individual zygotes survive to adulthood and reproduce. While a selective advantage may influence which genes are passed on to the next generation, there is always some probability that even nonselected genes rise in frequency, just by chance. This stochastic effect on allele frequencies is known as 'genetic drift.' We have gotten away with ignoring drift so far, because we assumed our population was almost infinitely large, but we will now turn to studying it in detail, as well as its effect on selection.

One way to analyze the frequency changes of a mutation due to drift is by means of the Wright–Fisher model (Figure 2). This model assumes that a population of individuals has a constant size N and is randomly mating; furthermore, it assumes that generations do not overlap. At each locus, only two alleles exist. We can visualize this model by imagining that we put all the alleles at a particular locus into a bag. Then, the frequency of an allele will be the number of copies of that allele divided by $2N$ because each individual has two chromosomes. In a two-allele model, the frequency of the two alleles will always add to one, so we need only track the frequency of one allele over time (Figure 2). At the next generation, we sample 'with replacement' from the bag of alleles from generation t in order to create a new generation of N individuals. The allele frequencies may increase or decrease, depending on how many individuals inherit each allele at generation $t+1$. One can show that the probability of eventual fixation for a new neutral mutation is $1/(2N)$. Conversely, the probability of its eventual extinction (removal from the population) is $1 - 1/(2N)$.

When we introduce selection into the Wright–Fisher model, the probability of fixation not only depends on the size of the population, but also on the strength of selection. If there is no mutation, either one of the two alleles at the site will eventually reach complete fixation, while the other will go extinct forever. However, the selected allele is not guaranteed to be the fixed allele. Kimura (1957) used a diffusion approximation to derive the probability of eventual fixation of A in an additive selection model, when the current frequency of A is p and the selection coefficients for the three possible genotypes (AA, Aa, and aa) are defined as in the previous section. He obtained the following equation:

$$p[A \text{ fixes}] = (1-\exp(-4Nsp))/(1-\exp(-4Ns))$$

This function is plotted in Figures 3(a) and 3(b), for different s and p. We see that, as we would expect, the fixation probability increases as the selection strength increases and also strongly depends on the current frequency of the allele. Even with a relatively high selection strength, newly arising alleles ($p=1/(2N)$) have a very low chance of fixation as they are much more likely to be lost by stochastic fluctuations.

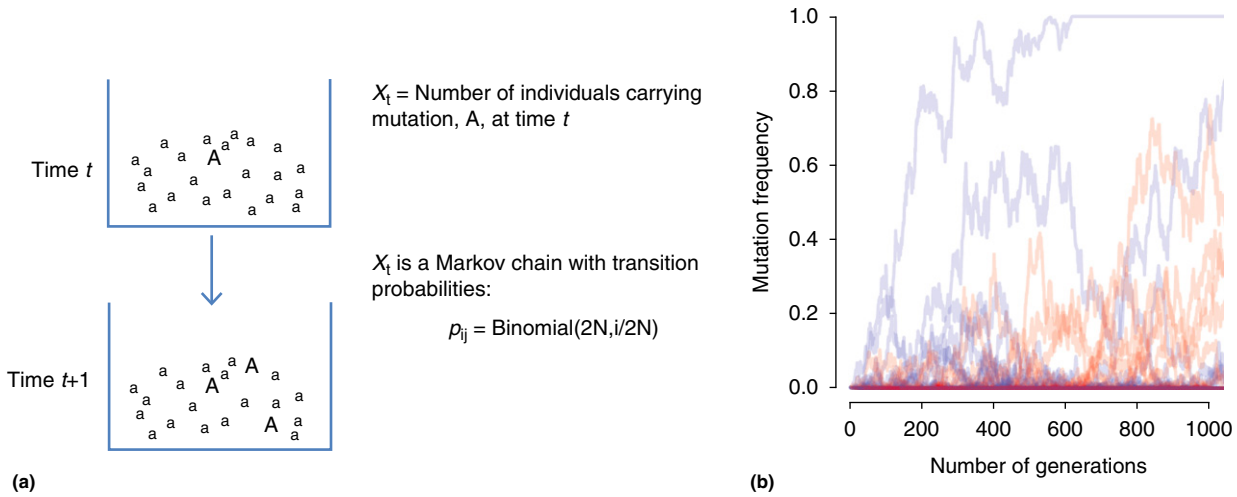

Figure 2 The Wright–Fisher model. (a) Schematic illustrating the Wright–Fisher model as random sampling with replacement from a population of alleles at time t to create the population at time $t+1$. X_t is a random variable defined as the number of chromosomes carrying the mutation A at time t. We use the Wright–Fisher model to simulate allele frequencies over time. (b) Example trajectories of a neutral Wright–Fisher model, the y-axis is the frequency of the allele in the population; the x-axis is time in generations. 2000 mutations were simulated, starting with one allele in the population, and were spaced uniformly in the time interval from 1 to 1000 generations, approximately two mutations per generation. The population size N is 500. We show example trajectories, evolving through 1000 generations. Notice that most mutations that arise over time are later lost from the population.

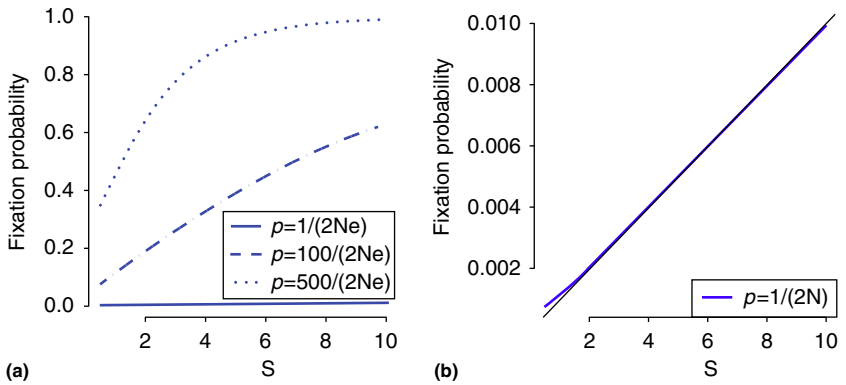

Figure 3 Fixation probabilities: Theoretical results from the diffusion approximation of the Wright–Fisher model. The panels are the probabilities of fixation as a function of the selection strength, $S=2Ns$, for different initial frequencies p. On the right is a zoom-in of the solid curve where $p=1/(2N)$, and the overlaid black line is the approximation $2s=S/N$, discussed in the text when $S\gg1$. The leftmost point on the curve of the zoom-in is $S=0.5$ and fixation probability $=0.0008$, the 'weakly advantageous' regime discussed in the text. By comparison, the neutral fixation probability is $1/(2N)=0.0005$. These functions can be obtained from formulas derived by Kimura and Ohta (1969), using a diffusion approximation to the Wright–Fisher model.

There is an approximation we can make for the case of a newly arising mutant A in a population made up entirely of a (i.e., when $p=1/(2N)$). If selection acts in favor of A ($s>0$) and $2Ns$ is much larger than 1, an allele will have a higher probability of fixation than under neutrality. In that case, the denominator will be approximately one, while the numerator will approximately equal $2s$, and so the probability that A eventually fixes will be close to $2s$ (**Figures 3(b)** and **4(a)**). When this occurs, we say that A is 'strongly advantageous.' However, in the case where the population or the selection coefficient are small (in particular, when $0<2Ns<=1$), then the probability of fixation will be only slightly larger than the neutral fixation probability ($1/(2N)$) (**Figure 3(b)**, right panel and **Figure 4(b)**) and we say that A is 'weakly advantageous.' An important determinant of whether a new mutant allele fixes in a population is whether it can make it past the initial low frequencies at which stochastic sampling (due to a low copy number) makes it highly probable that the allele goes extinct merely by chance, even when advantageous. For example, **Figure 4(d)** shows that the proportion of alleles that eventually go extinct is high, even when selection is strong ($s=0.1$) and the population is fairly large ($N=200$).

The time of fixation differs under the two regimes of weak and strong selection. When selection is strong and the population size is large, this time is approximately equal to the theoretical time at which an allele would get close to fixation in an infinitely sized population, where genetic drift does not affect the trajectory at all (**Figure 4(a)**). However, when the

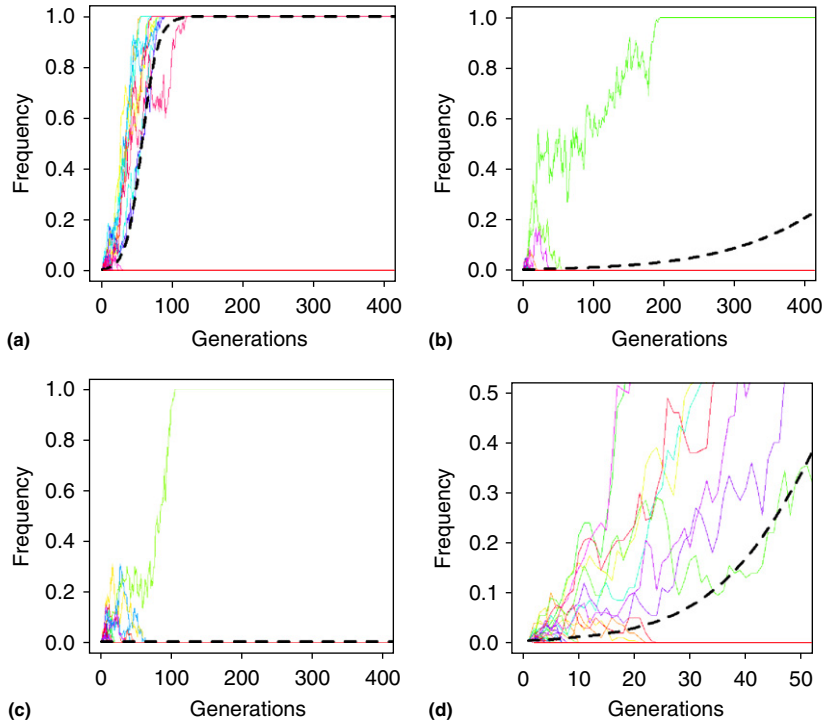

Figure 4 Examples of frequency trajectories of a newly arisen allele in finite populations. In each panel, we simulated 200 populations with size $2N=200$, and plotted the frequency trajectory of a new allele in each population. We also plotted the deterministic trajectory of an allele in an infinitely large population, where drift plays no role, and so there are no stochastic forces in the population (black dashed line). (a) Strongly advantageous allele ($s=0.1$). (b) Weakly advantageous allele ($s=0.01$). (c) Neutral allele ($s=0$). (d) Even under strong selection ($s=0.1$), alleles that fail to rapidly reach intermediate frequencies will likely go extinct.

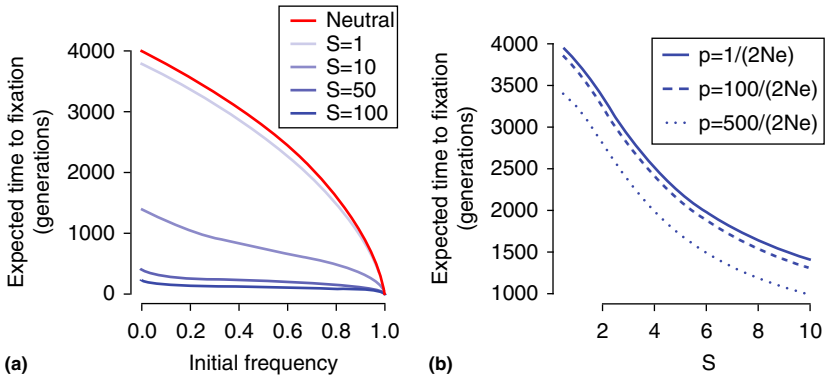

Figure 5 Expected time to fixation. Theoretical results from the diffusion approximation of the Wright–Fisher model. The panels are the predicted mean time to fixation of an allele, assuming a population size, N, of 1000. On the top left is the expected time is plotted as a function of the initial frequency. Curves are plotted for different scaled selection strengths, $S=2Ns$. The red curve is the neutral model prediction ($S=0$). The expected fixation time decreases as the selection strength increases or the initial frequency increases. The panel on the right is the same function plotted as a function of selection strength S, for three different initial frequencies, p.

population size is small relative to the selection coefficient, genetic drift will be a major determinant of the allele trajectory. In the few occasions in which the allele may manage to reach intermediate frequencies, genetic drift will contribute to push the allele to fixation faster. This will happen approximately at the speed at which neutral alleles fix, which is $2N$ generations (Figures 4(b) and 4(c)). In Figures 5(a) and 5(b), we plot the expected time to fixation of an allele under different selection regimes, as a function of the current frequency of the allele (left panel) and the selection strength (right panel).

Genetic Hitchhiking and Selective Sweeps

An additional assumption that we have been making for the Wright–Fisher model is that loci are independent and exist in

Figure 6 Different ways in which a beneficial mutation and its linked neutral variants may rise in frequency in a population. Each of the rectangles is a chromosomal segment that may undergo recombination. The green stars denote linked neutral variants, while the red, blue, and purple stars denote beneficial variants.

isolation, with each one changing frequency independently each generation. In reality, genes exist within chromosomes, and only over time will recombination uncouple allele frequencies between different sites. This creates an additional layer of complexity, but, as we will see later on, it also provides another way to detect positive selection along the genome.

Let us again imagine a new strongly beneficial allele that appears in a population for the first time. This time, however, we will keep in mind that the mutant sits on a chromosome with other genes that may have different alleles in different individuals (Figure 6(a)). As the allele rises in frequency, it also carries along with it the neutral variants that are physically linked to it in the same chromosome. At the same time, recombination works to break apart the chromosomes. If the selected allele rises in frequency fast enough, recombination may not have enough time to randomly shuffle the neutral alleles in the local neighborhood of the selected variant. Thus, the set of neutral variants that were originally linked to the beneficial mutant – its original 'haplotype' – will 'hitchhike' along with it (Maynard Smith and Haigh, 1974), replacing

other haplotypes that do not carry the mutant. This phenomenon will produce a recognizable footprint in the distribution of haplotypes in the population (**Figure 6(a)**) and is called a 'selective sweep.'

New Models: Soft Sweeps, Polygenic Selection, and Adaptive Introgression

While selective sweeps from new mutations (also called 'hard sweeps') are somewhat easy to detect (see section below) and appear to be prevalent in fruit flies (Sattath *et al.*, 2011) only a few examples of this phenomenon have so far been found in humans (Barreiro *et al.*, 2008; Pickrell *et al.*, 2009; Hernandez *et al.*, 2011, but see Enard *et al.*, 2014). This has prompted theoreticians to come up with alternative models by which positive selection may act, and to understand how they shape patterns of variation at linked variants (Hermisson and Pennings, 2005; Pritchard *et al.*, 2010; Messer, 2013; Messer and Petrov, 2013).

One of these models is called a 'partial sweep' and posits that sometimes a beneficial allele may not completely fix in the population, but remain at intermediate frequencies (Coop and Ralph, 2012; Voight *et al.*, 2006; Pritchard *et al.*, 2010; **Figure 6(b)**). Another model is called a 'soft sweep from standing variation' and posits that sometimes selection may not immediately act on a beneficial variant, which will therefore drift neutrally (or possibly subject to negative selection) for some time. Once the allele begins to be beneficial and rise in frequency, different copies of it will be linked to different alleles at nearby genes. This will generate a different pattern than the one observed during or after a selective sweep (Orr *et al.*, 2001; Hermisson and Pennings, 2005; **Figure 6(c)**). Additionally, if the mutation rate is high, different copies of the beneficial allele may arise in different haplotype backgrounds, before any particular copy has had a chance to fix. Selection may jointly favor these multiple copies, and so the distribution of haplotypes after selection may not look as uniform as in the case of a hard sweep. This is called a 'soft sweep from *de novo* mutation' (Pennings *et al.*, 2006a,b; Messer, 2013; Messer and Petrov, 2013; **Figure 6(d)**). Finally, the model of 'polygenic adaptation' posits that selection for a trait may act by subtly increasing the frequencies of alleles in different genes in coordination (Barton, 2000; Pritchard *et al.*, 2010; Berg *et al.*, 2014). In this case, no single locus may show a strong signature of a sweep (**Figure 6(e)**).

In recent years, there has also been increasing evidence in favor of selection acting by means of 'adaptive introgression' (Hedrick *et al.*, 2013; Racimo *et al.*, 2015). In this scenario, an advantageous mutation is passed on, via interbreeding, from one population or species to another, and afterwards is pushed to high frequencies by selection in the recipient population (**Figure 6(f)**). A striking example is the gene *EPAS1*, which is involved in the human response to hypoxia at high altitudes. A particular haplotype of this gene is uniquely found at an elevated frequency in Tibetans, while almost absent everywhere else in the world (Yi *et al.*, 2010; Huerta-Sanchez *et al.*, 2014). Moreover, the Tibetan haplotype is extremely different from other present-day human variants of the gene. Using ancient DNA sequences obtained from human fossil remains (Meyer *et al.*, 2012), Huerta-Sánchez *et al.* (2014) recently found that the haplotype is closest to the Denisovans, an extinct group of hominins that likely interbred with the ancestors of Tibetans.

Tests of Selection on Real Data

The Site-Frequency Spectrum

When studying natural populations, researchers often take the counts of all polymorphisms segregating in the population at a particular allele frequency, and build a histogram (**Figure 7**). This histogram is called the site-frequency spectrum (SFS). Under neutrality, if one assumes an infinite sites model of mutation (where each mutation creates a new biallelic site never before seen in the population), the expected number of polymorphic sites where the derived allele appears in i out of n chromosomes sampled is equal to θ/i. Here, $\theta = 4Nu$ is the per generation mutation rate (u) scaled by the population size (Fu, 1995). Departures from this expectation can be useful to infer both demography and selection.

Computing the SFS in a particular genomic region is a powerful tool for detecting departures from neutrality. However, the mutation rate parameter θ is often unknown, and so in practice the SFS is usually calculated from data as the proportion of polymorphic sites with a particular allele frequency, rather than the absolute number of such sites.

Figure 7 shows a schematic plot of an SFS for a sample of size 20 (10 diploid individuals) for a set of neutral mutations (in gray), a set of positively selected mutations (in red), a set of negatively selected mutations (in blue) and a set of neutral mutations subject to a selective sweep (in orange). Mutations that are positively selected tend to reach high frequencies more often than do neutral mutations. In contrast, negative selection pushes mutations toward low frequencies. Consequently, in the negative selection scenario, we see relatively more rare mutations and a deficit of high-frequency mutations compared to the neutral case. Finally, during a selective sweep, mutations that are near a selected site will hitchhike to high frequency if they are initially linked to the beneficial variant, while those that are not linked to it will be pushed to low frequencies. In this last case, we will see an excess of both low-frequency and high-frequency variants, relative to the neutral scenario.

There are two ways that the SFS can be used to study selection in real data. In one case, the SFS is computed over all sites that are putatively under selection in the genome. For instance, Boyko *et al.* (2008) examined the SFS of non-synonymous mutations in humans, which are putatively subject to both negative and positive selection. They then used population genetic theory to obtain estimates of the distribution of selection coefficients that would be consistent with the observed SFS in coding regions. Using the extensive functional genomics annotations available for humans, Racimo and Schraiber (2014) extended this work to estimate the distribution of negative selection coefficients genome-wide. This distribution can be informative to researchers interested in understanding the prevalence and intensity of selection throughout the genome.

Another advantage of the SFS is that it is useful for detecting particular regions of the genome that have recently experienced selection. In particular, the SFS can be used to look for the signature that the hitchhiking effect leaves on

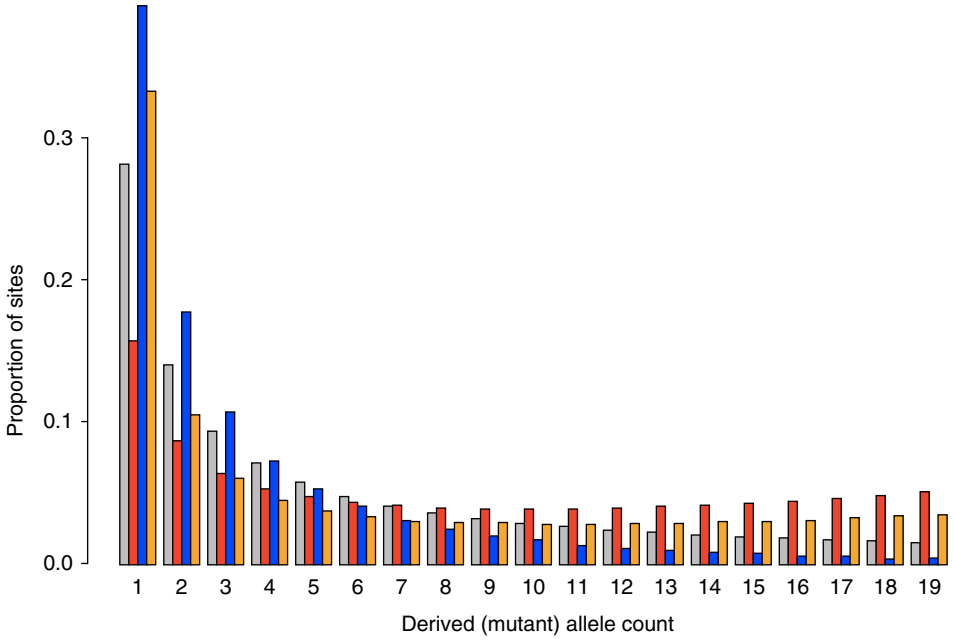

Figure 7 Representative site-frequency spectra (SFS) for a sample of size 20, under different selection regimes. The gray bars are a neutral SFS, the red bars are an SFS for positively selected sites, the blue bars are an SFS for negatively selected sites, and the orange bars are an SFS for sites that are affected by selective sweeps. The general shapes of the spectra are based on a figure from Nielsen, R., Williamson, S., Kim, Y., *et al.*, 2005. Genomic scans for selective sweeps using SNP data. Genome Research 15, 1566–1575.

neutral variation. In most cases, some test statistic summarizing the SFS is computed in sliding windows along the genome, and extreme departures from the neutral expectation of this statistic are used to identify regions under selection. The quintessential summary statistic of the SFS is Tajima's D (Tajima, 1989). Tajima's D compares two estimates of θ that are derived from the SFS: $\hat{\Theta}_\pi$ and $\hat{\Theta}_W$. Both are weighted averages over entries in the SFS, but $\hat{\Theta}_\pi$ gives more weight to middle frequencies, whereas $\hat{\Theta}_W$ weights all frequencies equally. Hence, Tajima (1989) defined a statistic called 'D,' which is proportional to $\hat{\Theta}_\pi - \hat{\Theta}_W$; under neutrality, $\hat{\Theta}_\pi$ should be equal to $\hat{\Theta}_W$, making Tajima's D equal to 0. A positive value of D indicates an excess of middle frequency alleles while a negative value of D indicates a deficit of middle frequency alleles.

A selective sweep will result in a negative value of this statistic. This is because alleles that are linked to the selected site will be dragged to high frequency along with the selected site, while alleles that are not linked to the selected site will be pushed to low frequencies. Hence, there will be a deficit of middle frequency alleles, resulting in a negative value of *D*. Unfortunately, historical demographic events, including bottlenecks, can also produce a negative value of *D*. Thus, to determine if the SFS in a region is actually shaped by hitchhiking, as opposed to other demographic forces, it is important to perform simulations under a demographic model that recapitulates the recent history of the species under study.

Haplotype Lengths

In addition to shaping the local SFS, genetic hitchhiking will cause patterns of haplotype sharing that are unexpected under neutrality. In particular, because a strong selective sweep occurs rapidly relative to neutral dynamics, there is less time for recombination to disrupt the haplotype upon which the selected site arose. Thus, haplotypes surrounding selected sites that recently fixed will be on average longer than haplotypes arising due to neutrality. In other words, the scale of linkage disequilibrium surrounding a recent selective sweep will be much larger.

Several test statistics exist to test for unusually long, high-frequency haplotypes. Among the first to be introduced was the Extended Haplotype Homozygosity (EHH) statistic (Sabeti *et al.*, 2002). Sabeti *et al.* (2002) applied EHH to genetic data from regions encompassing two genes thought to confer malaria resistance in humans: *G6PD* and *CD40L*. In both cases, they found that EHH was significantly higher than expected under neutrality. In later work, Bersaglieri *et al.* (2004) used EHH to show that lactase persistence was shaped by strong positive selection in some populations.

Because EHH was originally applied only to small regions, it did not provide a single test statistic that could be applied in a genome-wide scan. To remedy this problem, Voight *et al.* (2006) developed the integrated Haplotype Homozygosity (iHH) score. Roughly, iHH is expected to be higher in regions that underwent recent strong selection, because it captures the signal of higher identity-by-descent that is expected to be observed in a sample of selected haplotypes, but not in a sample of haplotypes evolving neutrally. Using iHH, Voight *et al.* (2006) found evidence of natural selection at several loci in humans, including *LCT*. This gene codes for the lactase enzyme and is thought to have allowed particular populations of humans to be able to digest milk as adults. This ability is hypothesized to have been especially advantageous during the origins of agriculture.

Population Differentiation

In many cases, selection acts as a response to a local environmental stress. In these cases, looking at patterns of population differentiation along the genome can serve to detect regions that experienced recent positive selection. Because positive selection can drive alleles to high frequency much faster than under neutrality, many approaches rely on looking for unusual differences in allele frequencies among populations. These methods are often fundamentally based on Wright's fixation index, F_{ST}. As commonly applied to genomic data, F_{ST} is calculated as the difference in heterozygosity (the proportion of sites that are heterozygous) as measured between and within populations, normalized by the between population heterozygosity. High values of F_{ST} indicate that there are substantial allele frequencies differences between populations, while low values indicate that the populations are close to randomly mating, as if they were a single population. By searching for genomic regions with particularly high F_{ST} relative to the genome-wide background, it is possible to find candidate regions of local adaptation between populations.

In a large-scale study, Coop *et al.* (2009) applied this approach to human genomic data. They found three common patterns of geographic differentiation: non-African sweeps (e.g., the *KITLG* variant conferring lighter skin color, which is at near fixation in all non-African populations), West Eurasian sweeps (e.g., the *SLC24A5* variant conferring lighter skin color, which is at high frequency only in west Eurasians), and East Asian sweeps (e.g., a variant of the *MC1R* gene of unknown consequence, which differentiates the Han Chinese from the French and Yoruba populations). Based on the prevalence of these patterns, they concluded that humans experienced novel selective pressures after expanding out of Africa.

When computed between two populations, F_{ST} alone cannot directly reveal which of the populations was the one that experienced selection. However, it is possible to leverage information from triplets of populations to determine this. This approach was used by Yi *et al.* (2010) to define the population branch statistic (PBS). Using PBS, they found evidence of recent, strong selection in the *EPAS1* locus in Tibetans when compared to the Han Chinese, while using a Danish population as a third outgroup. The selected variant of the gene is thought to confer an adaptation to high altitudes to Tibetans, making them less likely to suffer from hypoxia in their native mountainous environment.

Allele Frequency Time Series

All previously discussed methods of detecting selection explore patterns of variation in extant populations to infer the past action of selection. With the advent of high throughput sequencing, ancient DNA (aDNA) has made it possible to assay historical allele frequencies to look directly at selection in action. Using the theoretical framework of Bollback *et al.* (2008), Ludwig *et al.* (2009) found direct evidence that loci influencing coat color in horses evolved under natural selection, and estimated selection coefficients. More recently, using ancient DNA from several archeological sites in Europe, Mathieson *et al.* (2015) showed that several loci were subject to strong natural selection in Europeans, although most of the genome showed patterns of allele frequency changes consistent with neutral processes.

Conclusion

Evolution routinely carves seemingly designed adaptations out of the random starting material provided by mutation. Using mathematical models, it is possible to understand the dynamics and timescales of natural selection, as well as to look for its signature in genetic data. One of the most important lessons from the study of selection in finite populations is that even strongly beneficial alleles will frequently be lost very quickly. However, alleles that manage to survive their initial genetic drift at low frequency will rise to fixation by natural selection, leaving characteristic signatures of genetic variation within and between populations.

By examining the patterns of variation left by selectively favored alleles, we can elucidate the processes by which organisms adapt to their environment. Strongly selected, *de novo* mutations will drive one large haplotype to fixation. The signature of such mutants should be easy to detect in data on the basis of linked allele frequencies or haplotype lengths. On the other hand, other modes of adaptation, including soft and partial sweeps, will leave more diffuse, difficult to recognize signatures in the data. Nonetheless, new tools and models, including access to ancient DNA, may allow us to characterize the prevalence of each mode of adaptation in different species.

See also: Natural Selection, Introduction to. Neutral Evolution, Population Genetic Tests of

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Dispersal Biogeography

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Glossary

Colonization The biological process by which dispersing species become established in new areas.
Dispersal The movement of an individual away from its place of birth.
Dispersal biogeography An explanation for the geographical distribution of organisms based on processes of dispersal.
Invasion The expansion of a species into a new area.
Last glacial maximum (LGM) The time during the last glacial period when ice sheets were most extensive, roughly twenty thousand years ago.
Oceanic island An island that does not sit on continental shelf, typically volcanic in origin.
Phylogeography The study of geographic distributions of genetic lineages and processes that shape them.
Rafting The dispersal of organisms as, or in association with, floating material.
Refugia Geographical areas in which populations of glacially impacted organisms persisted during glacial periods.
Vicariance A biologically passive explanation for the geographical distribution of organisms, typically invoking geological processes.

Dispersal Biogeography

There are numerous factors potentially promoting organismal dispersal, including the avoidance of environmental change (Levin *et al.*, 1984) and opportunities for genetic diversification (Hamilton and May, 1977). Dispersal has been recognized as a key process underpinning biological distributions since the nineteenth century, when natural historians such as Darwin and Wallace proposed that many widespread species had likely dispersed long distances from regions of origin. These researchers also recognized that overwater dispersal events have played a particularly important role in establishing distinctive oceanic island biotas (Darwin, 1845, 1872; Wallace, 1876). Their proposed examples of dispersal included the suggested African origin for humans (Darwin, 1871) and circumpolar dispersal of southern freshwater fishes (Darwin, 1872; McDowall, 1970, 1978) – hypotheses subsequently corroborated by molecular evidence (Burridge *et al.*, 2012; Cann *et al.*, 1987). Indeed, human history provides many excellent examples of long-distance dispersal, even on recent timescales (Oppenheimer, 2012).

The acceptance of plate tectonic theory in the latter half of the twentieth century provided potential vicariant (nonbiological) explanations for many biological distributions. While there are indeed numerous cases in which biologically passive earth-history processes (rather than dispersal) have apparently led to biogeographic isolation of particular taxa (e.g., Burridge *et al.*, 2006; Knowlton and Weigt, 1998; Riddle *et al.*, 2000; Sanmartin and Ronquist, 2004), this focus on geological processes seemingly led some biogeographers to an extreme view of vicariance as the sole permissible explanation for biological distributions (Craw *et al.*, 1999; Croizat *et al.*, 1974; Nelson, 1974; Nelson and Platnick, 1981).

Over the last three decades, the incorporation of molecular data, along with a range of new technological and analytical approaches, have enhanced understanding of genealogical relationships among organisms, populations, and species, and have enabled dispersal events to be characterized. In particular, these studies have strengthened scientific understanding of biological dispersal processes and their role in promoting population connectivity and range expansion (de Queiroz, 2005; Gillespie *et al.*, 2012; Hewitt, 2000; Yoder and Nowak, 2006). Here we review evidence for biological dispersal and highlight key areas of dispersal research.

Dispersal as a Predictable Process: Evidence from Life History, Ecology, Biogeography, and Genetics

Although some early biogeographers dismissed dispersal as an untestable and therefore unimportant phenomenon (e.g., Croizat *et al.*, 1974; Platnick and Nelson, 1978), others recognized that, despite the difficulty in demonstrating its occurrence, it was surely a critical driver of many species' distributions (Carlquist, 1981). Today there is increasing recognition that dispersal is an important evolutionary biogeographic process that is often highly predictable based on ecological, life history, and abiotic data (Gillespie *et al.*, 2012). Indeed, over recent decades, population-genetic studies have provided compelling evidence of the key role of dispersal as a process that connects isolated populations and facilitates range expansion. Comparative studies and meta-analyses have repeatedly demonstrated that life history and ecology are strong predictors of gene flow between populations, with dispersive taxa typically showing low genetic structure relative to nondispersive taxa (Allibone and Wallis, 1993; Bohonak, 1999; Govindaraju, 1988; Nikula *et al.*, 2011; Ward *et al.*, 1994; but see Waters *et al.*, 2013). Additionally, taxa capable of long-distance dispersal (e.g., flighted insects, diadromous fishes, rafting invertebrates) often have substantially broader geographic ranges than those with more limited dispersal ability (e.g., flightless insects, freshwater-limited fishes, nonrafting invertebrates) (e.g., Beck and Kitching, 2007; Donald *et al.*, 2005; Fraser *et al.*, 2010; Lester *et al.*, 2007; McCulloch *et al.*, 2010; McDowall, 2002; Rundle *et al.*, 2002). Ecologically linked taxa, such as host–parasite and substrate–epifauna

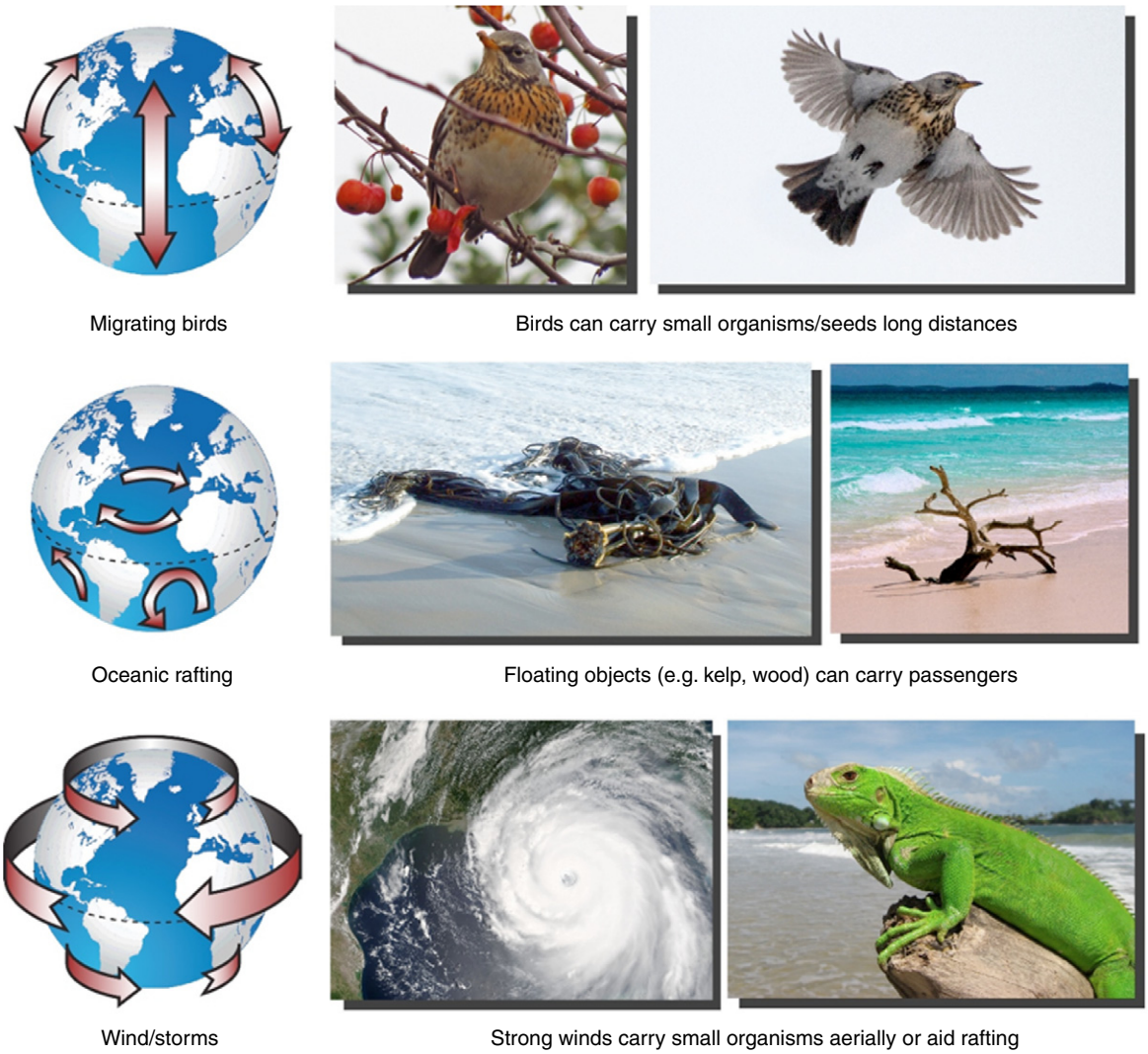

Figure 1 Dispersal of organisms can be mediated by wind, oceanic rafting, and the movement of animals. Migrating birds, for example, can carry seeds, insects, and other small organisms long distances, generally moving north–south or vice versa. Transoceanic dispersal of terrestrial or shallow water marine organisms can be facilitated by rafting on buoyant objects such as kelp, wood, and pumice, and generally follows the paths of ocean currents. Strong winds, such as the easterly equatorial winds and the westerly mid-latitude winds, can transport small organisms aerially or influence rafting events at sea; for example, green iguanas (*Iguana iguana*) were able to colonize a Caribbean island by rafting on driftwood in the path of a hurricane (Censky *et al.*, 1998). Photographs, from left to right, top to bottom, with credits in parentheses: the migratory bird *Turdus pilaris* ingesting seeds (T. Reynolds) and in flight (J. Strzelecki); the buoyant bull kelp *Durvillaea antarctica*, which can transport intertidal organisms across oceans (C. Fraser), and driftwood (Wlavallee); hurricane Katrina (J. Schmaltz, NASA) and a Caribbean iguana on driftwood (Plyco/dreamstime.com).

associations, often show similar dispersal histories (Fraser and Waters, 2013; Nikula *et al.*, 2010). Source and sink regions for dispersal are often predictable based on abiotic features such as glacial refugia (Fraser *et al.*, 2012; Hewitt, 2000), prevailing winds and storm tracks (Gillespie *et al.*, 2012; Monzón-Argüello *et al.*, 2012), and oceanographic features (Ali and Huber, 2010; Collins *et al.*, 2010; **Figure 1**).

Types of Dispersal

Dispersal can be broadly characterized by frequency and distance, with different types generating a range of distinctive genetic signatures. Dispersal that is 'leptokurtic' (with most individuals moving only small distances, but with rare long-distance dispersal), for instance, generates relatively patchy genetic patterns, relative to 'stepping stone' (where individuals only disperse between neighboring populations) (Ibrahim *et al.*, 1996). Genetic signatures of past range changes can be observed thousands of years after the changes occurred, as density-blocking by founders (the first lineages to recolonize an area) can limit the genetic diversity of recolonized versus refugial populations (Hewitt, 1993; Ibrahim *et al.*, 1996; Waters *et al.*, 2013).

Dispersal can also be classified as active or passive. Active dispersal events involve organisms moving to new regions through independent locomotion, such as flight. Passive long-distance dispersal events involve assistance and are usually

achieved via anemochory (dispersal with wind), hydrochory (disperal with water), or epi-/endo-zoochory (dispersal on/in animals) (Nathan *et al.*, 2008). Examples of direct evidence for these various modes of dispersal are provided below.

Transoceanic Dispersal and Island Colonization

Overwater dispersal has played a key role facilitating colonization of oceanic islands (Gillespie and Roderick, 2002), promoting distinctive and rapid radiations (with founder speciation) across geologically dynamic island archipelagos such as Hawaii (Fleischer *et al.*, 1998; Funk and Wagner, 1995; Mendelson and Shaw, 2005; Percy *et al.*, 2008; Shaw, 1996), the Galapagos (Parent *et al.*, 2008; Sequeira *et al.*, 2000), and the Canary islands (Emerson *et al.*, 2000; Juan *et al.*, 2000, 1995).

Long-distance dispersal also appears to have been a key process underpinning the evolution of 'continental' island biotas such as those of New Zealand, New Caledonia, and Madagascar. In New Zealand, a combination of molecular dating (e.g., Burridge *et al.*, 2012; Wallis and Trewick, 2009), geological and paleontological analysis (see Landis *et al.*, 2008; Waters and Craw, 2006) implies that most of the country's extant plant and animal lineages likely established following Oligocene inundation, probably via dispersal from the Australian region. Geological evidence similarly suggests New Caledonia emerged following the Eocene, with subsequent arrival of most of its biota via overwater dispersal (Grandcolas *et al.*, 2008; Neall and Trewick, 2008; Swenson *et al.*, 2014), and there is mounting support for transoceanic dispersal of many terrestrial vertebrates from Africa to Madagascar (Ali and Huber, 2010; Yoder and Nowak, 2006). Even many ratite birds, whose distributions have traditionally been assumed to be solely the result of vicariance, are now thought to have dispersed among landmasses since the breakup of Gondwana (Mitchell *et al.*, 2014).

Wind/Storm Dispersal

Strong winds have been shown to assist dispersal of small organisms such as insects (Compton, 2002) and plant seeds (Horn *et al.*, 2001). Indeed, long-distance, transoceanic aerial dispersal of plant propagules has been inferred to explain connectivity among Southern Hemisphere landmasses separated by thousands of kilometers of ocean (Muñoz *et al.*, 2004). Wind directions and strengths – and therefore dispersal sources and sinks – are often predictable according to geographic region; for example, storms in the north Pacific will normally transport organisms from east to west (Gillespie *et al.*, 2012).

Whereas most wind-assisted dispersal events are of small, easily airborne organisms, the extremely strong winds associated with storms such as hurricanes can also result in long-distance dispersal of larger organisms. For example, in the mid-1990s green iguanas (*Iguana iguana*) (**Figure 1**) colonized a Caribbean island (Anguilla) from a source population more than 200 km away (Censky *et al.*, 1998). The iguanas were first found amongst beached driftwood, shortly after several large hurricanes had passed through the region, and are inferred to have rafted between islands on uprooted trees driven by the strong storm winds (Censky *et al.*, 1998). Iguanas were not previously found on Anguilla.

Dispersal at Sea

Oceanic rafting events have been demonstrated for a range of terrestrial organisms including insects (Heatwole and Levins, 1972), mammals (e.g., rabbit on kelp: Prescott, 1959), and reptiles (e.g., iguanas: Censky *et al.*, 1998). Rafting can also explain the broad distributions of many coastal and marine species that lack inherent dispersal capacity, such as small, slow-moving, or sedentary organisms that brood their offspring or have short-lived larval phases (Donald *et al.*, 2005; Fraser *et al.*, 2011; Haye *et al.*, 2012; Johannesson, 1988; Nikula *et al.*, 2010; Thiel and Gutow, 2005b). Organisms can make use of a range of floating substrata for rafting, including macroalgae, pumice, wood, and man-made objects (see review by Thiel and Gutow, 2005a). The direction and distance of rafting events is driven by ocean currents and winds (**Figure 1**), whereas duration is influenced by the buoyancy and rate of deterioration/consumption of the floating substrate.

Dispersal with Animals

Small organisms such as plant propagules (Wheelwright and Gordon, 1982), insects (McClure, 1990), fungi (Johnson, 1996), parasites, and bacteria (Scott *et al.*, 2001) can be dispersed via the movement of animals. Migrating animals, in particular, can take passengers long distances (Gillespie *et al.*, 2012). Some seeds and parasites can be carried internally (epizoochory; e.g., fruit seeds, liver flukes), whereas others attach externally (endozoochory; e.g., barbed seeds, ticks, mites, moss, and fungal spores) (e.g., seeds: Costa *et al.*, 2014). Migrating birds tend to travel latitudinally (north to south and vice versa), resulting in primarily latitudinal transport of passengers (Gillespie *et al.*, 2012; **Figure 1**).

Dispersal Routes

Dispersal routes are often predictable based on particular oceanographic or geological features. Consistent ocean currents, for instance, may connect isolated populations in predictable ways (Ali and Huber, 2010; Nikula *et al.*, 2010; Gillespie *et al.*, 2012). Similarly, landbridge connections between previously isolated landmasses (e.g., Beringia, Panama) can represent clear pathways for parallel dispersal and range expansion events.

Past and Future Dispersal

Future dispersal trends can be predicted based on climate models, with anticipated range shifts toward the poles or to higher elevations (Chen *et al.*, 2011; Davis and Shaw, 2001; Walther *et al.*, 2002). Large range changes are, however, dependent on a species' ability to disperse, so will vary among taxa. Range changes can often be achieved by local propagule

dispersal, or by small movements of individuals at the edges of ranges, and by rare chance events that shift some individuals longer distances across barriers such as mountains or oceans. For example, intertidal shores of the subantarctic were scoured by sea ice at the last glacial maximum (LGM), causing local extinction of many coastal marine species, but recolonization of these shores by a wide range of species occurred after the ice receded, facilitated by long-distance dispersal, for example, via rafting with floating kelp (Fraser *et al.*, 2009; Macaya and Zuccarello, 2010; Nikula *et al.*, 2010).

Future analyses based on multidisciplinary approaches (e.g., oceanographic modeling and genetics) and emerging technologies (e.g., forensic genetics and remote sensing) promise to further enhance the scientific understanding of biological dispersal.

See also: Adaptation, History of. Biogeography, Marine. Evolutionary Biology, History of. Industrial Melanism, History of. Sociobiology, History of. Symbiosis, History of. Waddington's Epigenetic Landscape, History of

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Distance-Based Phylogenetic Inference

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Glossary

Additive distances A collection of pairwise distances δ_{ij} is additive if there exists a phylogenetic tree whose branch lengths determine distances between leaves coinciding with the δ_{ij}.

Branch lengths The branches in a phylogenetic tree usually have lengths representing the amount of evolutionary change that has occurred between the taxa at their endpoints. When the tree represents the evolution of a set of biological sequences, branch lengths are usually measured in terms of expected number of substitutions per site.

Computational complexity A measure of how a resource (typically time or memory) employed by an algorithm scales with the input data. An algorithm has complexity $O(f(n))$ if the resources grow at most proportionally to $f(n)$, a function of the input size n.

Distance matrix A square matrix, i.e., a table with an equal number of rows and columns corresponding to a set of taxa, where the entry in column i and row j, denoted δ_{ij} represents an estimate of the evolutionary distance between the i-th and the j-th taxon. The matrix is symmetric (i.e., $\delta_{ij}=\delta_{ji}$) and the elements on its diagonal are zero ($\delta_{ii}=0$).

Evolutionary distance A measure of the amount of change or divergence that has occurred between two taxa. When taxa are represented by biological sequences, 'change' is usually taken as the occurrence of substitutions along the evolutionary path connecting the sequences.

Least squares (LS) A general principle from regression analysis to adjust the parameters of a model function to best fit some observed data. In distance-based reconstruction, it is used to fit the branch lengths of a phylogeny to a set of distance estimates. Least squares methods aim to minimize a quadratic function of the differences between the distance estimates and the additive distances determined by the branch lengths. For 'ordinary least squares' (OLS) the goal is to minimize the sum of the squared differences, while for 'weighted least squares' (WLS) this sum is weighted by terms reflecting the variances of the distance estimates.

Minimum evolution (ME) A general distance-based principle, analogous to parsimony, to measure the plausibility of a phylogenetic tree whose branch lengths have been fitted to the data, typically by least squares (see above). If we define the length of a tree as the sum of its branch lengths, then shorter trees are deemed to be more plausible than longer ones.

Molecular clock The assumption of a constant substitution rate across a phylogeny, implying that branch lengths are directly proportional to time.

Molecular phylogenetics The study and reconstruction of phylogenies representing the evolution of molecular sequences, such as those of DNA or proteins.

Nearest neighbor interchange (NNI) A rearrangement of a tree topology swapping the position of two subtrees separated by exactly three branches. For example, one NNI can transform tree ((A,B),(C,D)) into ((A,C),(B,D)) or into ((A,D),(B,C)).

Sequence profile A table describing the general form of a collection of aligned sequences. For each site, the profile specifies the frequency of each possible character (including gaps) at that position.

Statistical consistency In statistics, a method of estimation is statistically consistent if it is guaranteed to converge, with probability 1, toward the correct value of the parameter, as the size of the input sample tends to infinity. In molecular phylogenetics, the central parameter is often taken to be the topology τ of the correct phylogenetic tree, so a method is said to be statistically consistent if the probability of reconstructing a tree of topology τ converges to 1 as the input sequence alignment becomes longer and longer.

Substitution model A probabilistic model describing the occurrence of substitutions in a DNA or protein sequence. Substitutions models are used to estimate the evolutionary distances between pairs of molecular sequences.

Subtree pruning and regrafting (SPR) A rearrangement of a tree topology, which removes a subtree (a clade) and reinserts it elsewhere in the tree topology.

Tree topology The discrete, structural information contained in a phylogenetic tree, besides branch length information.

Ultrametric distances The pairwise distances δ_{ij} are ultrametric if they are additive with respect to a tree whose leaves are all at the same distance from the root. In molecular phylogenetics, distances are approximately ultrametric only when the molecular clock assumption holds.

Introduction

Distance-based phylogenetic reconstruction rests on two steps. First, for each pair of taxa estimate the amount of change that has occurred along the evolutionary path connecting them. Such measure of change is called an 'evolutionary distance,' and in the case of biological (DNA or protein) sequences, it is proportional to the number of substitutions that have taken place in the two lineages since the last common ancestor of the two sequences. Tree inference is then based on these estimates – the goal being to find the tree that best accounts for the estimated distances.

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Using pairwise distances or similarities between taxa is arguably the oldest approach to investigate systematic relationships by means of a computer (Sneath, 1957; Sokal and Michener, 1958). Early work saw a very clear distinction between, on the one hand, hierarchical clustering techniques for taxonomic classification (Sokal and Sneath, 1963), and, on the other hand, optimization-based methods directly aimed at phylogenetic reconstruction (Cavalli-Sforza and Edwards, 1967; Fitch and Margoliash, 1967). These two lines of work eventually converged in the 1980s, leading to the hugely popular neighbor joining (NJ) method (Saitou and Nei, 1987) – which combined algorithmic ideas from classification (e.g., UPGMA, Sokal and Michener, 1958; ADDTREE, Sattath and Tversky, 1977) to optimization principles from phylogenetics (e.g., ME, Kidd and Sgaramella-Zonta, 1971; OLS, Cavalli-Sforza and Edwards, 1967; see below). From NJ onwards, phylogenetics has witnessed a renaissance of distance-based methods, often related to, or inspired by NJ (e.g., FastME, Desper and Gascuel, 2002; FastNJ, Elias and Lagergren, 2009; FastTree, Price *et al.*, 2009).

These methods are still widely used for their computational efficiency, an advantage that makes them particularly suited for the reconstruction of very large phylogenies, or large collections of phylogenies (e.g., for bootstrapping), or to provide a basis for progressive multiple sequence alignments (Larkin *et al.*, 2007), or even to construct initial trees for more sophisticated inference approaches such as those based on maximum likelihood (ML) (Guindon and Gascuel, 2003). More generally, using estimated distances between biological sequences is an obvious answer to cope with the massive datasets generated by the modern, ever faster and cheaper sequencing techniques – as testified by the ongoing success of NJ, which to date remains the most cited algorithm in phylogenetics.

In this article, we outline the main ideas that underlie the methodology for distance-based phylogenetics. We start by explaining the importance of estimating distances that reflect the number of changes that have actually occurred between two taxa, rather than the (smaller) number of differences between them. After describing concisely the task of estimating evolutionary distances, the main focus here is on the methods for tree inference proper, that is, to reconstruct a tree that fits well the estimated distances.

Preliminaries

A phylogenetic tree T over a set of taxa X has two components. First, a tree topology τ, i.e., an unrooted tree with no degree-two nodes, whose leaves are labeled by (and represent) the taxa in X, and whose internal nodes represent putative ancestors of these taxa. The second component consists of positive branch lengths $b(e)$ for every branch e in τ, which represent a measure of evolutionary change occurred along e.

Every phylogenetic tree T determines a collection of *tree distances* d_{ij}^T between the taxa $i,j \in X = \{1,2,\ldots,n\}$ labeling its leaves. They are defined by:

$$d_{ij}^T = \sum_{e \in \tau_{ij}} b(e)$$

where τ_{ij} denotes the set of branches between i and j in τ. In other words, the tree distance d_{ij}^T is the length of the path connecting i and j in T.

Now suppose that, for each pair of taxa $i,j \in X = \{1,2,\ldots,n\}$, an estimate δ_{ij} of their evolutionary distance is obtained. We say that the δ_{ij} are *additive*, if $\delta_{ij} = d_{ij}^T$ for all $i,j \in X$, for some phylogenetic tree T over X. Importantly, when such a tree exists, it is unique (Zaretskii, 1965; Simões Pereira, 1969; Buneman, 1971). Moreover, as we shall see in the following, it is algorithmically easy to reconstruct tree T from its tree distances d_{ij}^T. These observations provide the fundamental idea underlying distance-based methods: if we manage to obtain precise estimates of the distances d_{ij}^T for the phylogenetic tree we seek, then reconstructing this tree is easy.

The word additive comes from the fact that if we could observe a taxon i as it evolves into k at an intermediate stage, and then eventually into j, then the true evolutionary distances between i,j,k must satisfy

$$d_{ij} = d_{ik} + d_{kj} \qquad [1]$$

Distance Estimation

The first, fundamental component of a distance-based method is the definition of 'evolutionary distance.' It is important to understand that not any measure of distance can be adopted for phylogenetic reconstruction: the key requirement is that the parameters that we set out to estimate must be additive in the sense specified above. This ensures that, as the data become more and more abundant and the distance estimates more and more accurate, these estimates will determine the correct phylogenetic tree (Atteson, 1999).

As a consequence, in molecular phylogenetics, where the data are collections of DNA or protein sequences, simply counting the number of differences between each pair of sequences is not acceptable, because of the possibility of multiple substitutions at the same site (see Figure 1). The number of differences, or mismatches, between sequences is sometimes referred to as their 'uncorrected distance.'

A much better approach is to define the distances as (proportional to) the number of substitutions that have occurred between the two sequences, which clearly leads to an additive measure. As this number is unobservable, the general approach is to estimate it using nucleotide or amino acid substitution models. Note that uncorrected distances do not

```
i: GAATACTCAAA
   ||.|.|.|.||
k: GACTGCCCGAA
   ||.|||.||.|
j: GATTGCTCGGA
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Figure 1 Uncorrected distances are not additive. We assume that i, k and j are realizations of the same sequence at three successive times. If distances were defined as the number of differences, we would have $d_{ij}=4<d_{ik}+d_{kj}=7$, contradicting eqn [1], and showing that the distances would not be additive. Note that both d_{ij} and $d_{ik}+d_{kj}$ potentially underestimate the number of substitutions occurred between i and j.

account for unobserved changes – such as multiple substitutions at the same site – and therefore underestimate this number (see Figure 1). We will now describe, in very general terms, the ML approach to solve this estimation problem. The interested reader is referred to more advanced textbooks (e.g., Felsenstein, 2004; Yang, 2006) for a detailed treatment of distance estimation.

Substitution models allow us to calculate a substitution probability matrix $P(d)=(p_{xy}(d))$, where x and y denote nucleotides, amino acids or other biological characters, and $p_{xy}(d)$ denotes the probability that an x becomes a y after evolving along a branch of length d. Note that d is not expressed in units of time. Instead, the rate of substitution models is usually scaled so that d equals the expected number of substitutions per site along a branch of that length. Also recall that π_x denotes the stationary probability of x. It can be defined as $\pi_x = \lim_{d\to\infty} p_{yx}(d)$, and is sometimes estimated using the frequency of x in the sequences being analyzed.

The evolutionary distance between two sequences x and y is estimated on the basis of a pairwise alignment of these two sequences. Denote by x_i and y_i the i-th aligned character of x and y, respectively. Assuming that the substitution model is time-reversible, the likelihood is given by:

$$L(d) = \prod_{i=1}^{m} \pi_{x_i} p_{x_i y_i}(d)$$

where the product is over the m aligned sites. Then, the ML estimate of the distance between x and y is the value of d that maximizes $L(d)$ above, and can be obtained numerically or analytically, depending on the model.

For illustration, we consider the simplest model of nucleotide substitution, the JC model (Jukes and Cantor, 1969). For this model, we have, $\pi_A=\pi_C=\pi_G=\pi_T=1/4$ and, assuming $x\neq y$,

$$p_{xx}(d) = \frac{1}{4}\left(1+3e^{-\frac{4}{3}d}\right), \quad p_{xy}(d) = \frac{1}{4}\left(1-e^{-\frac{4}{3}d}\right)$$

The likelihood is then given by:

$$L(d) = \frac{1}{4^{2m}}\left(1-e^{-\frac{4}{3}d}\right)^{m_{\neq}}\left(1+3e^{-\frac{4}{3}d}\right)^{m-m_{\neq}}$$

where $m_{\neq}$ is the number of mismatches. It is then easy to calculate that $L(\delta)$ is maximized for

$$\delta = -\frac{3}{4}\ln\left(1-\frac{4}{3}\frac{m_{\neq}}{m}\right)$$

That is, the distance estimate δ between the two sequences is a simple, strictly increasing function of the proportion of mismatches – the uncorrected distance we mentioned above. Note that $\delta \geq m_{\neq}/m$, corresponding to the fact that there are more substitutions than observed differences.

Other models cause the ML estimates of the distances to be functions of multiple features of the pairwise alignment, so strictly speaking it is not always accurate to describe distance estimates as transformations of the uncorrected distances. As ML distance estimation is the same as ML phylogenetic reconstruction of a two-taxon tree, the numerical techniques for distance estimation are largely the same as those employed for ML branch length optimization.

Tree Reconstruction

We organize our brief survey of tree reconstruction methods around three well-defined components. Any choice with respect to them defines a possible distance-based method.

C1 Branch Length Estimation: a method to assign lengths to the branches of any fixed tree topology, so that the resulting tree distances are as close as possible to the estimated distances δ_{ij}. This is usually achieved using least squares techniques from regression analysis (Cavalli-Sforza and Edwards, 1967; Fitch and Margoliash, 1967).

C2 What to Optimize: a criterion assigning a score to all the trees of different topologies that can be obtained with component C1, reflecting the biological plausibility of a phylogenetic reconstruction given the estimated distances. An obvious choice for this is the least squares criterion used to assign branch lengths, but as we describe below a lot of recent methodology is based on a different criterion, 'minimum evolution' (ME) (Kidd and Sgaramella-Zonta, 1971). Some methods (e.g., ADDTREE; Sattath and Tversky, 1977) directly optimize topological criteria, and thus bypass C1.

C3 How to Optimize: an algorithm to seek the optimal tree with respect to the criterion in C2. Since this is a computationally hard optimization problem, algorithms are usually heuristic, and based on simple but effective ideas such as stepwise addition, iterative agglomeration, or hill climbing (Swofford *et al.*, 1990; Felsenstein, 2004).

In the following, we start by surveying the methodology for C1 (Section Least Squares Branch Length Estimation). Then we describe one of the most popular criteria for C2, ME (Section Optimization Criteria: ME), which is at the foundation of what is still the best known distance-based method, NJ. We illustrate this algorithm along with other approaches that are based on the same ideas (Section NJ and Related Algorithms). Finally, we briefly describe a few promising approaches which, strictly speaking, are not distance-based methods, but which share with them several ideas and the same emphasis on computational efficiency (Section Beyond Distances).

Least Squares Branch Length Estimation

Given the estimated distances δ_{ij} for all $i,j\in X$, the goal of least squares phylogenetic reconstruction (Cavalli-Sforza and Edwards, 1967; Fitch and Margoliash, 1967) is to find a phylogenetic tree T that minimizes the gap between the estimates δ_{ij} and the tree distances d_{ij}^T, measured in terms of a quadratic function $Q(T)$ of the residuals $\delta_{ij}-d_{ij}^T$. Different statistically motivated choices for $Q(T)$ are possible, and are detailed below. While many versions of this problem have been proven computationally hard (Day, 1987), here we focus on the simpler problem of assigning branch lengths to a tree of fixed topology τ. As we show below, exact, analytic, and polynomially computable solutions are available for this task.

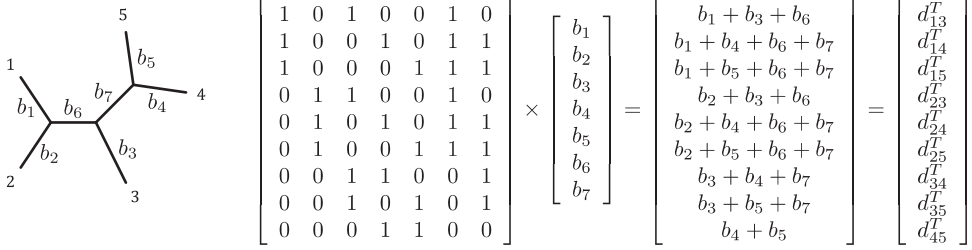

Figure 2 The usefulness of the topological matrix. The tree distances for the 5-taxon tree on the left are expressed here as $A_\tau \boldsymbol{b} = \boldsymbol{d^T}$.

The method of least squares was first introduced in phylogenetics in the mid-1960s. The proposed objective function was

$$Q(T) = \sum_{i<j} w_{ij}\left(\delta_{ij} - d_{ij}^T\right)^2$$

with $w_{ij}=1$ (Cavalli-Sforza and Edwards, 1967) and $w_{ij} = 1/\delta_{ij}^2$ (Fitch and Margoliash, 1967). The former is often referred to as 'ordinary least squares' (OLS), and both approaches are particular cases of 'weighted least squares' (WLS), where various choices are possible for w_{ij}. From a statistical standpoint, the weight w_{ij} represents the degree of confidence that we can attach to the distance estimate δ_{ij}: ideally w_{ij} should be inversely proportional to the variance of δ_{ij}, but in practice setting the weights is a delicate art, because the variances are difficult to evaluate. One particular choice on which we will come back a few times in the following is that of 'balanced' weights – with w_{ij} proportional to $2^{-|\tau_{ij}|}$ ($|\tau_{ij}|$ denotes the number of edges on the path between i and j in τ) – which assigns less confidence to the distances between topologically distant taxa.

WLS approaches ignore the correlations between the distance estimates δ_{ij}, δ_{kl} for different pairs of taxa, which may be significant when the paths τ_{ij} and τ_{kl} share many branches. In order to take these correlations into account, 'generalized least squares' (GLS) (Chakraborty, 1977; Bulmer, 1991) minimizes

$$Q(T) = \sum_{i<j}\sum_{k<l} w_{ij,kl}\left(\delta_{ij} - d_{ij}^T\right)\left(\delta_{kl} - d_{kl}^T\right)$$

where the $w_{ij,kl}$ should be set as the entries of the inverse of the variance–covariance matrix for the distance estimates δ_{ij}. Just as OLS is a particular case of WLS, WLS is a particular case of GLS, obtained by setting $w_{ij,kl}=0$ whenever $\{i,j\} \neq \{k,l\}$. In practice GLS is rarely used for phylogenetic inference, because of the difficulty of evaluating the covariances, and because of its higher computational costs relative to those of OLS and WLS.

The branch lengths that are optimal with respect to the $Q(T)$ criteria above can be simply expressed in matrix notation. To this end, let $\boldsymbol{\delta} = (\delta_{ij})$ denote the distance estimates and $\boldsymbol{d^T} = (d_{ij}^T)$ the tree distances for a tree T, in vector form. Moreover, we represent any topology τ with a binary matrix $A_\tau = (a_{ij,e})$ – whose rows correspond to pairs of taxa $\{i,j\}$ and whose columns correspond to branches of τ – defined by setting $a_{ij,e}=1$ if e is on the path between i and j in τ, and 0 otherwise. Given these notations, we can write

$$\boldsymbol{d^T} = A_\tau \boldsymbol{b}$$

where $\boldsymbol{b} = (b(e))$ denotes the branch lengths of T in vector form. See **Figure 2** for an example illustrating these notations.

The objective functions of OLS, WLS, and GLS can then be written concisely in matrix form:

$$Q(T) = (\boldsymbol{\delta} - A_\tau \boldsymbol{b})^t W (\boldsymbol{\delta} - A_\tau \boldsymbol{b})$$

where $W = (w_{ij,kl})$ contains the GLS weights and the superscript t denotes the matrix transpose. When W is diagonal, the identity matrix, WLS and OLS are obtained, respectively. The branch lengths for τ that minimize $Q(T)$ can then be expressed as:

$$\boldsymbol{b} = \left(A_\tau^t W A_\tau\right)^{-1} A_\tau^t W \boldsymbol{\delta} \qquad [2]$$

The matrix calculations in eqn [2] are computationally expensive, but a number of properties of the matrices involved can be exploited to speed up the solution (Gascuel, 1997a; Bryant and Waddell, 1998). For WLS, computational complexity is dominated by the matrix inversion – or equivalently by linear system resolution – which standard algorithms achieve in $O(n^3)$ time, where we recall that $n = |X|$ denotes the number of taxa. For OLS, the complexity can be reduces further to $O(n^2)$. In fact, the OLS branch lengths can be expressed with simple combinatorial formulae (Vach, 1989, Rzhetsky and Nei, 1993). More recently it was discovered that similar formulae exist for WLS with balanced weights (Desper and Gascuel, 2004), and in fact for a whole new class of special cases of WLS, those with 'multiplicative weights' (Mihaescu and Pachter, 2008).

We conclude by remarking that none of the approaches described above guarantees that the assigned branch lengths are all positive. Least squares branch length estimates are sometimes negative, which does not correspond to any biological reality. Constraining branch lengths to be nonnegative leads to nonnegative least-squares (NNLS) regression (Lawson and Hanson, 1974), an approach that however further increases computational costs.

Optimization Criteria: ME

Once a way to assign branch lengths to any fixed topology has been determined, the next question (C2) is how to measure the plausibility of the trees we obtain. One possibility is to adopt the same least squares criterion $Q(T)$ used for branch lengths, implying that a tree topology is considered plausible if its tree distances can be fitted very closely to the estimated distances. This approach is known to perform best when negative branch lengths are disallowed (Kuhner and Felsenstein, 1994), a constraint that can be imposed in popular

programs such as FITCH (Felsenstein, 1997) and PAUP* (Swofford, 2003).

A different approach – ME – scores a tree using the sum $L(T)$ of the fitted branch lengths: the shorter the tree, the better. The intuition underlying ME is the same as that of maximum parsimony for character-based tree reconstruction: similarly to the general principle that simple explanations are preferable to complex ones, in phylogenetics shorter trees are often considered more plausible than longer ones.

ME can deal with negative branch lengths in a variety of ways: they can be simply excluded from the sum in $L(T)$ (Swofford *et al.*, 1990), or $L(T)$ can be defined as the sum of the absolute values of the branch lengths (Kidd and Sgaramella-Zonta, 1971). To date, the most common approach is to define $L(T)$ as the sum of all branch lengths, irrespective of their sign (Saitou and Imanishi, 1989; Rzhetsky and Nei, 1993), which would seem to favor negative branch lengths, but in practice works quite well.

The first theoretical foundation of ME was provided by Rzhetsky and Nei, who showed that, if the distance estimates are unbiased, and branch lengths are assigned with OLS, then the mathematical expectation of $L(T)$ is minimized for the correct tree topology (Rzhetsky and Nei, 1993). This means that, when the distance estimates equal the tree distances for a tree T – that is when $\boldsymbol{\delta} = \boldsymbol{d}^T$ – then the optimal tree with respect to OLS + ME is T itself. Note that in the following we will use the short form 'C1 + C2' to denote the optimization principle based on a particular choice for components C1 and C2 (see above).

If we assume that $\boldsymbol{\delta}$ converges to $\boldsymbol{d}^T$ as more and more data are available, the result above implies that OLS + ME is 'statistically consistent' – meaning that the probability of reconstructing T (within any given approximation) converges to 1 – which is an essential property of any phylogenetic inference method. Unfortunately ME does not always have this property (Gascuel *et al.*, 2001) – an observation that casts serious doubts on the general applicability of the ME principle in phylogenetics. To date, statistical consistency has been proven to hold for all instances of WLS + ME with multiplicative weights (Pardi and Gascuel, 2012).

One special case of this is WLS + ME with balanced weights, which is also known as BME (Pauplin, 2000; Desper and Gascuel, 2002, 2004). This optimization principle has several interesting mathematical features (Semple and Steel, 2004), including the fact that for fully resolved trees its objective function can be expressed very concisely – and elegantly – as a function of the estimated distances:

$$L(T) = \sum_{i<j} 2^{1-|\tau_{ij}|}\delta_{ij} \qquad [3]$$

BME is of key importance to interpret some of the most central methods in distance-based phylogenetics, including NJ, as we explain below.

NJ and Related Algorithms

NJ is an agglomerative clustering algorithm, that is, it constructs a tree in a bottom-up fashion by alternating the following two steps until the tree is complete:

'Selection step': based on the distances between taxa, choose two 'active' taxa i and j to agglomerate. That is, connect them to a new taxon (ij) representing their direct common ancestor. (Initially all taxa are 'active.')

'Reduction step': remove i and j from the list of active taxa, and define new distances between the new active taxon (ij) and all remaining active taxa.

The two steps above are common to all agglomerative algorithms. For NJ, there are a number of equivalent (Gascuel, 1994) ways to specify the two steps above (Saitou and Nei, 1987; Studier and Keppler, 1988). Here we describe the most efficient computationally (Studier and Keppler, 1988). In the selection step, NJ agglomerates the taxa i and j that minimize

$$q_{ij} = (r-2)\delta_{ij} - \sum_{k=1}^{r}\delta_{ik} - \sum_{k=1}^{r}\delta_{jk} \qquad [4]$$

where the sums run on the set of remaining active taxa, and r is their number. As for the reduction step, the new distance between (ij) and any other taxon k is defined by

$$\delta_{(ij)k} = \frac{1}{2}\left(\delta_{ik} + \delta_{jk} - \delta_{ij}\right)$$

A third step, defining branch lengths for the reconstructed tree, is also usually described for NJ, but here we omit it for simplicity.

Different choices for the two steps above lead to other well-known agglomerative algorithms: single-linkage clustering (Sneath, 1957), average-linkage clustering, also known as UPGMA (Sokal and Michener, 1958), WPGMA (Sokal and Sneath, 1963), ADDTREE (Sattath and Tversky, 1977), UNJ (Gascuel, 1997a), and BIONJ (Gascuel, 1997b). The last method is a special case of the MVR approach (Gascuel, 2000a), which adapts the reduction step above so as to account for the variances and covariances of the distance estimates δ_{ij}. It is similar in spirit to another agglomerative algorithm, Weighbor (Bruno *et al.*, 2000), which also modifies the selection criterion, and uses a different formula (Bulmer, 1991) to evaluate the variances of the distances.

Of the algorithms above, the fastest are single-linkage clustering, UPGMA and WPGMA, as they manage to construct a tree in $O(n^2)$ time (Sibson, 1973; Murtagh, 1984; Gronau and Moran, 2007). However, they are only accurate when the distances are approximately ultrametric (see Glossary), and thus they are little used for phylogenetic inference, where the molecular clock is the exception rather than the rule. Apart from ADDTREE and MVR, which have a time complexity of $O(n^4)$, all other agglomerative algorithms mentioned above reconstruct a tree in $O(n^3)$ time. This includes NJ, where each of the n-3 selection steps is carried out in $O(r^2) = O(n^2)$ time via precalculation of the sums $\sum_{k=1}^{r}\delta_{ik}$ (Studier and Keppler, 1988). One of the reasons for the continued success of NJ is the fact that it has long been considered to achieve a very good tradeoff between reconstruction accuracy and running times.

Recently, a lot of work has gone into crafting computationally efficient implementations of NJ, both in terms of running time and memory usage. These include QuickTree (Howe *et al.*, 2002), QuickJoin (Mailund and Pedersen, 2004), the bucket-based method of Zaslavsky and Tatusova (2008), NINJA (Wheeler, 2009), RapidNJ, and ERapidNJ

(Simonsen *et al.*, 2011). A general idea is to speed up the selection of the pair of taxa that minimizes q_{ij} in eqn [4], by limiting the search to a subset of pairs guaranteed to contain the best pair. Moreover, external (disk) memory is used to cope with large datasets (standard implementations can only deal with a few thousand taxa because of internal memory limitations). Although the worst-case time complexity remains $O(n^3)$ these approaches permit a dramatic improvement in efficiency, allowing the reconstruction of trees with more than 50 000 taxa in a few hours on a normal PC (Wheeler, 2009; Simonsen *et al.*, 2011).

Even faster NJ-like algorithms can be obtained by employing heuristics – instead of exact algorithms – to select the pair of taxa to join on the basis of q_{ij}. These include FastNJ (Elias and Lagergren, 2009) and RelaxedNJ (Evans *et al.*, 2006), which is implemented in Clearcut (Sheneman *et al.*, 2006). In the case of FastNJ, the taxa to join are selected among a list of $O(n)$ pairs, implying a running time of $O(n^2)$. For both methods, some loss of accuracy is to be expected, as in general these approaches do not reconstruct the same tree as NJ. Despite this, like NJ, these methods are statistically consistent, as they are guaranteed to reconstruct a tree T when the input distances are additive with respect to T, or nearly additive (Atteson, 1999; Elias and Lagergren, 2009).

A question that puzzled phylogeneticists for some time is whether NJ is related to any of the optimization criteria we discussed above. It was often suggested that "NJ has some relation to OLS and some to ME, without being definable as an approximate algorithm for either" (Felsenstein, 2004). These connections come from the fact that the original selection criterion q_{ij} can be obtained as a sum of OLS estimates for a certain subset of branch lengths (Saitou and Nei, 1987; Gascuel, 1994). However, if the good performance of NJ were due to its relation to OLS + ME, then we would expect that better (shorter) trees with respect to this criterion would also be phylogenetically more accurate than NJ trees, something that is actually contradicted by experience (Saitou and Imanishi, 1989; Kumar, 1996; Gascuel, 2000b; Desper and Gascuel, 2002).

More recently, it was shown that the real optimization criterion behind NJ is BME (Desper and Gascuel, 2005; Gascuel and Steel, 2006). To briefly illustrate this, we note that the formula $L(T)$ in eqn [3] (which only applies to bifurcating trees) can be generalized to unresolved trees by replacing $2^{1-|\tau_{ij}|}$ by a factor $p(i \to j)$ expressing the probability of ending up in j when following a suitably defined random walk starting at i. The resulting generalized BME formula can then be seen as the guiding principle behind the selection step in NJ: at each of these steps, the agglomeration performed by NJ is the one that results in the tree with the smallest BME length.

Motivated by the observation that NJ can be seen as a greedy algorithm for BME, other methods guided by BME have been proposed (Desper and Gascuel, 2002; Catanzaro *et al.*, 2012). These methods differ from NJ in their choice for component C3, that is, the algorithm to seek the BME-optimal tree. FastME (Desper and Gascuel, 2002; Lefort *et al.*, 2015) implements heuristics including stepwise addition to construct an initial tree, and common tree topology rearrangements (NNI, SPR) to perform a local search in tree space. Although the running time of FastME is comparable to that of NJ, its reconstruction accuracy is superior (Desper and Gascuel, 2002, 2004; Vinh and von Haeseler, 2005), thus confirming the suitability of BME as an optimization principle in distance-based phylogenetics.

Beyond Distances

An important computational bottleneck of all the methods we presented so far is that they require the initial estimation and storage of distances for all pairs of taxa, which takes $O(\ell n^2)$ time – assuming that distances are estimated from sequences of length ℓ – and $O(n^2)$ memory. It is intuitive, however, that not all distances are necessary to reconstruct a phylogeny, meaning that these bounds can be improved. Some distances – those with large variances – may even be misleading for tree inference. Moreover, for large datasets with hundreds of thousands taxa, reducing memory usage may be a necessity: just storing the entire distance matrix for 100 K taxa typically requires 20 GB of memory, which may be problematic for many users.

In the last few years a number of approaches have been proposed to bypass the bottleneck above. The most widely used is FastTree (Price *et al.*, 2009), whose strategy to construct an initial tree is inspired by NJ, but with two fundamental differences – one aimed at improving reconstruction accuracy, and the other for computational efficiency. We now briefly describe the main distinctive points of FastTree, as they underlie its good performance and thus its popularity.

The first difference with NJ and other classical distance-based methods is that FastTree stores 'sequence profiles' for the active taxa (see Glossary) – instead of a distance matrix – and only computes the distance between two profiles if the corresponding pair is a candidate for joining. After each agglomeration, the sequence profile for the new node joining i and j is computed as the arithmetic average of the profiles for i and j. The pair of taxa to join is selected on the basis of a distance-based criterion formally similar to eqn [4], but where distances are uncorrected and defined on the basis of the profiles stored for the active taxa. The second difference consists of maintaining a list of $O(\sqrt{n})$ 'top-hits' for each taxon (i.e., putative closest neighbors), which is combined to the strategies of FastNJ (Elias and Lagergren, 2009) and RelaxedNJ (Evans *et al.*, 2006) to reduce the pairs of taxa to consider for agglomeration: at the end of its execution FastTree will only have considered $O(n^{1.5}\log n)$ pairs – as opposed to $O(n^3)$ for NJ, and $O(n^2)$ for FastNJ. The NJ-like reconstruction of an initial tree – with a claimed complexity of $O(\ell n^{1.5}\log n)$ time and $O(\ell n + n^{1.5})$ memory – is then followed by a local search based on common tree topology rearrangements (NNI, SPR), using techniques similar to those implemented in FastME (Desper and Gascuel, 2002).

FastTree is faster and more memory-efficient than the distance-based methods discussed so far, and it can easily cope with datasets with hundreds of thousands of sequences. Given the central role of sequence profiles, it can be argued that FastTree is not a distance-based method. It shares ideas with with character-based methods such as parsimony, benefitting in particular from information about ancestral sequences – something that is not available to purely distance-based methodology.

Other methods that arguably lie beyond the frontier of distance methods – but which share with them several ideas and the same emphasis on computational efficiency – have recently been proposed by Brown, Truszkowski, and collaborators (Truszkowski *et al.*, 2012; Brown and Truszkowski, 2012). Particularly promising as a basis for future methodology is LSHTree (Brown and Truszkowski, 2012), which like NJ proceeds in a bottom-up fashion by joining subtrees – although not necessarily at their roots. To do so, it uses 'locality-sensitive hashing' exploiting ancestral sequence reconstructions, to rapidly find candidate pairs of close sequences to join. Just as FastTree, the running time of LSHTree is sub-quadratic.

Conclusion

Despite the simplicity of their approach, and the loss of information that is necessarily entailed by summarizing sequence data into a numerical matrix, distance-based methods are not just computationally efficient, but also remarkably accurate. The reconstruction accuracy of distance-based methods has been the subject of many simulation studies in the past (e.g., Saitou and Imanishi, 1989; Kuhner and Felsenstein, 1994; Kumar, 1996; Gascuel, 2000b; Nakhleh *et al.*, 2002; Desper and Gascuel, 2004). The general idea of these studies is to generate sequences using standard substitution models and known model trees, and then compare the trees reconstructed by a number of competing methods to the model trees employed to generate the sequences. From these works it transpires that although not comparable to that of likelihood-based methods, the reconstruction accuracy of distance-based methods is competitive with that of maximum parsimony (MP) with MP superior for trees with short branches, and inferior when the effect of multiple substitutions at the same site becomes important. The reason for this is largely intuitive: while distance-based methods naturally account (or 'correct') for multiple substitutions in the way they estimate distances, MP does not even model branch lengths, leading to serious problems such as statistical inconsistency in extreme cases (Felsenstein, 1978). Another important conclusion that empirical studies have helped to reach is the importance of improving the tree reconstructed initially, via topological rearrangements such as NNI and SPR (e.g., Vinh and von Haeseler, 2005). This is why most modern tree reconstruction methods include this important step, as we have seen for FastME (Desper and Gascuel, 2002) and FastTree (Price *et al.*, 2009) in a distance-based context.

Another important advantage of distance-based methods is their versatility: they can be employed not just with sequence data, but in every context where pairwise comparisons are possible. For example, they have been used to infer phylogenies from morphological characters (Sokal and Michener, 1958), immunological data (Sarich and Wilson, 1967), gene frequencies (Cavalli-Sforza and Edwards, 1967), DNA–DNA hybridization data (Sibley and Ahlquist, 1984), and more recently from gene content (Snel *et al.*, 1999) or gene order (Wang *et al.*, 2006) within genomes. Another area where distance methods may prove useful is phylogenomics, where large collections of genomic alignments may be summarized into multiple distance matrices. Their combined analysis may provide an efficient alternative to traditional supertree and supermatrix approaches (Lapointe and Cucumel, 1997; Criscuolo *et al.*, 2006).

See also: Bayesian Phylogenetic Methods. Maximum Likelihood Phylogenetic Inference. Molecular Evolution, Models of. Parsimony Methods in Phylogenetics. Phylogenetic Invariants

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Divergence and Diversification, Quantitative Genetics of

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Glossary

Adaptive landscape G.G. Simpson's phenotypic adaptive landscape which can be conceptualized as a surface describing the relationship between a population's mean values for a set of continuous traits and relative fitness (cf. Wright's adaptive landscape, in which population fitness is a function of allele frequencies).

Additive genetic variation The variance in breeding values (mean genetic values of offspring for a given population) for a quantitative trait in a population. Additive genetic variation is the component of genetic variation that leads to resemblance between parents to offspring and is therefore most relevant for evolutionary change.

Brownian motion A simple continuous-time stochastic model often used to model quantitative trait evolution. The model describes the random movement of trait means through phenotypic space.

Constraints hypothesis The hypothesis that patterns of divergence among lineages are constrained by the amount and structure of genetic variation within populations, with divergence being limited by genetic constraints along certain axes of trait space.

Correlational selection Selection that favors combinations of traits rather than acting on traits independently.

Demographic constraints A demographic constraint is an inability for a population to maintain a viable population size while approaching an adaptive peak. Thus, a demographic constraint on adaptation occurs when the population becomes extinct before the peak is reached, despite some additive genetic variation in the direction of selection.

Diversifying selection Natural selection that drives divergence and increases phenotypic variation among populations or lineages.

Evolvability A measure of the amount of additive genetic variation in a trait or along an axis of multivariate trait space. Formally, evolvability is the additive genetic coefficient of variation (Houle, 1992).

Genetic constraints Insufficient genetic variation in the direction of selection to produce an evolutionary response. An absolute genetic constraint exists if no genetic variation exists along a certain axis of trait space.

Key innovations Novel traits that allow or trigger diversification and/or divergence of phenotypes and often accompany adaptive radiations and the colonization of novel niches.

M-matrix A matrix describing the variance and covariance of mutational effects of new mutants affecting a set of phenotypic traits in a population.

Phenotypic integration A term used to describe the state of having a set of correlated phenotypic traits, generally integrated together as a functional unit. Phenotypic de-integration then represents the loss of genetic correlations that allows traits to evolve independently from one another in the population.

Qst A measure of population differentiation that describes the partitioning of quantitative genetic variation in continuous traits within and among populations.

Stabilizing selection Natural selection that favors intermediate values and selects against extreme values.

Wright's F-statistic A measure of population differentiation that describes the partitioning of genetic variation within and among populations.

Introduction

Disentangling the evolutionary processes that shape the divergence and diversification of lineages requires an understanding of the inheritance of quantitative traits. While quantitative genetics provides a powerful and effective framework for understanding short-term response to selection and genetic drift (Lande, 1979; Lande and Arnold, 1983), it is less clear how the complex interplay between selection, standing genetic variation, mutation, migration, and demographic stochasticity results in realized patterns of divergence and diversification among lineages over longer evolutionary timescales (Arnold *et al.*, 2001). This article examines to what extent the properties of quantitative genetic variation within populations can inform our understanding of the evolutionary processes influencing lineage divergence and diversification over 'macroevolutionary' time. Our goal is not to predict the exact course of evolution in natural populations over such scales, as this is likely infeasible; instead, we seek to understand whether and how the variational properties in evolving populations bias or constrain divergence and diversification.

As described in previous articles, the structure of genetic variation within populations can be summarized for quantitative traits by the genetic variance–covariance matrix (the G-matrix, Lande, 1979). The G-matrix describes the set of genetic constraints present in a population (Arnold, 1992). The amount of additive genetic variation for a given phenotypic trait determines a population's response to selection (Lande, 1979; Lande and Arnold, 1983) and the rate at which variation among lineages accumulates in response to genetic drift (Lande, 1976). Furthermore, the genetic covariation between traits resulting from pleiotropy and linkage disequilibrium determines correlated responses to selection that can skew the trajectory away from the direction of selection (Lande, 1979; Schluter, 1996).

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The G-matrix is therefore a key parameter for predicting patterns of evolution for specific evolutionary models and, more generally, for understanding whether genetic constraints bias patterns of divergence among lineages. Furthermore, when phenotypic traits are tied to reproductive or ecological isolation, quantitative genetic variation may influence the process of speciation and help explain disparate patterns of diversification across lineages. Here we address the effect of quantitative genetic variation on among-lineage evolutionary patterns at three distinct levels: (1) Can the observed divergence patterns among lineages be attributed to a specific evolutionary process (e.g., drift or selection)? (2) Does the structure of genetic variation bias patterns of phenotypic divergence across lineages? (3) Does the amount of genetic variation within or among populations affect speciation and diversification?

Is Divergence Among Populations the Result of Drift or Selection?

The quantitative genetic model of genetic drift provides a neutral model of phenotypic evolution (Lande, 1976; Lynch, 1990) in much the same manner as Kimura provided a neutral expectation for molecular evolution (Kimura, 1968; Kimura, 1983). Just as Kimura's model allows for tests of selection from gene sequences, the neutral model of quantitative genetics generates predictions for patterns of divergence expected under genetic drift. Significant deviations from this expected pattern provide a useful tool for detecting adaptive trait differentiation and determining the nature of selection.

A neutral expectation for phenotypic divergence of a single character under genetic drift can be obtained by first assuming constant genetic variation. Each generation drift erodes genetic variation, while mutation (and, potentially, migration) increases genetic variation, resulting in mutation–drift equilibrium (Phillips *et al.*, 2001). Assuming that the effective population size remains constant, genetic drift is expected to shift the phenotypic mean of the population each generation at a rate proportional to the additive genetic variation in the population, and inversely proportional to effective population size (N_e, Lande, 1976). Under such a model of drift, divergence among lineages is expected to evolve via Brownian motion and the variance among replicate population's mean phenotypes is expected to increase linearly with time. One can also relax the assumption of constant genetic variation, since the amount of genetic variation itself is expected to depend on N_e. Specifically, Lynch and Hill (1986) showed that at mutation–drift equilibrium, genetic variance is expected to approximately equal $2\,V_m N_e$, where V_m is the mutational variance added each generation. Under these assumptions, effective population size cancels out when predicting divergence among replicate populations, and variance increases at a rate proportional only to the mutational variance.

These models allow rough estimates for the amount of divergence expected under neutrality in typical natural populations. Lynch (1990) and later Estes and Arnold (2007) showed that most macroevolutionary divergence between species occurs at a rate that is substantially slower than what is expected under genetic drift. Comparing across a wide range of paleontological divergence estimates, Lynch (1990) found that only hominid divergence in cranial capacity fell within the range predicted by genetic drift. Of course, this does not indicate that hominid cranial capacity is a neutrally evolving trait, but rather shows that most macroevolutionary divergence is considerably slower than predictions from genetic drift, indicating that stabilizing selection is prevalent in nature (but see Hansen and Houle, 2004). This observation is consistent with the paleontological observation of stasis (Eldredge and Gould, 1972; Gingerich, 1983; Estes and Arnold, 2007; Uyeda *et al.*, 2011), or little-to-no accumulation of evolutionary change over long timescales (whereas genetic drift predicts steady increase in divergence over time).

Similarly, neutral expectations for patterns of divergence for multiple, covarying traits can be modeled under drift as a multivariate Brownian motion process. Estimates of the G-matrix and effective population sizes can be used to generate predictions for the amount of divergence among lineages. This provides a null expectation for the amount of divergence that is consistent with genetic drift (Rogers *et al.*, 2002; Marroig and Cheverud, 2004). In addition to predicting the amount of expected divergence among populations, it is also possible to test whether correlations among lineages' multivariate trait values reflect within-population genetic covariance between traits (McGuigan *et al.*, 2005). Building on these methods, Hohenlohe and Arnold (2008) developed a phylogenetic comparative approach for testing whether among-species patterns of divergence are consistent with expectations under genetic drift, as well as evaluating whether the pattern of covariation is consistent with within-population structure. This latter question is discussed in more detail in the Section Does Genetic Architecture Bias the Direction of Evolution (or Vice Versa)?

Frequently, it is difficult to obtain reliable estimates of relevant parameters (e.g., effective population size, time since divergence) to make specific predictions about the amount of divergence under drift. Furthermore, the assumptions necessary to make direct predictions are frequently violated in natural populations (e.g., no migration). Studies below the species level in natural populations commonly encounter complex population histories of gene flow, population fission, and fusion. These processes greatly complicate direct prediction of expectations under genetic drift using quantitative genetic models. An alternative approach is to compare the amount of phenotypic divergence to divergence at putatively neutrally evolving genetic loci (Wright, 1951; Lande, 1992; Spitze, 1993). This approach compares Wright's F-statistic for a neutral genetic locus with an analogous quantity for a quantitative trait (Qst). Both quantities describe how variation is partitioned within and among populations. A value of 0 is indicative of no divergence among populations in allele frequencies, while the theoretical maximum value of 1 would indicate that all variation is partitioned between populations (with no variation shared between populations). Under the neutral model, Qst is expected to behave as a single neutrally evolving molecular locus (Whitlock, 2008). Assessing significance of a given value of Qst can be obtained by comparing its value to the distribution of Fst for neutrally evolving loci (Whitlock, 2008; Whitlock and Guillaume, 2009). A Qst significantly higher than Fst would indicate a phenotypic trait

under diversifying selection, while a Qst significantly lower than Fst would indicate stabilizing selection. Multivariate extensions of Qst–Fst tests allow exploration of the relationship between orientation and direction of divergence in quantitative traits within and among populations (Kremer *et al.*, 1997; Chenoweth *et al.*, 2008).

Empirical studies reveal that at the relatively short timescales of population differentiation, it is quite common for phenotypic traits to exhibit diversifying selection (Merilä and Crnokrak, 2001; Leinonen *et al.*, 2013). Given that the traits under study are often assumed to be ecologically relevant, it is perhaps unsurprising that these traits evolve and adapt to local conditions faster than neutral expectation. However, note that this is in contrast to macroevolutionary comparisons of trait divergence relative to neutrality that were discussed above, which typically show rates of phenotypic divergence slower than predicted under drift. This disparity likely reflects a difference in timescales. By analyzing a large database of divergence estimates collected from generational to macroevolutionary timescales, Estes and Arnold (2007) showed that empirical estimates of phenotypic divergence in body-size related traits across a large range of taxa are generally larger than neutral expectation at microevolutionary and population divergence timescales. However, at longer timescales divergence lags behind neutral expectation. These differences underscore that traits such as body size are not likely to evolve neutrally for very long, but rather, have long-term dynamics driven by the evolution of adaptive landscape (Hansen, 2012).

Does Genetic Architecture Bias the Direction of Evolution (or Vice Versa)?

Under most circumstances, complex phenotypic traits that are closely tied to organismal fitness are not expected to be selectively neutral. A more interesting question is whether within-population genetic variance and covariance biases the pattern of phenotypic divergence among lineages. This so-called constraints hypothesis posits that the pattern of divergence among lineages will reflect the evolutionary constraints present within populations (Schluter, 1996).

Schluter (1996) conjectured that divergence among lineages proceeds, at least temporarily, along 'lines of least resistance' in phenotypic space. In particular, he posited that the 'genetic line of least resistance' – or the combination of traits with the most available genetic variation in a population – would bias divergence to occur primarily in this direction. Mathematically, this axis is described by the leading eigenvector of the G-matrix, or g_{max} . The hypothesis may be viewed from two sides. First, it may be viewed as a hypothesis about genetic constraints, in which divergence is limited because of low levels of genetic variation, particularly along multivariate axes of trait variation (**Figures 1(a)** and **(b)**; Arnold, 1992; Blows and Hoffmann, 2005; Walsh and Blows, 2009). On the other side of the coin, one may view genetic covariance among traits as not limiting divergence, but rather by positively channeling divergence along lines of least resistance (**Figure 1(c)**; Lande, 1979; Gould, 1989; Brakefield, 2006; Eroukhmanoff, 2009; Agrawal and Stinchcombe, 2009). Patterns of divergence among lineages can be described in a manner analogous to within-population divergence, via the divergence matrix (D-matrix, a variance–covariance matrix of phylogenetically-weighted lineage-specific phenotypic means; Lande, 1979; Blows and Higgie, 2003). Schluter's model predicts that the D-matrix and the G-matrix will be proportional, providing a test for whether the genetic architecture of populations affects the structure of phenotypic diversification. Indeed, under both drift and selection, genetic covariance will bias both divergence generated by genetic drift and the short-term selection response of covarying traits (**Figures 1(a)–(d)**).

Empirical tests of the constraints hypothesis commonly find a relationship between multivariate axes of high genetic variance and divergence (Schluter, 1996; Mitchell-Olds, 1996; Chenoweth *et al.*, 2010; Hohenlohe and Arnold, 2008; Hansen and Houle, 2008; Blows and Higgie, 2003; Bégin and Roff, 2004; McGuigan *et al.*, 2005; Bolstad *et al.*, 2014). A smaller but still notable number of studies report a lack of a relationship (Merilä and Björklund, 1999; Chenoweth *et al.*, 2008; Kimmel *et al.*, 2012). Even so, a number of statistical and methodological issues have yet to be fully addressed. Bolstad *et al.* (2014) argue that the majority of these statistical issues will weaken the observed relationship between g_{max} and divergence, and that few studies can be claimed to convincingly demonstrate a lack of effects of genetic constraints on patterns of divergence. Furthermore, a strict alignment between d_{max} (the leading eigenvector of the D-matrix) and g_{max} is not necessary to validate the more general hypothesis that genetic constraints bias long-term evolutionary trends, as phenotypic differentiation may occur along one of many axes with higher than average genetic variance and evolvability (Hansen and Voje, 2011; Bolstad *et al.*, 2014).

Of course, G-matrices themselves evolve over time (Turelli *et al.*, 1988; Roff, 2000; Steppan *et al.*, 2002). However, the G-matrix must be relatively constant over the timeframe of divergence in order to make quantitative predictions. While it is a certainty that the G-matrix will evolve over time, the overall structure of genetic constraints may often remain relatively constant over the timescales of interest (Bégin and Roff, 2004). However, exceptions do occur, and some closely related populations or species have diverged substantially in g_{max} (Berger *et al.*, 2013). Ultimately, the constancy of the G-matrix over long timescales is an open empirical question.

The constraints hypothesis suggests that lineages are constantly chasing their adaptive optima, or under weak selection. Otherwise, biases induced by trait covariance are expected to be temporary so long as the constraints are not 'absolute constraints' (all eigenvalues of the G-matrix are nonzero) and populations are given sufficient time to reach an adaptive peak. In fact, many studies find an abundance of genetic variation along all axes of variation (Mezey and Houle, 2005; Walsh and Blows, 2009). Strong directional selection is also frequently observed (Hereford *et al.*, 2004; Siepielski *et al.*, 2009; Kingsolver and Diamond, 2011). In combination, these two patterns predict near instantaneous adaptation to adaptive peaks (relative to macroevolutionary time) and would seem to suggest limited potential for genetic constraints to shape divergence (Hansen and Houle, 2004). Naively, these patterns suggest that among-lineage patterns of divergence may be driven primarily by the dynamics of adaptive peaks over time,

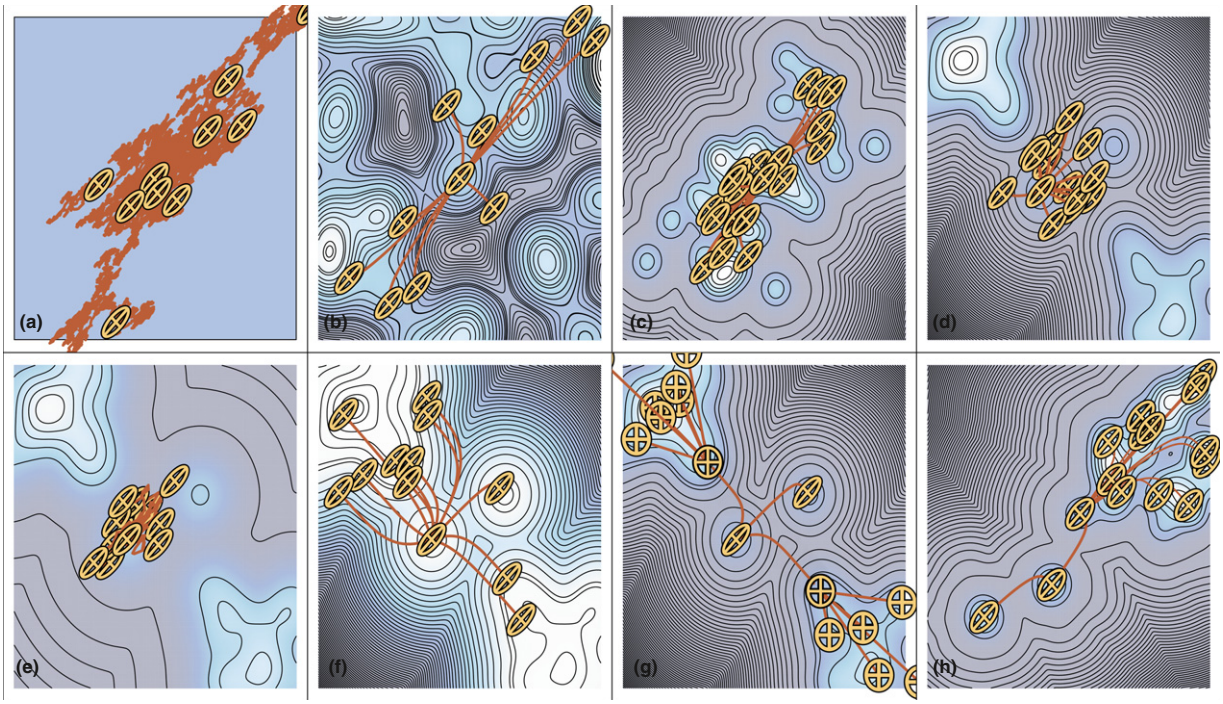

Figure 1 Conceptual figure depicting scenarios depicting the relationship between the adaptive landscape and the G-matrix. The G-matrix is indicated by the orange ellipses, which represent the 95% confidence interval for the bivariate distribution of genetic values in the population. X- and Y-axes indicate mean values for two phenotypic traits, while the contour lines and color indicates mean fitness of the population. Note that we cannot depict spatially or temporally varying adaptive landscapes in only two dimensions. Hence, we show here adaptive peaks varying across a landscape, but in a given population, we assume a single adaptive peak. Trajectories therefore illustrate paths between the ancestral adaptive peak (center) and novel adaptive peaks in surrounding regions. (a) Genetic drift – A flat adaptive landscape allows diverging species to freely explore phenotypic space. Divergence is biased along genetic lines of least resistance. (b) Randomly oriented adaptive peaks – In this scenario, no region of the adaptive landscape is favored over any other. However, peaks along g_{max} are reached more quickly, leading to greater divergence along this axis. Nevertheless, given sufficient time for selection, all peaks will reach their optimum. Thus, the correspondence between g_{max} and d_{max} erodes over time. Nevertheless, if demographic constraints prevent species from reaching distant peaks along constrained axes of variation, a correspondence between g_{max} and d_{max} may remain. (c) Positive constraints – This scenario depicts so-called 'positive constraints,' where the correlation between traits channels variation in directions likely to be adaptive in novel habitats. (d) Negative constraints – This scenario depicts an ill-formed G-matrix. High fitness 'adaptive ranges' are located nearby, but approach to these peaks is slow, and combined with demographic constraints, prevents populations from colonizing these areas of phenotypic space. Scenarios (e)–(h) depict different types of ecological release and key innovations leading to an increase in phenotypic divergence. (e) Decrease in stabilizing selection – In accordance with the hypothesis of Yoder *et al.*, 2010, stabilizing selection can decrease the valleys between adaptive peaks and thus allow lineages to traverse to novel adaptive peaks. However, this occurs at a slower rate due to weaker selection favoring novel peaks. This scenario comes to resemble genetic drift. (f) Increase in absolute fitness – Demographic constraints may prevent species from reaching distant peaks. Therefore, an increase in absolute fitness in accordance with ecological release can allow lineages to traverse fitness valleys to new adaptive peaks without risking extinction. In this scenario, relative fitness remains the same, and thus divergence proceeds at the same rate as in (d), but with a relaxation of demographic constraints. (g) Loss of phenotypic integration – Key innovations are thought to allow the rapid differentiation of species into novel regions of phenotypic space. Here a loss of genetic constraints and phenotypic integration allows lineages to more rapidly colonize novel adaptive peaks which were always present and accessible, but could not be reached due to genetic constraints. (h) Novel area of phenotypic space – This type of key innovation can result simply from a shift in the mean that allows access to novel habitats with an abundance of adaptive peaks. This is typical when, for example, a species invades a novel niche or is introduced to a novel bioregion.

with most populations residing close to their adaptive optima. If we accept this view, the primary determinant of evolutionary divergence among lineages is the long-term dynamics of adaptive peaks themselves with the specifics of genetic architecture playing little role in the pattern of divergence (Simpson, 1944; Arnold *et al.*, 2001; Hansen, 2012). Alternatively, the trajectory of evolution may still be biased by the G-matrix if the landscape has multiple peaks, and local peaks in certain directions of phenotypic space are favored over others, leading to biased exploration of the adaptive landscape (Hine and Blows, 2006; McGuigan and Blows, 2007).

What, then, explains the commonly observed relationship between the G-matrix and the D-matrix? The dual observation of abundant genetic variation in most measured directions and the existence of strong directional selection is not a death blow to the constraints hypothesis. Multivariate genetic constraints with unmeasured traits experiencing stabilizing selection may limit adaptation while simultaneously allowing limited but rapid short-term evolutionary responses (Walsh and Blows, 2009; Hansen, 2012; Bolstad *et al.*, 2014). This latter pattern is widely observed for phenotypic divergence across timescales, which find that although divergence is often quite rapid in the short

term, it is bounded and does not accumulate until much longer, million-year timescales (Uyeda *et al.*, 2011). Such a pattern may arise by considering the concept of 'conditional evolvability' (Hansen and Houle, 2008), which is defined as the amount of available genetic variation along the vector of selection that is uncorrelated to variation along perpendicular axes (i.e., the amount of evolutionary change possible in a given direction without change in other traits). Because only a small subset of traits are typically measured, this may mean that conditional evolvabilities are considerably lower than evolvabilities measured in natural populations (Bolstad *et al.*, 2014). Furthermore, genetic constraints need not be absolute if populations are constrained by finite population sizes. If populations are maladapted and off their fitness optima, they may incur demographic costs and decrease in population size. Even temporary lags resulting from insufficient genetic variation could impose 'demographic constraints' on adaptation, preventing colonization of peaks along axes of low evolvability despite an absence of absolute genetic constraints (Gomulkiewicz and Houle, 2009).

Alternatively, alignment between the G-matrix and the D-matrix may result secondarily from the evolution of the G-matrix to conform to the axis of divergence and the shape of the adaptive landscape. Thus, rather than genetic architecture biasing divergence, divergence along adaptive ridges in phenotypic space may shape genetic architecture to orient g_{max} along so-called selective lines of least resistance (Arnold *et al.*, 2008). Indeed, simulation studies have shown that the G-matrix evolves as a balance between the shape and orientation of the adaptive landscape – which may be skewed by correlational selection – and the shape and orientation of the mutation matrix (M-matrix), which describes the phenotypic effects of new mutations that enter into the population (Jones *et al.*, 2003, 2007, 2012). Directional selection and migration between populations can likewise orient the G-matrix along the axis of divergence between populations by introducing alleles with correlated effects on phenotypic traits – albeit weakly and with transient effects (Guillaume and Whitlock, 2007). Finally, recent simulation studies suggest that the M-matrix itself can evolve, especially in the presence of epistatic interactions between genes, to conform to the shape of the adaptive landscape, resulting in triple alignment between the M-matrix, G-matrix, and the axis of divergence among populations (Jones *et al.*, 2007; Hether and Hohenlohe, 2014; Jones *et al.*, 2014).

These alternative explanations are not mutually exclusive and may be reinforcing in natural populations. While this general condition of alignment seems likely under many circumstances, instances of misalignment between the G-matrix and the D-matrix certainly do occur. Furthermore, it seems likely that genetic constraints are underestimated in natural populations. Nonetheless, understanding to what extent the genetic architecture drives patterns of divergence remains an outstanding question in scaling between microevolutionary processes and macroevolutionary patterns.

Does Genetic Architecutre Affect Rates of Diversification?

Until now, we have focused on phenotypic divergence and ignored speciation and diversification. Yet, a vast disparity exists between rates of diversification among clades. Furthermore, radiations can both encompass dramatic diversifications in phenotype, as well as 'cryptic radiations' in which species diversify without phenotypic change. To what extent does the structure of quantitative genetic variation within a species affect these patterns of diversity and phenotypic divergence?

Under some evolutionary processes, the structure and amount of genetic variation can play a key role in driving phenotypic divergence in traits important for reproductive isolation. For example, quantitative genetic models of sexual selection predict that amounts of genetic variation for sexual preference and ornament traits under a variety of circumstances can affect the rate of evolution of reproductive isolation (Lande, 1981; Mead and Arnold, 2004; Gavrilets, 2000; Gavrilets and Hayashi, 2005; Uyeda *et al.*, 2009). Similar arguments can be made for other types of coevolution, so long as a mechanistic link exists between phenotypic divergence and the evolution of reproductive isolation (Felsenstein, 1981; Kiester *et al.*, 1984, but see Yoder and Nuismer, 2010).

Adaptive radiations characterized by large amounts of phenotypic divergence and lineage diversification have long captured the attention of evolutionary biologists. This correspondence between lineage diversification and phenotypic divergence in these clades suggests a potential role for changes in genetic architecture, or of the adaptive landscape, in driving these patterns. Yoder *et al.* (2010) argue that ecological opportunity drives these patterns and can be conceptualized as a weakening of the strength of stabilizing selection, allowing populations to traverse fitness valleys more easily (**Figure 1(e)**). However, such a model is somewhat inconsistent with the seemingly rapid pace of adaptation in many adaptive radiations, as weakened stabilizing selection results in pattern akin to expectations under genetic drift rather than the rapid, niche-based divergence of lineages typically envisaged in such radiations (**Figure 1(a)**). Alternatively, 'ecological release' of populations can be defined as an overall increase in the absolute fitness of populations, resulting in relaxation of demographic constraints while still maintaining stark differences in relative fitness among peaks and strong selection (cf. Wellborn and Langerhans, 2014; **Figure 1(f)**).

Adaptive radiations are sometimes attributed to 'key innovations' that allow colonization of previously unattainable niches. Two types of key innovations are possible: (1) an innovation resulting from novel phenotypic values (**Figure 1(h)**) and (2) a change in the genetic architecture that removes genetic constraints (i.e., a loss of phenotypic integration, **Figure 1(g)**). This latter type of innovation is of primary interest to the subject of this article, as it would indicate that the potential for adaptive radiation may be predicted from genetic architecture of trait variation. A change in the effective dimensionality of trait covariance can affect the overall rate of phenotypic divergence along novel axes of phenotypic space (Nosil and Sandoval, 2008). If these traits are related to mechanisms of speciation and diversification, adaptive radiation may result. This is because higher-dimensionality in trait variation results in more axes along which divergence can occur, and sexual isolation can result if the trait is tied to reproductive isolation (Hohenlohe and Arnold, 2010; Kemp, 2007; Wainwright, 2007; Doebeli and Ispolatov, 2010). For example, Maia *et al.* (2013) showed key innovations in

pigmentation cells resulted in an increase in dimensionality of color phenotypes and are simultaneously correlated with both increased rates of phenotypic evolution and diversification. Nonsexually selected key innovations with de-integrated phenotypes have met with more equivocal effects on diversification (Alfaro *et al.*, 2009; Frédérich *et al.*, 2014). Instead, a common pattern is for the increase in diversification to lag behind the evolution of the key innovation or require a sequence of innovations (Alfaro *et al.*, 2009; Near *et al.*, 2012; Sanderson and Donoghue, 1996; Kemp, 2007). These patterns suggest that diversification rate shifts likely result from a shift into novel regions of the adaptive landscape itself (**Figure 1(g)**), rather than changes in genetic architecture directly influencing diversification rates (**Figure 1(h)**; Wainwright, 2007).

Conclusion

Bridging the divide between micro- and macroevolution requires understanding the degree to which the structure of genetic variation influences macroevolutionary patterns of divergence and diversification among lineages. Quantitative genetics provides a means to generate quantitative and empirically testable predictions that help disentangle the evolutionary processes of drift and selection in explaining divergence. Furthermore, the evolutionary constraints hypothesis posits that among-lineage patterns of divergence are constrained by patterns of genetic variation within lineages. In general, an emerging picture is one in which divergence among lineages generally correlates with within-lineage patterns of trait variation. Naive extrapolation of measured levels of genetic variation, however, appear inconsistent with absolute constraints on divergence. Nevertheless, genetic constraints may still play a role and empirical estimates of evolvability may be affected by unobserved trait correlations limiting divergence in certain directions. Additional empirical research is also necessary on the evolution of genetic architecture itself in a comparative framework to establish the degree to which divergence G-matrix itself evolves among lineages and in response to patterns of selection (Steppan *et al.*, 2002).

While the degree of integration among phenotypes has long been suggested to impact rates of species diversification, relatively few macroevolutionary tests of the effect of phenotypic integration on rates of diversification have been conducted. Increased dimensionality and evolvability for some phenotypic traits may increase diversification rates. However, as with phenotypic diversification, the relationship may be more complex than naive extrapolation would indicate. Rather, the specific means by which patterns and processes can be reconciled across scales and macroevolutionary time require significant further exploration.

In general, connecting 'microevolution' (the variational properties of populations) and 'macroevolution' (the divergence and diversification of lineages) remains a challenging but fertile research avenue. The strong predictive power of quantitative genetic models combined with increasing availability parameter estimates obtained from empirical studies provides a powerful framework for pursuing these important goals.

See also: Macroevolution, Quantitative Genetics and. Multivariate Quantitative Genetics

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E

Ecological Evolutionary Developmental Biology

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Glossary

Canalization The situation that arises when a trait is 'fixed' or expressed constitutively regardless of normal changes in the environment.

Cryptic genetic variation Variation that is expressed only under atypical conditions.

Developmental plasticity See 'phenotypic plasticity.'

Differential gene expression The situation that arises when different genes are activated, or expressed, in different cell types or organisms; nearly always accompanies 'phenotypic plasticity.'

Ecomorphs (morphs) Alternative phenotypes induced by the environment that occupy different ecological niches.

Enhancer It is involved in 'differential gene expression'; the region of a gene (i.e., a sequence of DNA) that binds 'transcription factors'; the resulting complex interacts with the gene's 'promoter,' thereby activating the gene and beginning transcription.

Epigenetics Any mechanism of development that generates phenotypic variation without altering the base-pair nucleotide sequence of DNA; phenotypic variation that involves changes in the expression of genes rather than their sequence.

Epigenetic inheritance Heritable phenotypes that are not encoded by the genome.

Genes as followers The hypothesis that the most important role of genetic mutations in evolution may be to contribute not so much to the origin of phenotypic novelties as to the pool of genetic variation that makes 'genetic accommodation' possible.

Genetic accommodation A mechanism of evolution wherein a novel phenotype, generated by either a mutation or environmental change, is refined into an adaptive phenotype through quantitative genetic changes; can result in either increased or decreased environmental sensitivity of an induced phenotype; when environmentally induced phenotypes lose their environmental sensitivity, they undergo 'genetic assimilation.'

Genetic assimilation An extreme form of 'genetic accommodation' that occurs when environmentally induced phenotypes lose their environmental sensitivity over evolutionary time and become 'canalized' or expressed constitutively.

Lamarckianism The hypothesis, attributable to the French naturalist Jean-Baptiste Lamarck (1744–1829), which holds that an organism can pass on characteristics that it acquired during its lifetime to its offspring.

Maternal effects The situation that arises when a female's phenotype influences its offspring's phenotype, independent of the direct effects of the female's coding sequences on its offspring's phenotype.

Phenotypic plasticity The ability of an individual organism to change its phenotype in direct response to stimuli or inputs from the environment (often used synonymously with 'developmental plasticity').

Promoter The region of a gene (i.e., a sequence of DNA) that binds the enzyme RNA polymerase, thereby activating the gene and beginning transcription (see also 'enhancer' and 'transcription factor').

Reaction norm A graphical representation of the sensitivity of a group of organisms of the same genotype to some specific environmental variable.

Transcription factor A protein that binds to a specific DNA sequence, thereby activating the gene and beginning transcription (see also 'promoter' and 'enhancer').

Introduction

Ecological evolutionary developmental biology ('eco-evo-devo') seeks to understand how interactions between an organism's genome and environment shape its development, and how such environmentally responsive development, in turn, impacts ecology and evolution (Sultan, 2007; Gilbert and Epel, 2009). This focus on environmentally contingent development is a departure from how developmental and evolutionary biology have been studied in the past. For example, until relatively recently, developmental biology has focused on a few species ('model organisms') reared in uniform environments. This approach has fostered the view that environmentally contingent development is rare or unimportant. Similarly, evolutionary biology has traditionally regarded environmental responsiveness as simply

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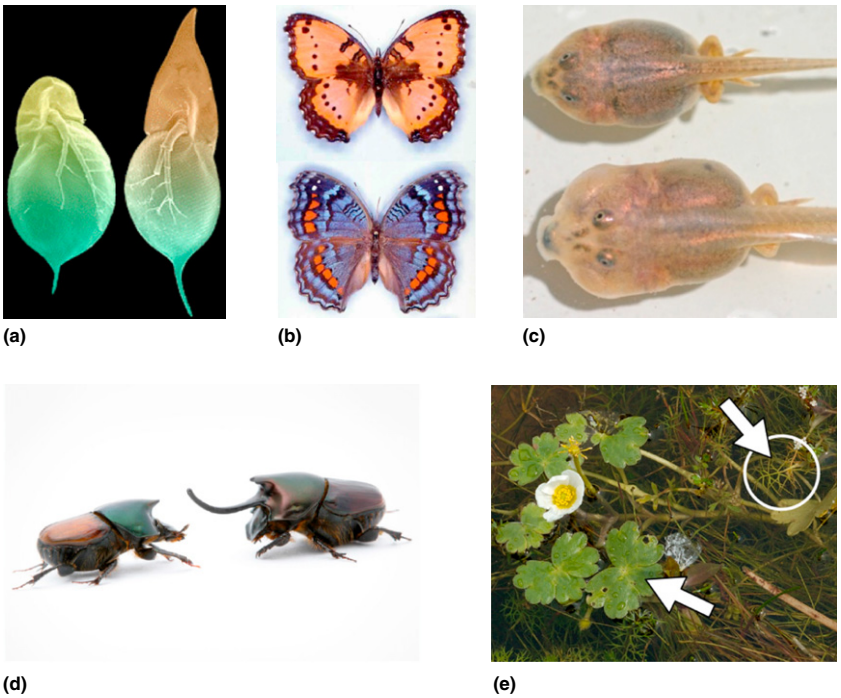

Figure 1 Examples of environmentally contingent development (i.e., 'phenotypic plasticity'). (a) Normal (left) and predator-induced (right) morphs of water fleas, *Daphnia cucullata*; (b) wet-season (top) and dry-season (bottom) gaudy commodore butterflies, *Precis octavia*; (c) omnivore (top) and carnivore-morph (bottom) spadefoot toad tadpoles, *Spea multiplicata*; (d) small-horned (left) and large-horned (right) dung beetles, *Onthophagus nigriventris*; (e) broad, aerial leaves and narrow, submerged leaves (circled) on the same water crowfoot plant, *Ranunculus aquatilis*. Reproduced with permission from Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., *et al.*, 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution 25, 459–467.

developmental 'noise' that has no long-term evolutionary consequences (Orr, 1999). Indeed, the architects of the modern synthesis of evolutionary biology explicitly cho se not to incorporate environmentally responsive development into the field's conceptual framework, partly as a reaction against Lamarckianism (reviewed in Pigliucci, 2007).

Yet, it has become increasingly apparent that an organism's environment can profoundly alter its phenotype (**Figure 1**). Moreover, it has even been suggested that such environmentally initiated phenotypic change might often precede, and even facilitate, genetic evolution and therefore plays a key role in generating biodiversity. Additionally, a growing body of evidence has revealed that environmentally induced phenotypic change can be transmitted between generations, which challenges our basic assumptions of how inheritance works (reviewed in Jablonka and Lamb, 2010; see also below).

This article explores these issues in greater detail. As described below ongoing research in eco-evo-devo promises to provide fresh insights into the evolutionary process.

Phenotypic Plasticity

For much of the twentieth century, genes were the dominant paradigm for explaining biodiversity. Yet, scholars have long suspected that genes alone do not determine which traits organisms produce (Bonduriansky, 2012). Indeed, it is now abundantly clear that the environment is a normal and necessary agent in phenotype production (Schlichting and Pigliucci, 1998; Gilbert and Epel, 2009). Nowhere is this point more clearly illustrated than by the widespread occurrence of phenotypic plasticity (Nijhout, 2003; West-Eberhard, 2003).

'Phenotypic plasticity' (often used synonymously with 'developmental plasticity') is the ability of an individual organism to change its phenotype in direct response to stimuli or inputs from the environment (sensu West-Eberhard, 2003). As it turns out, many (perhaps all) organisms can alter their phenotype in response to a diverse array of environmental factors, such as temperature, nutrition, light, pressure, or gravity, and the presence of predators, parasites, or competitors (Gilbert and Epel, 2009). For instance, temperature can influence phenotype production, because nearly all enzyme activity is temperature-dependent (**Figure 1(b)**); food often contains potent chemical signals that induce phenotypic change (**Figure 1(d)**); light can stimulate plants to produce different-shaped leaves and shoots (**Figure 1(e)**); and pressure can cause muscle and bone to grow differently. Moreover, predators can release chemicals that induce defenses in their prey (**Figure 1(a)**) and competitors can cause stress (which releases stress hormones) and alter resource abundance (and thereby an individual's nutritional state), which can trigger an alternative phenotype (**Figure 1(c)**). Regardless of the precise environmental factor, a feature common to all the above is that phenotypic plasticity is nearly always accompanied by changes in gene expression (Gilbert and Epel, 2009; e.g., see **Figure 2**).

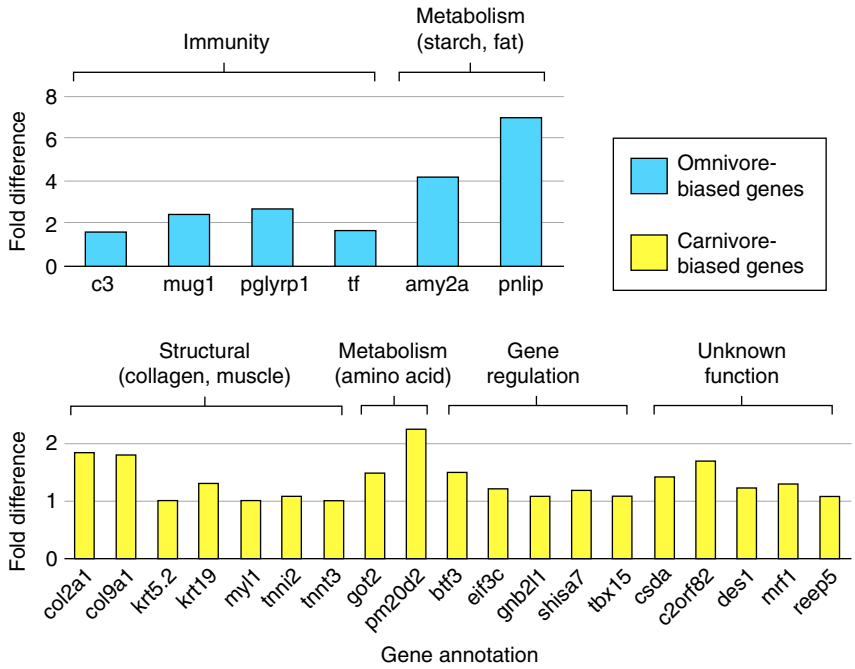

Figure 2 Differential gene expression in alternative, environmentally induced, larval ecomorphs of spadefoot toads (genus *Spea*). Depending on their diet, spadefoot tadpoles develop into either an omnivore morph, which is a dietary generalist, or a distinctive, large-headed carnivore morph, which specializes on shrimp and other tadpoles (see **Figure 1(c)**). Shown are fold differences (a measure of the change in expression level of a particular gene) for each of six genes expressed at significantly higher levels in omnivores than in carnivores and 19 genes expressed at significantly higher levels in carnivores than in omnivores; above each gene is its putative function (where known). Data from Leichty, A.R., Pfennig, D.W., Jones, C.D., Pfennig, K.S., 2012. Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. Intergrative and Comparative Biology 52, 16–30.

Considerable effort has gone into identifying the molecular mechanisms of differential gene expression and, thus, of phenotypic plasticity (Gilbert and Epel, 2009). One model (based on studies of how different cells in a multicellular organism that share the same genome can assume different shapes/functions) is that differential gene expression is mediated by the differential binding of proteins called 'transcription factors' to a gene's enhancer region. To understand how this process works, consider that for a gene to be activated (specifically, for RNA polymerase to attach to a gene's promoter and begin transcription), RNA polymerase must be held in place on the promoter site. Transcription factors play a key role in stabilizing RNA polymerase on the gene's promoter. Importantly, different transcription factors bind different enhancers and thereby alter expression of different genes. Moreover, which transcription factors are present in any particular cell can be influenced by signals from outside the cell (e.g., in the organism's external environment). Thus, one proposed mechanism of phenotypic plasticity is that the neuroendocrine system transduces sensory information from the external environment by recruiting different transcription factors, thereby activating different genes (Nijhout, 2003) which, in turn, produce different gene products (e.g., see **Figure 2**) and ultimately, different phenotypes (**Figure 1**).

Other mechanisms can alter phenotype production, however. For instance, gene expression changes can be induced directly, as when bacteria in an animal's gut induce changes in the expression of intestinal genes (Gilbert and Epel, 2009). Future research is needed to identify additional molecular mechanisms of phenotypic plasticity.

Phenotypic Plasticity and Evolution

Although phenotypic plasticity is commonplace, its evolutionary significance remains controversial (Pfennig *et al.*, 2010; Moczek *et al.*, 2011). On the one hand, evolutionary biologists have long held that plasticity has no relevance for the evolutionary process other than to perhaps impede it by dampening the effects of selection (reviewed in Schlichting, 2004). On the other hand, several prominent, early evolutionists, such as Weismann, Goldschmidt, Schmalhausen, and Waddington, maintained that phenotypic plasticity plays a central role in the origins of new traits and phenotypic differences between species (Jablonka and Lamb, 1995).

Indeed, for over a century, researchers have hypothesized that environmentally induced phenotypic change might facilitate genetic evolution and thereby fuel the origins of new, ecologically relevant traits (Baldwin, 1902; Schmalhausen, 1949 [1986]; Waddington, 1953; West-Eberhard, 2003). Although various mechanisms have been proposed (dubbed the 'Baldwin effect,' 'genetic assimilation,' 'stabilizing selection,' and 'genetic accommodation'), all such mechanisms assume that: (1) environmentally induced phenotypes evolve first; and (2) selection favors those phenotypes (whether induced or not) that are the most adaptive (West-Eberhard, 2003).

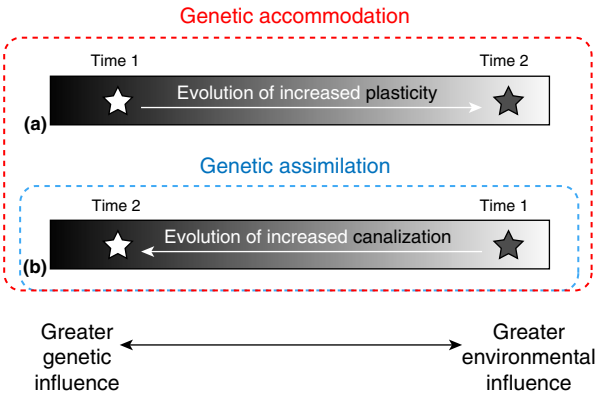

Figure 3 A diagram illustrating the distinction between genetic accommodation and genetic assimilation. Genetic accommodation is any adaptive genetic change in the regulation and form of a phenotype. For example, a trait may evolve either (a) increased or (b) decreased environmental sensitivity (i.e., phenotypic plasticity). The complete loss of phenotypic plasticity (i.e., increased canalization) is an extreme form of genetic accommodation known as genetic assimilation.

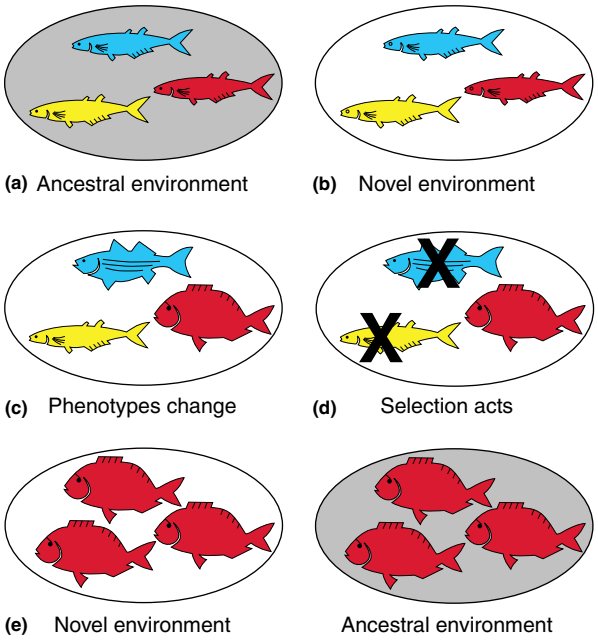

Figure 4 Phenotypic plasticity, along with genetic accommodation/assimilation, may facilitate the evolution of a new, canalized trait through the following steps (here, different fish shapes are alternative ecomorphs; different colors are different genotypes). (a) A genetically variable population (b) experiences a novel environment (indicated here as a change from a shaded to an unshaded background). (c) Consequently, novel phenotypes are induced by the environment, but different genotypes respond differently. (d) Selection disfavors those genotypes that produce maladaptive phenotypes in the new environment (indicated here by an 'X'). (e) Such selection may result in the evolution of a novel, canalized trait (e.g., a novel ecomorph) that is expressed regardless of the environment (i.e., even if the environment changes back to the original, ancestral state).

According to one prominent version of this theory (West-Eberhard, 2003), when selection acts on quantitative genetic variation regulating the expression of an initially environmentally induced trait, it can promote the evolution of either increased or decreased plasticity through an evolutionary process known as 'genetic accommodation' (**Figure 3**). If the affected trait evolves decreased plasticity to the point of becoming constitutively expressed, 'genetic assimilation' occurs (sensu Waddington, 1953; **Figure 3**). The outcome of genetic assimilation is a novel, canalized trait (i.e., a trait that is 'fixed,' or expressed constitutively regardless of normal changes in the environment).

To illustrate this process, imagine a population that experiences a novel environment (**Figure 4**). Individuals in such populations often cope with stress associated with unfamiliar environments by producing new phenotypes through phenotypic plasticity (Badyaev, 2005). If such an induced trait improves fitness, then selection will favor alleles or genotypes that enhance the trait's expression (different genotypes within the same population often vary in the degree to which they respond to any particular environmental stimulus; Gupta and Lewontin, 1982). As these alleles/genotypes accumulate in the population, individuals may express the trait without the original environmental stimulus; i.e., genetic assimilation occurs. In this way, a novel canalized trait emerges from a trait that was originally environmentally induced.

With genetic accommodation/assimilation, the origin of a complex new trait need not require the evolution of new genes. Instead, selection acts on existing genes and converts a plastic trait into a canalized trait via an evolutionary adjustment in the regulation of trait expression (West-Eberhard, 2003). A plausible mechanism whereby such an adjustment could occur is through changes in gene expression (Gilbert and Epel, 2009; Renn and Schumer, 2013; see **Figure 5**). Recall from above that gene expression is typically environmentally sensitive, and that changes in gene expression often (possibly, always) underlie phenotypic plasticity. If selection is persistent and coarse-grained (i.e., individuals encounter only one selective environment), then induced differences in gene expression may evolve to become less environmentally responsive until they become fixed (e.g., via allelic substitutions at regulatory loci; Grishkevich and Yanai, 2013).

Although genetic accommodation can occur whether a novel trait is mutationally or environmentally induced, environmentally triggered novelties likely have greater evolutionary potential than mutationally induced ones for at least two reasons. First, changes in the environment often impact many individuals simultaneously (e.g., consider how exposure to sudden cold temperatures or a new competitor or predator can affect a population). By contrast, a new mutation initially affects only one individual (and its immediate descendants). The widespread impact of environmental change enables a newly induced trait to be tested in diverse genetic backgrounds, thereby increasing the chances that genetic accommodation will occur (see above). Second, an environmentally triggered novelty is automatically associated with a particular environmental situation – the one that induced it. Therefore, such traits are more subject to consistent selection and

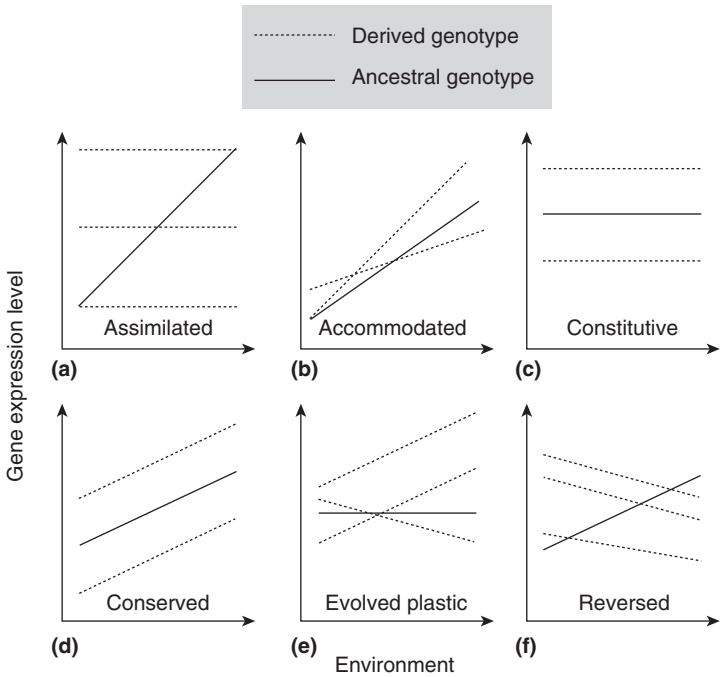

Figure 5 Alternative patterns by which gene expression might evolve. Each panel depicts the 'reaction norms' of ancestral and derived genotypes (a reaction norm is a graphical representation of the sensitivity of a group of organisms of the same genotype to some specific environmental variable). Redrawn, with permission from Renn, S.C.P., Schumer, M.E., 2013. Genetic accommodation and behavioural evolution: Insights from genomic studies. Animal Behaviour 85, 1012–1022.

directional modification than are mutationally induced novelties, for which expression is more likely to be random with respect to the environment.

Phenotypic plasticity also promotes the accumulation of 'cryptic genetic variation' – variation that is expressed only under atypical conditions (Paaby and Rockman, 2014) – that makes genetic accommodation possible. Phenotypic plasticity facilitates the build up of such variation, both because the effects of novel genetic variants are buffered by compensatory plastic responses (Moczek, 2008), and because environment-specific genes experience relaxed selection in the non-inducing environment (Lahti *et al.*, 2009; Leichty *et al.*, 2012). This accumulation of cryptic genetic variation can lead to further phenotypic novelty when it is revealed through a subsequent change in the environment or genome. Such unmasking of standing genetic variation may play an important role in fueling evolutionary changes (Moczek *et al.*, 2011).

Thus, the most important role of genetic mutations in evolution may be to contribute not so much to the origin of phenotypic novelties as to the pool of genetic variation that makes genetic accommodation possible. Or, as West-Eberhard (2003, p. 158) put it, genes may be 'followers, not necessarily leaders, in phenotypic evolution.'

Although laboratory studies have demonstrated that genetic accommodation can occur (Waddington, 1953; Rutherford and Lindquist, 1998; Suzuki and Nijhout, 2006), relatively little is known about whether this process is responsible for ecologically and evolutionarily relevant traits in natural populations (but see Schlichting and Wund, 2014). Generally, if plasticity has preceded – and facilitated – the evolution of a particular novel trait, then there should be evidence that: (1) ancestral species express the trait only conditionally; (2) novel environments uncover cryptic genetic variation; and (3) trait expression has been refined in derived species. A number of examples appear to satisfy these conditions (**Table 1**). Moreover, a recent meta-analysis has uncovered several convincing cases in which genes appear to be 'followers' in the origins of novel traits (Schwander and Leimar, 2011). Additionally, there is evidence that genetic accommodation might even play a role in speciation: there are numerous examples in which a formerly plastic trait has undergone canalization (i.e., lost its plasticity) in a particular lineage, and such shifts are typically accompanied by speciation (Schwander and Leimar, 2011). Yet, additional tests from natural populations are needed to evaluate plasticity's role (if any) in speciation.

Phenotypic Plasticity and Ecology

Phenotypic plasticity also has important implications for ecology, which, in turn, have additional evolutionary consequences. Recall from above that an organism's phenotype is shaped by its ecology. Yet, the reciprocal is also true: an organism's ecological interactions – and thus the selective regimes that it experiences – can be influenced by its developmental responses.

For instance, the individuals of many species respond adaptively to interspecific competition by facultatively modifying their resource-use traits through phenotypic plasticity (Pfennig and Pfennig, 2012). Species that can alter their phenotype in this way may persist in the face of novel

Table 1 Possible examples of genetic accommodation in natural populations of animals. For possible examples in plants, see Schlichting and Wund (2014)

Organism	*Trait(s) undergoing adaptive evolution*	*Reference(s)*
Water fleas (*Daphnia melanica*)	Pigmentation	Scoville and Pfrender (2010)
Stickleback fish (*Gasterosteus aculeatus*)	Benthic and limnetic ecomorphs	Wund *et al.* (2008)
Stickleback fish (*Gasterosteus aculeatus*)	Growth rate	Robinson (2013)
Spadefoot toads	Adult body/leg length and larval duration	Gomez-Mestre and Buchholz (2006)
Spadefoot toads (genus *Spea*)	Carnivore and omnivore ecomorphs	Ledón-Rettig *et al.* (2008); Pfennig and Martin (2010)
Australian tiger snakes (*Notechis scutatus*)	Head size	Aubret and Shine (2009)
House finches (*Carpodacus mexicanus*)	Sex determination and sex-specific resource allocation	Badyaev (2009)

competitive interactions because they can immediately (i.e., within a single generation) switch to a selectively favored phenotype. In the absence of such plasticity, species may be driven locally extinct through competitive exclusion (Pfennig and Pfennig, 2012). Thus, environmentally responsive development can reduce extinction risk, thereby influencing the composition of ecological communities (similar arguments can be made regarding the ability to respond rapidly to any sort of change in an organism's biotic or abiotic environment). Of course, populations that do not go extinct should ultimately be more likely to diversify. This may explain, at least in part, why clades in which conspicuous phenotypic plasticity (e.g., **Figure 1(c)**) has evolved are more species rich than sister clades lacking such plasticity (Pfennig and McGee, 2010).

Epigenetic Inheritance and Evolution

Lastly, research in eco-evo-devo is changing our view of inheritance, and indeed, our very definition of evolution. Because inheritance is a prerequisite for evolution to occur, clarifying how inheritance works has long been a goal of evolutionary biology (Bonduriansky and Day, 2009). Generally, most evolutionary biologists assume that inheritance occurs exclusively through alterations in the base-pair nucleotide sequence of genes; by contrast, phenotypic changes induced directly by the environment are assumed to be incapable of being inherited and to therefore play no role in mediating evolutionary change (Futuyma, 2013). Indeed, evolution is typically defined as change in a population's genes (Futuyma, 2013).

Yet, a growing body of evidence has conclusively shown that various extra- (or epi-)genetic factors that influence phenotype production – and that were initially induced directly by changes in the organism's environment – can be transmitted from one generation to the next (Jablonka and Raz, 2009). Such transmissible 'epigenetic' changes (Gilbert and Epel, 2009) thereby constitute a form of inheritance – distinct from that based on changes in DNA base-pair sequence – known as 'transgenerational epigenetic inheritance' (Jablonka and Lamb, 2010).

Transgenerational epigenetic inheritance is underlain by two main mechanisms. The first involves 'chromatin marking,' which occurs when small chemical groups are added to the DNA strand. For instance, for RNA polymerase to attach to a gene's promoter and begin transcription, the DNA strand must be unwound and untangled from the proteins that surround it (this DNA/protein complex is the 'chromatin'). The addition of a methyl group (CH_3) condenses the chromatin more tightly (Gilbert and Epel, 2009), which can prevent RNA polymerase from finding the promoter, thereby inactivating the affected gene. Importantly, the addition of a methyl group to a DNA strand can be triggered by a change in the organism's environment (Gilbert and Epel, 2009, pp. 43–46). Once a gene has been inactivated in this manner, the gene's inactivated state can be inherited when special enzymes – DNA methyltransferases – recognize a methylated sequence on the parent strand and then methylate the same region on the newly synthesized daughter strand (Gilbert and Epel, 2009).

Thus, the altered state of activation of a gene – as well as any changes to the phenotype – can thereby be transmitted across generations in the absence of any changes in base-pair nucleotide sequence of the DNA. Although the degree to which such epigenetic marks mediate the transmission of ecologically relevant traits across generations is unclear, there are some compelling examples (reviewed in Jablonka and Raz, 2009). Moreover, because chromatin marking mediates inheritance of cellular epigenetic variants (e.g., different cell types within a multicellular organism), the potential for transgenerational epigenetic inheritance is therefore present in all multicellular organisms (Maynard Smith and Szathmáry, 1995).

The second major mechanism of transgenerational epigenetic inheritance involves soma-to-soma transmission of epigenetically based variations (Jablonka and Lamb, 2010). Soma-to-soma transmission encompasses many different processes, all of which entail reconstructing the parental phenotype during somatic development in successive generations without direct involvement of the germline. Three main (non-mutually exclusive) processes mediate such soma-to-soma transmission.

First, phenotypes can be inherited via a 'maternal effect.' Maternal effects arise when a female's phenotype influences its offspring's phenotype, independent of the direct effects of the female's coding sequences on its offspring's phenotype. For instance, in many animals, larger females produce larger young, simply because they produce larger eggs, more milk, and/or have larger wombs. Subsequently, the large daughters of these large females may perpetuate the trend of producing

large offspring (Jablonka and Lamb, 2010). Likewise, females of many species differentially endow their seeds, eggs, or offspring with acquired information or materials (e.g., RNA transcripts, cytoplasm, and hormones) that can influence their offspring's phenotype (Mousseau and Fox, 1998). These effects may endure for many generations, even persisting long after the original environmental stimulus that created the maternal effect disappears (Kirkpatrick and Lande, 1989). Moreover, such maternal effects have been shown to mediate the inheritance of divergent phenotypes in natural populations, often in a manner indistinguishable from a phenotypic shift stemming from a change in DNA base-pair nucleotide sequence (e.g., Badyaev *et al.*, 2002; Pfennig and Martin, 2009).

Second, soma-to-soma transmission of epigenetic variants can occur via transmission of symbionts (smaller organisms that live inside a host organism as parasites or mutualists). Many symbionts alter their host's phenotype, and are transmitted primarily or even exclusively from parent to offspring (Werren *et al.*, 2008). Differential transmission of symbionts may mediate phenotypic divergence between populations and possibly even speciation, especially when different symbionts are incompatible within the same host (Brucker and Bordenstein, 2013).

Third, soma-to-soma transmission of epigenetic variants can occur via learning. For example, in animals, feeding (Papaj and Prokopy, 1989), mating (Crews *et al.*, 2007), and habitat (Slagsvold *et al.*, 2013) preferences can be transmitted from parent to offspring exclusively through learning. The transmission of fitness-enhancing information via learning may play an important role in both ecology and evolution by facilitating speciation and reducing extinction risk (Beltman *et al.*, 2004; Verzijden *et al.*, 2012).

Note, however, that the long-term stability of epigenetically based variations – whether mediated by chromatin marks or soma-to-soma transmission – remains uncertain. Unlike with DNA replication – where accuracy is largely insensitive to the environment – the stability of epigenetic changes depends on an organism's current environment; a change in the environment can modify, and even reverse, epigenetically based variations (Jablonka and Lamb, 2010). Yet, even if most epigenetic variants are found to last only a few generations, epigenetic inheritance mechanisms may still be important if they decrease the chances of extinction and/or increase the likelihood of genetic changes (Pfennig and Servedio, 2013).

Concluding Remarks

Eco-evo-devo is an emerging field that seeks to understand the causes and consequences of a common feature of development – its tendency to be responsive to changes in an individual's environment. Research in this field has at least three important implications for evolutionary biology. First, as described above, an understanding of the interconnectedness between an organism's environment and its developmental responses can illuminate how the environment not only selects among diverse phenotypes, but how it also creates those phenotypes in the first place (essentially, the environment can dictate both the 'survival' as well as 'arrival of the fittest'; Gilbert and Epel, 2009). Second, this phenotypic plasticity might play a critical, and often underappreciated, role in initiating evolutionary innovation and diversification. Finally, such phenotypic change induced by the environment can form the basis of an alternative inheritance system, which might – by itself – mediate evolutionary change. This shift in emphasis on environmentally initiated phenotypic change in evolution should not be seen as a threat to modern evolutionary theory (Orr, 1999); rather, it represents an opportunity to expand the theory (Pigliucci, 2007; Pfennig *et al.*, 2010).

Additional studies are required, however, especially those utilizing new model organisms (Collins *et al.*, 2007). Ideally, these new models would include related groups of species whose phylogenetic relationships are resolved; whose ecology is well known; that experience diverse ecological (and thus, selective) regimes; that display different levels of phenotypic plasticity; and that are amenable to experimental manipulation (e.g., Emlen, 2000; Ledón-Rettig and Pfennig, 2011). Using such new model organisms, greater effort is especially needed to ascertain: (1) the conditions and frequency with which phenotypic plasticity facilitates, rather than impedes, evolution; (2) the degree to which phenotypic plasticity impacts large-scale (i.e., macroevolutionary) change; and (3) the long-term stability and efficacy of epigenetically based variations in mediating evolution. Answers to these and other questions in eco-evo-devo research promise to continue to provide fresh insights into the evolutionary process.

See also: Developmental Plasticity and Phenotypic Evolution. Epigenetic Inheritance. Epigenetics and Genome Evolution. Genotype-by-Environment Interaction. Maternal Effects. Robustness and Evolvability in Molecular Evolution. Waddington's Epigenetic Landscape, History of

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Ecological Fitting and Novel Species Interactions in Nature

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Glossary

Adaptive syndrome An organism's long-term adaptation and response to various biotic and abiotic stresses in the environment, such as pathogenic infection and temperature.
Ecophysiological equivalence In evolutionary terms, the sharing of important traits, such as immunity or morphology, in allopatric species that exhibit close phylogenetic affinity. In fitting equivalence is important because it enables one species to exploit novel resources that nevertheless are similar to resources on which they coevolved.
Phenotype-environment matching Phenotypic variation in traits such as body color, morphology, and behavior in a species that provide adaptation to local conditions in the habitat.

Introduction

The theory of coevolution underpins our understanding of interspecific interactions in nature (Ehrlich and Raven, 1964; Rosenzweig, 1973; Abrams, 1986; Marrow and Cannings, 1993; de la Peña *et al.*, 2011). Moreover, it has provided an essential link that connects disparate fields in biology, such as organismal behavior, community ecology, and studies of larger scale ecosystem-related processes such as productivity, stability, and resilience (Levin, 1999). Many classic studies on coevolutionary arms races and adaptive syndromes have been based on interactions between plants and insect herbivores (Ehrlich and Raven, 1964; Benson *et al.*, 1975; Berenbaum and Zangerl, 1992; Pilson, 1996; Janz and Nylin, 1998; Becerra *et al.*, 2009), as well as between herbivorous insects and their natural enemies, such as predators and parasitic wasps (Godfray, 1994). These and other studies have demonstrated that host–parasite interactions are often based on long and intimate periods of coevolution (Carius *et al.*, 2001).

One of the drawbacks of coevolutionary theory, however, is that it leads investigators to assume a long history of tight, reciprocal selection between contemporaneously interacting species. In contrast, it is now well established that many organisms interact quite diffusely in both space and time, and some interspecific relationships have little or no coevolutionary history (hence invocation of the term evolutionary 'hotspots' (Thompson, 2005) to describe this phenomenon). As an example, some herbivores feed on many closely related plant species in nature but are rarely found on any individual plants of a given species at a given time. When this happens, selection can be weak in one respect (at the level of interactions between the herbivore and any individual plant), but highly reliable in other respects (at the species or population levels of the plant or herbivore). This also implies that there are many evolutionary 'colds-spots' in nature, where little selection is being generated, and that it is in the 'hotspots' – where interactions occur with much higher regularity or frequency – that selection for various adaptations and counter-adaptations is strong.

An extreme, though potentially common, type of false coevolutionary relationship is 'ecological fitting' (**Figure 1**), wherein organisms colonize and persist in novel environments, use novel resources, and form novel associations with other species as a result of a suite of traits that they evolved under different circumstances (Janzen, 1985; Agosta, 2006; Brooks *et al.*, 2006; Agosta and Klemens, 2008). Fitting underpins many key ecological processes, including community assembly and structure and is broadly relevant to many fields of biology and environmental sciences, including invasion ecology. Recent opinion articles by Agosta (2006) and Agosta and Klemens (2008) propose three different mechanisms to explain the phenomenon of ecological fitting: (1) exaptation, which

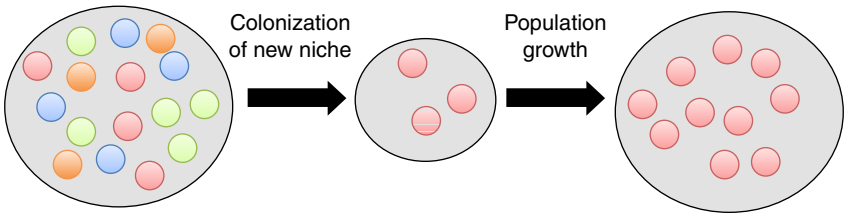

Figure 1 The ecological fitting concept in an invasive species. In this figure, each differently colored circle represents a different species which compete for the same resources; the left circle represents the native habitat in which these species coexist. The red species, through some process (natural or man-aided dispersal) is able to colonize a new habitat elsewhere containing novel resources with which it has not coevolved but nevertheless which it successfully exploits. This fulfills the criteria of 'ecological fitting.' Having escaped from coevolved enemies as well, in time, the population of this species grows, enabling it to become dominant in its new habitat.

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argues that an existing trait may be co-opted for a new function that enables an organism to survive and reproduce in a new environment. For example, feathers and wings likely used for insulation or display in therapod dinosaurs later enabled the evolution of flight in birds (Agosta and Klemens, 2008); (2) the correlated evolution of traits that preadapt organisms to novel conditions. This involves selection for parallel changes in multiple traits rather than a single one (for instance, physiological and morphological characteristics), which, working in concert, enhance the ability of a species to colonize or exploit novel habitats or resources; and (3) the process of 'phylogenetic conservatism,' whereby the evolutionary history of a species is related to its ability to exploit novel resources that have a close ecophysiological affinity to the resources in the environment where it evolved (Agosta, 2006). Agosta and Klemens (2008) also developed the term 'sloppy fitness space,' which they defined as the range of niches – both realized (actual niches) and fundamental (potential niches), in which a species can have realized fitness (survival to reproduction) (**Figure 2**). A niche is not a static entity but changes over time in response to changes or shifts in local (and global) conditions. Thus, sloppy fitness space may include components of a fundamental niche that are incorporated into the realized niche as conditions become more favorable for an organism. Some of the resources in the fundamental niche may actually be novel but are incorporated into the diet of the organism, representing an example of ecological fitting.

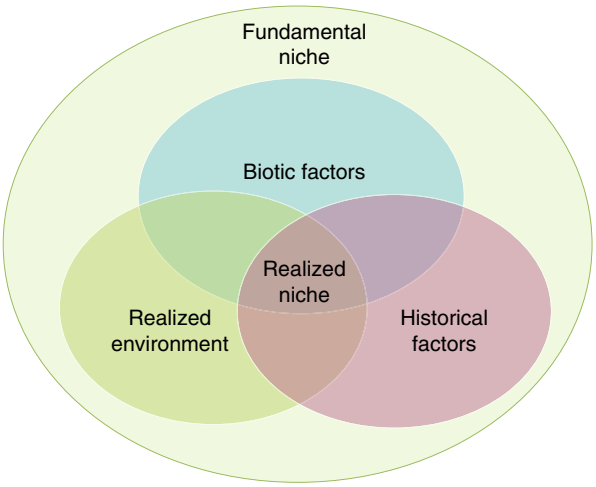


Figure 2 The habitat/niche concept. Factors that facilitate the potential (fundamental) and actual (realized) niches of a species are shown. Niches are not linear but encompass many dimensions (hence the definition of a niche as an 'n-dimensional hypervolume') but for reasons of clarity it is simplified here. The fundamental niche is often large, encompassing a broad range of habitats and resources. On the other hand, the realized niche is reduced due to the effects of biotic factors (predation, pathogenic infection, and competition), historical factors (constraints imposed in the distant and recent past), and realized environment, in which the populations actually exist in the field or/and in which individuals are not present, but could survive and reproduce. Ultimately, these factors in concert generate a realized niche which is the actual boundary of a habitat at different scales occupied by a species.

The Role of Evolution in Ecological and Evolutionary Fitting: Ecophysiological Equivalence and the Expression of Complementary Traits

In a broad sense, evolution is results in adaptation to a range of environmental constraints. In that context, ecological fitting is not 'evolution-free' but 'evolution-light.' A species may possess traits in habitats in which it evolved that may also give it high fitness in novel environments if the two habitats are alike in some way. In other words, provided the new conditions are benign or equivalent to those in which an organism evolved, then 'phenotype-environment' matching can occur. There are many examples of species that thrive in habitats (or on resources) with which they have no coevolutionary history. Evolution has outfitted these species with phenotypes that enable them to exhibit realized fitness in new habitats or on new resources. In a seminal study, Brooks *et al.* (2006) showed that a parasitic flatworm that had coevolved with North American frogs (Ranidae) was able to infect and reproduce in frogs native to Costa Rica if the habitats in which both were found were equivalent (e.g., wetlands) and if the Costa Rican and North American host frogs exhibited close phylogenetic affinity. Similarly, Harvey *et al.* (2012) showed that two species of closely related parasitic wasps from North America and Europe which attack closely related moths in their native ranges were highly successful in developing inside each other's hosts. These studies show that successful fitting is at least partially based on the possession of evolved traits that enable a species to exploit a particular set of novel resources or conditions.

Invasive Species: Highly Visible Examples of Ecological Fitting

Some of the best examples of successful ecological fitting in nature involve invasive organisms that have been introduced into nonnative ecosystems and which have established, spread, and in many cases become highly disruptive pests (Lowe *et al.*, 2000; **Figure 3**). The most visible and ecologically dramatic examples of invasive species involve plants and animals introduced to new regions separated by immense distances of land or water (Mooney and Hobbs, 2000; Lockwood *et al.*, 2013). When an exotic species enters a nonnative ecosystem it encounters abiotic and biotic conditions with which it has not evolved. The majority of successful invasive species are habitat generalists (Marvier *et al.*, 2004) possessing suites of traits that enable them to fit into many novel habitats ('broad-spectrum fitting'). These traits allow exotic organisms to outcompete native species which have to deal with coevolved enemies and competitors. Invaders are often able to escape their coevolved natural enemies, such as pathogens and predators (Keane and Crawley, 2002), enabling them to invest limited metabolic resources toward other functions, such as growth and competitiveness (Blossey and Notzold, 1995). Still other invasive species possess novel traits, such as secondary metabolites in plants, that are toxic to native herbivores that have not coevolved to deal with them (Callaway and Ridenour, 2004; Cappuccino and Arnason, 2006). For these reasons invasion ecology is a field that is strongly suited to the study of false coevolution and ecological fitting.

Alliaria petiolata

Salenopsis invicta

Rhinella marina

Pacifastacus leniusculus

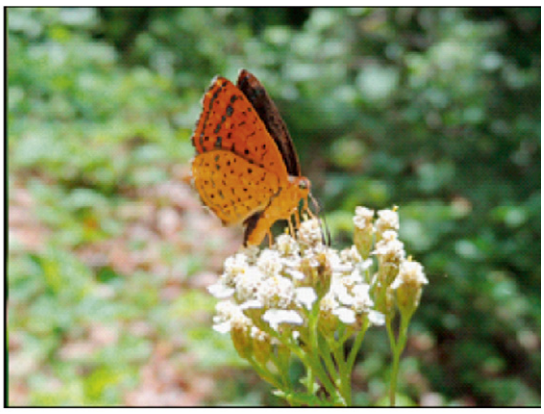
Figure 3 Examples of highly invasive species that are habitat generalists and possess traits that they evolved in their native habitats that have enabled them to establish and spread (broad-spectrum fitting) in nonnative ecosystems. The garlic mustard (*Alliaria petiolata*) is native to Eurasia but has spread across much of eastern North America where it is dispalcing native vegetation; the fire ant (*Solenopsis invicta*) is native to South America, but was introduced into Florida, spread north and has now displaced many native ants from the southeastern United States; the cane toad (*Rhinella marina*) is native to central and South America but has been introduced to several other countries, including Australia, where it is now spreading and decimating local fauna; the signal crayfish (*Pacifasticus leniusculus*) is a North American freshwater crustacean species that was introduced into Europe in the 1960s. It has since spread and dispalced many natives in its new range.

Bis Interimitur, Qui Suis Armis Perit (What Goes Around, Comes Around)

Important insights into the conditions that permit (or do not permit) ecological fitting can be elucidated by comparing the habitat and dietary breaths of organisms (plants or animals). Organism–organism interactions are often assumed to be ecologically specialized, tightly coevolved systems driven by mutual modification (Brooks *et al.*, 2006). However, many interactions are of a far more generalist nature. Generalists can exploit multiple resources or occupy multiple niches, whereas many specialists have evolved much more highly refined traits that enable them to successfully exploit a much narrower range of niches but with more effectiveness than generalists (Loxdale *et al.*, 2011). Generalist organisms may be able to adjust their behavior to exploit novel conditions much more effectively than specialists, and food or other resources can be less limited by resource availability for generalists than for specialists. It follows, then, that dietary and habitat generalists may be more prone to ecological fitting.

Alternatively, although specialists may exploit local resource types or habitats more effectively than generalists, this may lead some specialist species down evolutionary dead-ends. Much coevolutionary research involving specialist consumers and their resources have thus far been based on parasite–host interactions, where speciation in one lineage causes speciation in another lineage (e.g., Hafner and Nadler, 1990). Hafner and Nadler (1988, 1990) describe this process as 'synchronous co-speciation' where adaptive radiation in one species depends on another species. In this scenario, the consumer loses the ability to evolve independently of the resource it is exploiting or the habitat in which it lives. Consequently, the conditions required for survival and reproduction become so incredibly narrow that they do not allow for a switch to other diets or habitats (**Figure 4**). These species are evolutionarily 'frozen' in space and time and therefore are not

Ailuropoda melanoleuca



Calephelis borealis

Phascolarctos cinereus

Rostrhamus sociabilis

Figure 4 Examples of species that have become so specialized that they exhibit exceedingly narrow niches ('super-fitting-dead-end'). The giant panda (*Ailuropoda melanoleuca*), which is not only constrained by one narrow type of sufficient food (25 species of bamboo) but also by abiotic conditions; the Northern metalmark butterfly (*Calephelis borealis*) whose larvae are only able to digest the round-leafed ragwort (*Aster umbellatus*); the koala (*Phascolarctos cinereus*) with a monophagous diet of eucalyptus trees; and the snail kite (*Rostrhamus sociabilis*), a raptor which has evolved a beak that is adapted solely for a diet of aquatic snails.

able to respond to even potentially minor environmental changes and thus adapt to them. Extreme specialist organisms are therefore unlikely to experience ecological fitting.

Unwittingly Fitting, and How Manufactured Interactions are Often Used to Explain Biological Processes

In spite of the clear importance of local interspecific interactions in driving trait evolution, many studies in the laboratory from both an applied and fundamental perspective have been based on facetious interactions involving species that have little or no coevolutionary history. These studies have included a wide range of biota, from plant–herbivore to herbivore–natural enemy interactions. Indeed, the preponderance of plant–herbivore research using the model plant system *Arabidopsis* is an example of researcher-mediated ecological fitting. Some species studied in the lab are not even sympatric, but originate from different biogeographical regions. Such studies are possible because the interacting species are able to exploit novel resources based on phylogenetically conserved traits or convergent evolution. This enables them to form immediate and sometimes highly intimate associations with completely novel species. Some theories in life-history (co)evolution – such as the efficacy of indirect plant defences via the release of herbivore-induced plant volatiles or selection for plant defences – are based on studies involving species that probably do not (or at best only rarely) interact in nature (e.g., Turlings *et al.*, 1990; Züst *et al.*, 2012). This is not necessarily a criticism of such studies – an intimate evolutionary history between two or more species is not an essential prerequisite for addressing important biological questions and exploring mechanisms. However, the results of these studies must be interpreted with caution because the ways in which selection plays out under conditions involving coevolving species may be profoundly different when independently evolving species are fitted together in a laboratory.

Summary

Although it is still largely not recognized, the concept of ecological fitting is broadly relevant to many fields in ecology and evolutionary biology. Novel ecological interactions arise constantly in nature, and when these enable a plant to establish in

a new habitat, or an herbivore to successfully incorporate a new plant into its diet, they become examples of ecological fitting. A necessary prerequisite for ecological fitting between interacting species is a coevolutionary history of interactions involving closely related or convergent species exhibiting physiological equivalence. Importantly, many lab-based biological studies are based on interactions between species that have been selected for convenience rather than their coevolutionary history.

Acknowledgments

The authors would like to thank Arnaud Gaillard, Fernando Revilla, Annette Allor, Jean M. Hoogacker, Sannse, Alex Wild, Philip Bowles and David Perez, via Wikimedia Commons for their photographs of organisms shown in **Figures 3–4**.

See also: Coevolution, Introduction to. Invasive Species, Evolution and

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Ecological Speciation and Its Consequences

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Glossary

Divergent natural selection Selection that acts in contrasting directions in between two populations (e.g., large body size confers high survival in one environment and low survival in another), including the special case in which selection favors opposite phenotypes within a single population (i.e., disruptive selection).
Eco-evolutionary dynamics Interactions between ecology and evolution on contemporary timescales (i.e., generally driven by rapid evolution and rapid ecological change).
Ecological speciation The process by which barriers to gene flow evolve between populations as a result of ecologically based divergent selection between environments.
Epistasis Interactions between alleles found at different loci such that the phenotypic effect of an allele at one gene relies on an allele at one or more other genes.
Linkage disequilibrium Nonrandom statistical associations between alleles at two or more loci.
Mutation-order speciation The evolution of reproductive isolation by the fixation of different advantageous mutations in separate populations experiencing similar selection pressures (i.e., 'uniform selection').
Pleiotropy Multiple phenotypic effects of a single gene.
Reproductive isolation Biological barriers to interbreeding between populations, which can occur before or after mating.
Speciation continuum Quantitative variation in the degree of speciation (i.e., reproductive isolation).

Biologists have long sought to explain the origin and maintenance of biological diversity within and among species. Natural selection is generally recognized as a central mechanism driving evolutionary change within species (Kingsolver *et al.*, 2001; Darwin, 1859; Endler, 1986). Thus, natural selection plays a key role in producing the array of phenotypic and genetic diversity we see in nature. But to what extent is selection, the process driving adaptation within species, also responsible for the formation of new species? In other words, to what extent do phenotypic and species diversity arise via the same process?

Renewed efforts to address these questions have emerged, based on the following general scenario. Populations living in different ecological environments (e.g., desert vs. forest habitats) might undergo divergent and adaptive evolutionary change via divergent natural selection. These same evolutionary changes can also result in the populations evolving, perhaps incidentally, into separate species. For example, adaptation to different environments, via divergent selection, might cause the evolution of genetically based differences between populations in the way that individuals tend to behave, look, or smell. These differences might then in turn cause individuals from different populations to dislike mating with one another, or for hybrids to be unfit if mating occurs. Thus, the populations would cease exchanging genes, thereby diverging into separate species because of the adaptive changes that occurred via divergent selection. This is a simple description of the 'ecological speciation' hypothesis (Juan *et al.*, 2010; Funk, 1998; Schluter, 1996a,b, 1998, 2000, 2001, 2009; Rundle and Nosil, 2005; Nosil *et al.*, 2002; Nosil, 2012).

More specifically, 'ecological speciation' is defined as the process by which barriers to gene flow evolve between populations as a result of ecologically based divergent selection between environments (Nosil, 2012). This will often occur because traits under divergent selection, or those genetically correlated with them, incidentally cause reproductive isolation (Muller, 1942; Mayr, 1963). Under such a scenario, speciation occurs as a 'by-product' of adaptive divergence. In some instances of ecological speciation, divergent selection might operate directly on reproductive isolation itself. This occurs, for example, if the evolution of mating signals and preferences is dependent on the ecological environment such that ecologically differentiated populations diverge in signals and preferences, resulting in premating sexual isolation (Boughman, 2001; Seehausen *et al.*, 2008).

Selection itself is considered ecological when it arises as a consequence of the interaction of individuals with their external environment during resource or mate acquisition, or from the interaction of individuals with other organisms. Selection is divergent when it acts in contrasting directions in the two populations (e.g., large beaks in a bird species confers high fitness in one environment and low fitness in the other). This includes the special case of disruptive selection in which selection favors opposite extremes of the trait distribution within a single population (Rundle and Nosil, 2005). The agents of divergent selection during ecological speciation are thus extrinsic and can include abiotic and biotic factors, such as food resources and habitat, and interspecies interactions, such as parasitism and competition.

Ecological speciation thus has three necessary components (Rundle and Nosil, 2005; **Figure 1**). The first is a source of divergent selection, such as differences between environments (e.g., in aridity, temperature, etc.), interactions between species, or ecologically based sexual selection. Examples of all these sources of selection in ecological speciation now exist. The second component is a form of reproductive isolation,

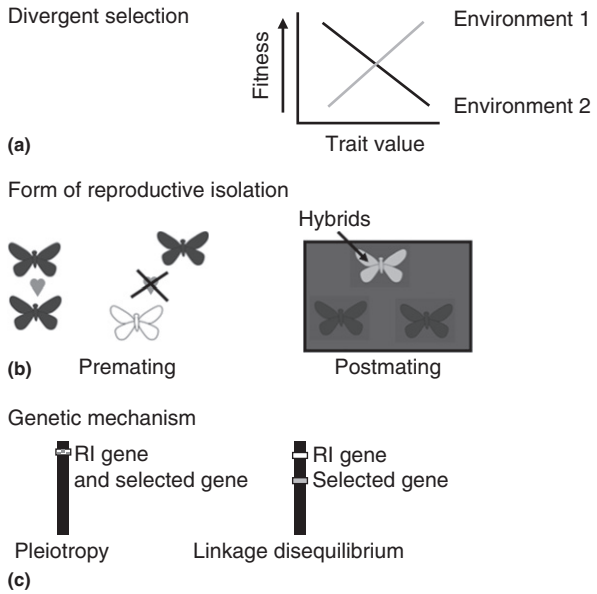

Figure 1 The three components of ecological speciation (c.f., Rundle and Nosil, 2005). (a) A source of divergent selection. The commonly recognized sources are differences between environments, interactions between populations, and sexual selection. (b) A form of reproductive isolation, which might act before or after mating. The example of premating isolation here concerns divergent mating preferences such that there is assortative mating based upon color. The example of postmating isolation concerns environment-dependent selection against hybrids such that the intermediate phenotype of hybrids renders them more vulnerable to visual predation in the environment of each parental species. (c) A genetic mechanism linking selection to reproductive isolation. The genes under selection and conferring reproductive isolation may be one and the same (pleiotropy) or physically different (but statistically associated via linkage disequilibrium). Modified from Nosil, P., 2012. Ecological Speciation, Oxford: Oxford University Press with permission from Oxford University Press.

which can occur before or after mating. Examples of premating isolation are habitat isolation resulting from divergent habitat preferences, immigrant inviability due to selection against migrants between environments, and sexual isolation due to divergent mating preferences. The prime example of postmating isolation is hybrid inviability, which can occur for ecological reasons or due to intrinsic genetic incompatibilities between the genomes of parental species when they are brought together in a hybrid. All of these forms of reproductive isolation can evolve as an incidental by-product of genetic divergence created by divergent adaptation, and each has been implicated in ecological speciation. For example, ecotypes of stickback fish and host-associated beetles have adapted to different environments via divergent selection and exhibit multiple forms of reproductive isolation as a consequence (Egan and Funk, 2009; Matsubayashi and Katakura, 2009; Mckinnon and Rundle, 2002). The third component of ecological speciation is a genetic mechanism to link divergent selection to the genes causing reproductive isolation. Two main such mechanisms are distinguished by whether the genes affected by divergent selection are one and the same as those causing reproductive isolation (e.g., pleiotropy) or the genes under selection being different than those causing reproductive isolation. In the latter case, statistical associations (i.e., linkage disequilibrium) between the different sets of genes can cause selection on genes involved in divergent adaptation to be transmitted to the genes causing reproductive isolation. Additionally, interactions between genes (i.e., epistasis) involved in ecological speciation, be they those under selection or those causing reproductive isolation, can affect observed levels of reproductive isolation (Arnegard *et al.*, 2014).

With this description of the process of ecological speciation in place (see Nosil, 2012 for details), we now turn to outlining: (1) alternatives to ecological speciation, (2) evidence and support for ecological speciation, (3) the genomic basis of ecological speciation, (4) constraints on speciation, and (5) the broader consequences of ecological speciation for communities and ecosystems. We conclude by discussing promising avenues for further research.

Alternatives to Ecological Speciation

Ecological speciation can be distinguished from other models of speciation in which the evolution of reproductive isolation involves key processes other than deterministic and ecologically based divergent selection (Nosil, 2012; Schluter, 2009). These alternatives tend to involve stochastic events, such as random changes in alleles frequencies and differences among populations in the specific mutations that arise. Such alternatives can be classified into two main categories. The first category considers mechanisms of speciation that do not involve selection, such as speciation via genetic drift, perhaps involving population bottlenecks and founder events. The second considers mechanisms that do involve selection, but selection is not divergent between ecological environments. One such alternative to ecological speciation is thus 'mutation-order speciation,' defined as the evolution of reproductive isolation by the fixation of different advantageous mutations in separate populations experiencing similar selection pressures, i.e., 'uniform selection' (Schluter, 2009; **Figure 2**). In essence, different populations find different genetic solutions to the same selective problem (Mani and Clarke, 1990). In turn, the different genetic solutions (i.e., mutations) are incompatible with one another, causing reproductive isolation (Price, 2007; Schluter, 2001, 2009; Nosil and Flaxman, 2011). During mutation-order speciation, the same alleles would be favored in both populations, but divergence occurs anyway because, by chance, the populations do not acquire or fix the same mutations. Divergence therefore involves stochasticity, but the process also involves selection, and thus is distinct from speciation via genetic drift. Selection can be ecologically based under mutation-order speciation, but ecology does not favor divergence as such, and a correlation between ecological divergence and reproductive isolation is not expected. Notably, these models that do not involve divergent selection are explicit alternatives to ecological speciation, but are not mutually exclusive to it (i.e., several different models could be operating simultaneously).

Support for Ecological Speciation

The process of ecological speciation makes some explicit and simple predictions. We outline below five approaches for

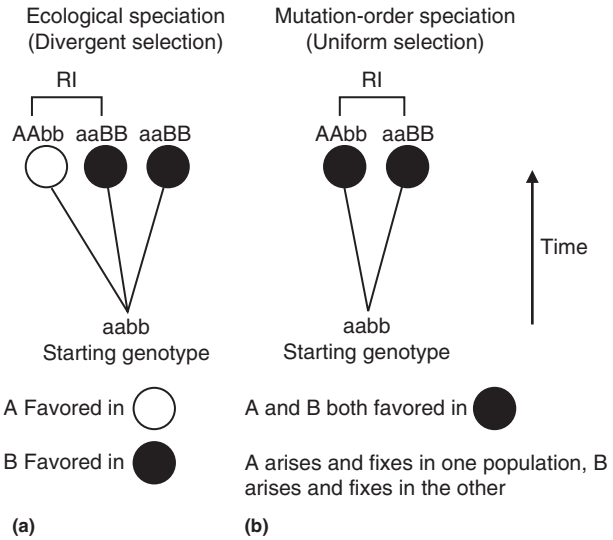

Figure 2 Schematic diagram of (a) Ecological speciation vs. (b) Mutation-order speciation (following Schluter, 2009). Distinct environments are indicated by open vs. filled circles. Genetic drift is ignored and change occurs via selection. Genetic changes are shown at two bi-allelic loci, with the derived alleles A and B being incompatible with one another. 'RI' denotes the types of population crosses that result in reproductive isolation. The essential distinction depicted is that under ecological speciation only populations in different environments evolve reproductive isolation, whereas under mutation-order speciation populations adapted to the same environment evolve reproductive isolation. Modified from Nosil, P., 2012. Ecological Speciation, Oxford: Oxford University Press with permission from Oxford University Press.

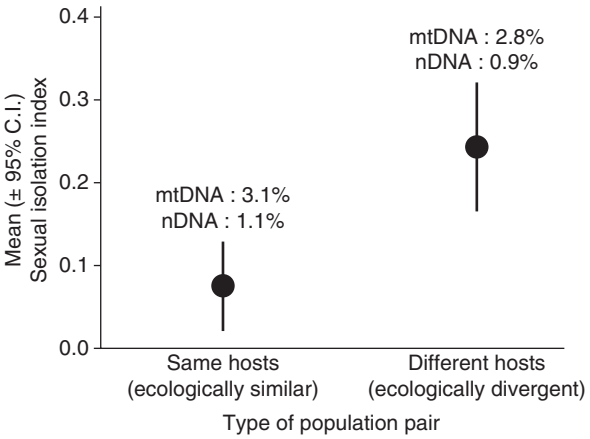

Figure 3 Evidence for ecological speciation in host-plant associated populations of *Timema cristinae* walking-stick insects (individual populations feed on either the host plant *Ceanothus spinosus* or on *Adenostoma fasciculatum*). Pairs of populations feeding on the same host plant species, but in different geographic localities, are ecologically similar. In contrast, pairs of populations feeding on different host plant species are ecologically divergent and subject to host-associated divergent selection. Different-host pairs (n=15 pairs) exhibit significantly greater reproductive isolation due to divergent mating preferences (i.e., sexual isolation) than do same-host pairs (n=13 pairs). This pattern is independent from neutral genetic divergence, a proxy for time since divergence. Mean divergence is shown for the mitochondrial COI gene (mtDNA) and for the nuclear IT-2 gene (nDNA). Modified from Nosil, P., 2012. Ecological Speciation, Oxford: Oxford University Press with permission from Oxford University Press.

testing for ecological speciation, all of which have received some empirical support (Nosil, 2012). The first class of 'comparative' approaches examines multiple taxon pairs and tests for a positive association between levels of reproductive isolation and levels of ecological divergence, independent from time since population divergence (following Funk *et al.*, 2002, 2006; Funk, 1998; **Figures 3** and 4). Such correlations between ecological divergence and reproductive isolation have been detected across disparate plant, vertebrate, and invertebrate taxa (Funk *et al.*, 2006). Second, when analysis of multiple taxon pairs in a comparative framework is not possible, 'trait-based' approaches can test if the same traits involved in divergent adaptation also affect reproductive isolation ('magic traits', c.f., Gavrilets, 2004; Servedio *et al.*, 2011). For example, butterfly wing patterns can be under ecological selection for mimicry but also contribute to premating isolation (Jiggins *et al.*, 2001; Merrill *et al.*, 2012). Likewise, body size can contribute to adaptive divergence and to premating isolation, as observed in fish (Langerhans *et al.*, 2007; Mckinnon *et al.*, 2004).

Sometimes it will not be possible to know *a priori* which traits are subject to divergent selection. In this case, one may use a third class of 'fitness-based' approaches to test if reproductive isolation is a direct consequence of ecologically based selection against immigrants and hybrids. These approaches involve measuring fitness in different habitats, rather than selection on specific phenotypic traits. Fitness-based methods predict a reduction in the fitness of individuals placed into

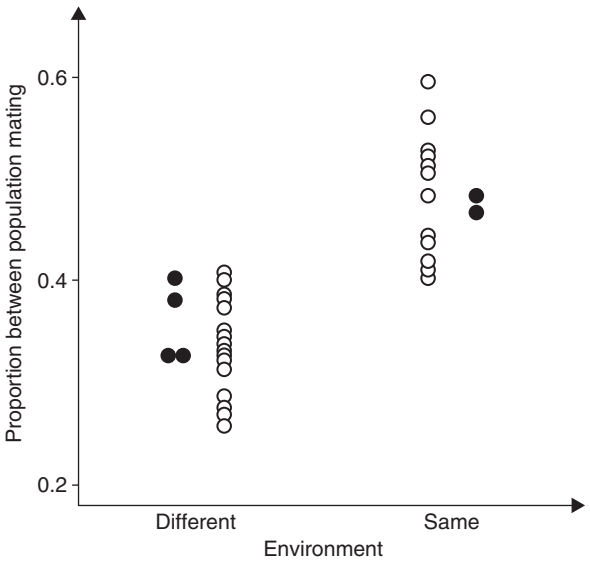

Figure 4 Evidence for ecological speciation from experimental evolution studies in the lab. Each dot represents a replicate experimental line. Shown is the proportion of matings occurring between independently evolved lines of *Drosophila* as a function of the dissimilarity of their environments. Mating between populations is less common when populations have been adapted to different environments. Open circles are from Dodd (1989) and closed circles from Kilias *et al.* (1980).

a novel environment relative to resident individuals. When experimental data cannot be collected, 'gene-flow based' approaches can test whether gene flow at molecular markers is reduced as adaptive divergence increases. Finally, a fifth 'phylogenetic shift' approach can ask whether branching (i.e., speciation) events in a phylogenetic tree coincide with ecological shifts.

Although evidence for ecological speciation is abundant and stems from each of the aforementioned approaches, almost all of it is correlative and indirect, or from lab studies. Manipulative field experiments testing whether adaptive divergence reduces gene flow in nature are lacking and represent a 'final frontier' in tests of ecological speciation.

Genomic Basis of Ecological Speciation

Although there are clear examples of ecological speciation, we are only beginning to understand the genomic basis of the process. For example, we know little about the number, distribution, and effect sizes of genes driving ecological speciation. Moreover, the nature of genomic divergence during ecological speciation is unclear because empirical evidence is mixed. Although there are examples of incipient speciation involving divergence in only a few genomic regions (Heliconius Genome, 2012; Nadeau *et al.*, 2012; Poelstra *et al.*, 2014), there is accumulating empirical evidence that ecological speciation can involve divergence in many regions of the genome, even at its earliest phases (Michel *et al.*, 2010; Soria-Carrasco *et al.*, 2014; Jones *et al.*, 2012; Ellegren *et al.*, 2012; Lawniczak *et al.*, 2010). However, theoretical models often predict that when fewer loci control a trait, speciation with gene flow is easier, because each locus experiences a stronger per-locus selection coefficient (Gavrilets, 2004; Yeaman and Whitlock, 2011).

How can widespread genomic divergence be reconciled with the aforementioned theoretical predictions? These findings can be readily reconciled by considering the important distinction between: (1) the number of loci affecting a given, individual trait versus (2) the total number of loci across the entire genome experiencing divergent selection (i.e., summed across all traits subject to selection). In the former case, fewer loci affecting the trait can aid divergence of the specific trait. However, if more loci overall experience selection and can overcome gene flow (perhaps aided by synergistic effects across loci on reducing gene flow), this increases genetic divergence and the chances of extrinsic or intrinsic genetic incompatibilities which cause reproductive isolation (Flaxman *et al.*, 2013, 2014). Thus, ecological speciation with gene flow might be easiest when selection acts on many traits influenced by few loci each, and hardest when selection acts on a single trait influenced by many loci. What do empirical data say concerning the numbers of loci involved in ecological speciation?

One approach taken by empirical studies of the genetic basis of ecological speciation is to determine the genetic architecture of traits that play a role in adaptation to divergent ecological environments. By definition, these are the same loci that are involved in ecological speciation. Studies taking this approach have shown that the genetic architectures of such traits fall along a continuum ranging from traits controlled by a few genes each with large phenotypic effects (Bradshaw and Schemske, 2003; Hoekstra *et al.*, 2006; Jiggins and Mcmillan, 1997; Peichel *et al.*, 2001; Rosenblum *et al.*, 2010; Van't Hof *et al.*, 2011) to those controlled by many genes that each have modest effects on phenotypic variation (Arnegard *et al.*, 2014; Ellison *et al.*, 2011; Gompert *et al.*, 2013; Atwell *et al.*, 2010; Brachi *et al.*, 2010). Thus, there are examples of ecological speciation driven by strong divergent selection acting on 'simple' traits controlled by few loci with large phenotypic effects, such as wing coloration traits in butterflies (Heliconius Genome, 2012), as well as by selection acting on complex suites of traits controlled by many loci with small phenotypic effect, such as foraging traits involved in niche adaptation of stickleback fish (Arnegard *et al.*, 2014). But to what extent do these different genetic architectures influence the likelihood of ecological speciation? How common are each? It is too early to answer these questions because data are currently available only on a case-by-case basis. A major goal in ecological speciation research is thus to determine the relative roles that either strong selection acting on a few loci versus 'multifarious' selection acting on many loci play in generating reproductive isolation, and the conditions under which each is expected to drive speciation. Another major question is how strongly physical linkage of loci involved in ecological speciation promotes the process (Feder *et al.*, 2012). It might be the case that simple genetic architectures comprised of a few loci are required for ecological speciation with high gene flow, but not otherwise.

A second approach to understanding the genomic basis of ecological speciation relies on what has been termed 'reverse ecology' (Li *et al.*, 2008). Specifically, genome-wide surveys of molecular variation (so-called 'genome scans') that do not rely on *a priori* candidate traits or loci can identify outlier loci with elevated levels of differentiation relative to the rest of the genome. Such outlier loci can be identified in the absence of knowledge concerning the traits under selection, and are candidates for regions harboring genes under divergent selection. Frequently, genome scans identify hundreds or even thousands of outlier loci spread across the genome (Hohenlohe *et al.*, 2010; Parchman *et al.*, 2013; Soria-Carrasco *et al.*, 2014; Lawniczak *et al.*, 2010). At face value, this pattern is consistent with ecological speciation being the result of selection affecting many loci. However, the situation is much more complex than this. Many processes other than divergent selection, such as demographic history, mutation, recombination, and drift, can also generate outlier loci (Bierne *et al.*, 2011; Ellegren *et al.*, 2012; Roesti *et al.*, 2014; Feder *et al.*, 2012). In particular, background selection in regions of low recombination can create outlier loci in genomic regions not involved in divergent adaptation (Noor and Bennett, 2009; Cruickshank and Hahn, 2014). Therefore, it is important to move beyond genome scans alone to disentangle the contribution of divergent selection versus other processes in generating observed patterns of differentiation (Feder *et al.*, 2012).

One possibility in this regard is to use experiments that can quantify selection's effect on genomic variation, for example, by exposing experimental populations to selection in different environments and then obtaining genome sequence data from them. The main premise is that selection on phenotypic traits

is transmitted to causal genetic variants affecting the traits (direct selection) as well as to additional genetic loci correlated via linkage disequilibrium with these functional variants (indirect selection). Thus, the genome-wide response to selection (i.e., total selection = direct selection + indirect selection) can be measured experimentally (Gompert *et al.*, 2014). Indirect targets of selection may be spread across the genome, and while these loci may not functionally control adaptive phenotypes, their evolutionary response to selection is real, and they may still contribute to the evolution of reproductive isolation. Recent studies adopting such experimental approaches have shown that: (1) divergent natural selection can have a widespread effect on the genome that involves multiple genomic regions, and/or (2) outlier loci in genome scans of natural populations harbor loci affected by divergent selection in experiments (Gompert *et al.*, 2014; Anderson *et al.*, 2014; Pespeni *et al.*, 2013; Soria-Carrasco *et al.*, 2014). For example, experimental populations of stick insects transplanted between two host-plant species showed evidence for host-plant associated selection, and this selection affected regions of the genome that differ between natural populations on different hosts (Soria-Carrasco *et al.*, 2014). However, these studies quantified total selection and thus did not isolate the effects of direct selection from indirect selection. Isolating the specific loci that are under direct selection and driving the process of ecological speciation will be more difficult, and likely require combining ecological, mapping, experimental, genomic, and functional data (Barrett and Hoekstra, 2011). In addition to experiments, the geographic setting of populations may help infer the role of divergent selection versus other factors in promoting genetic differentiation. For example, gene flow is less likely to affect differentiation between sets of geographically isolated populations (where gene flow is reduced by geographic barriers for all populations), reduced recombination aids adaptive divergence more strongly for populations that are exchanging genes, and high gene flow can negate the effects of genetic drift (Ortiz-Barrientos *et al.*, 2002; Nosil, 2012).

Constraints on Ecological Speciation

Once ecological speciation is initiated, there is often quantitative variation in how far it proceeds. Thus, divergence among populations tends to span a continuum ranging from weakly reproductively isolated populations, to moderately divergent ecotypes, to distinct species (i.e., this variation is referred to as the 'speciation continuum') (Nosil *et al.*, 2009). In this context, many examples exist in populations for which reproductive isolation remains incomplete (Alcaide *et al.*, 2014; Peccoud *et al.*, 2009; Renaut *et al.*, 2013; Soria-Carrasco *et al.*, 2014). These examples beg the question, what are the processes that impose limits on ecological speciation?

One obvious factor is time: 'incomplete' speciation might simply require more time to complete the speciation process. However, other factors may constrain speciation. Gene flow between populations has arguably received the most attention in terms of imposing constraints on speciation because of its homogenizing effects on population divergence (Slatkin, 1973, 1985). The nature of selection can also influence the likelihood of ecological speciation. For example, temporal variation in selection can constrain ecological speciation by counteracting divergent selection that results from spatial heterogeneity in the environment. In addition, selection can vary among traits. If universally favored phenotypes evolve, they can facilitate gene flow and maintain connectivity among populations. Finally, genetic correlations among traits can impose constraints on speciation if the quantitative genetic variation underlying adaptive traits is not coincident with the direction of divergent selection (Agrawal and Stinchcombe, 2009). One example of such constraints on speciation due to gene flow, variable selection, and genetic architecture concerns a melanistic phenotype of the stick insect *Timema cristinae* (Comeault *et al.*, 2015). This phenotype is cryptic on the stems of both host plant species used by *T. cristinae*, has a high dispersal rate, and exhibits a universal mating advantage. These factors increase gene flow between populations adapted to different host plants, counteracting divergent between populations. Gene flow is also affected by genetic architecture, because, for example, the locus affecting melanism is physically linked to a locus under divergent selection, counteracting divergence at this locus.

Consequences of Ecological Speciation

Much recent research in evolutionary ecology focuses on how different genotypes can differentially influence community structure, and ecosystem function (Whitham *et al.*, 2006). Because adaptive divergence affects genetic diversity, this begs the question whether and how ecological speciation itself can influence ecology. Research in the field of eco-evolutionary dynamics investigates how rapid evolutionary change can influence the ecology of populations, communities, and ecosystems (Pelletier *et al.*, 2009; Schoener, 2011), and often uses systems that exemplify adaptive divergence between populations. Such systems allow well-controlled 'common gardening' experiments, whereby two or more ecologically divergent populations (i.e., ecotypes) are transplanted to a common environment, and the effects of different ecotypes on ecological properties are evaluated (Matthews *et al.*, 2011). Demonstrations of the ecological consequences of adaptive divergence and/or ecological speciation for populations, communities, and ecosystems exist using fish (Harmon *et al.*, 2009; Post and Palkovacs, 2009; Bassar *et al.*, 2010), insects (Farkas *et al.*, 2013), and plants (Agrawal *et al.*, 2012; Johnson *et al.*, 2009). For example, adaptive divergence of stickleback ecotypes that are undergoing ecological speciation influences the composition of invertebrate prey in a community, which further affects nutrient flux at the ecosystem level (Harmon *et al.*, 2009). More recently, studies of stick-insect ecotypes have shown how quantitative variation in the degree of adaptive divergence affects community-level dynamics by mediating the attraction of bird predators that eat stick insects, but also other cohabiting arthropod species (Farkas *et al.*, 2013).

Studies like those described above make a strong case that rapid evolutionary dynamics during ecological speciation can be a powerful driver of contemporary ecological processes. An extension of one-way effects of evolution on ecology is the

idea of eco-evolutionary feedbacks (Schoener, 2011), whereby modified ecology subsequently influences the trajectory of evolutionary change (Yoshida *et al.*, 2003; Farkas and Montejo-Kovacevich, 2014). Though understudied, eco-evolutionary feedbacks could influence the fate of ecological speciation, whereby the ecological effects of adaptive divergence in turn constrain or further promote subsequent divergence. Further work on eco-evolutionary feedbacks is highly warranted.

Conclusions

Much progress has been made in understanding ecological speciation. Numerous examples of the process now exist and the occurrence of ecological speciation is now fairly well accepted. However, the relative importance for ecological speciation of different sources of divergent selection, forms of reproductive isolation, and genetic architectures of traits under selection remains unclear. Major questions also remain concerning the importance of ecological speciation relative to its alternatives, and the genomic basis and ecological consequences of ecological speciation. Furthermore, the point in the speciation continuum achieved during ecological speciation is poorly understood, and represents a major avenue for further research. Like in most rapidly growing fields, evidence for ecological speciation tends to be indirect, relying on observational and comparative studies. In some study systems, at least some stages of the process might be recreated experimentally in replicate field or lab populations, with experimental results then compared to patterns in natural populations providing some of the strongest evidence and insight possible. The study of ecological speciation is yet to enter a truly experimental phase, where manipulations are commonly employed to isolate causal associations between the factors driving and constraining the process. Thus, much will likely be learned in the future, particularly as genomic methodologies continue to be integrated with classical ecological approaches.

See also: Sequential Speciation. Speciation Continuum. Speciation, Sexual Selection and. Speciation-with-Gene-Flow

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Effective Population Size

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Glossary

Eigenvalue The eigenvalues of a matrix describe some of the important essential features of that matrix.

Markov chain A Markov chain is a process occurring in time (or in space) such that the state of some process (in the examples in this article the genetic constitution of some population) at any time point (here at the time of an offspring generation) depends in some prescribed random way on the state at the previous time point (here the time of the parental generation).

Matrix A matrix is a rectangular array of numbers that can be manipulated in several well-defined mathematical operations.

Introduction

The effective population size concept is one of the most misunderstood in population genetics theory. Most of the misunderstandings arise in areas largely outside population genetics, for example, in non-genetic evolutionary studies. It is therefore important to describe what the meaning and derivation of the concept are.

In the early days of population genetics theory many simplifying assumptions were made in deriving results. This was especially true of the stochastic theory, in which random changes in gene frequency due to random sampling in small populations were considered (random genetic drift). The theory is difficult enough without taking into account the existence of two sexes, the geographical dispersal of a population, changing population sizes over generations, and so on. The earliest analyses upon which population genetics theory was largely built, in particular those of Fisher (1930) and Wright (1931), were of necessity over-simplified, in which, for example, individuals were assumed to be monoecious (in effect one sex only allowed), random mating is assumed, and the population size is assumed to remain the same from one generation to the next, and so on. Even with such simplifications the stochastic theory rapidly becomes formidable.

The Early Theory

In order to get some idea of the evolutionary properties of a biological population under the Mendelian hereditary scheme, Fisher (1930) and Wright (1931) implicitly used a simple Markov chain model in assessing the frequency changes of a given allele A_1 at some gene locus with two alleles A_1 and A_2 (It was Wright who introduced the concept of an effective population size.). A diploid population of unchanging size N is assumed (so that in any generation there are $2N$ genes at this locus, some of allelic type A_1 and some of allelic type A_2). The genes in any offspring generation are assumed to be derived by random sampling, with replacement, from the genes of the parental generation. This implies that if there are i A_1 genes in the parental generation, the probability p_{ij} that there will be j A_1 genes in the parental generation is given by the binomial formula

$$p_{ij} = \binom{2N}{j}\left(\frac{i}{2N}\right)^{j}\left(\frac{2N-i}{2N}\right)^{2N-j} \qquad [1]$$

This will be recognized as defining the transition matrix of a Markov chain. This Markov chain has two absorbing states, one where there are no A_1 genes and one where there are $2N$ A_1 genes, and eventually one of these two states is entered, implying loss of genetic variation.

We call the model defined by [1] the 'simple' Wright–Fisher model. It fundamentally involves binomial-type form of random sampling in obtaining the genes in the offspring generation from those in the parental generation. In this sense it follows a simple process of obtaining the genes in one generation from those in the previous generation. It is also simple because there is no concept of two genders, no real acknowledgment of the diploid nature of the population, no concept of geographical dispersion of the population, and so on. Further, the size of the population is assumed to be the same from one generation to the next. Models generalizing [1] will be discussed below.

If the model [1] is so unrealistic, why even consider it? There are two answers to this question. First, this model was proposed at the very beginning of the re-writing of the Darwinian paradigm in Mendelian terms. It was always understood that this simple model would be followed by more realistic models. Second, evolution by natural selection requires genetic variation in the population. In the deterministic theory parallel to the model [1], gene frequencies do not change from one generation to another, so that any genetic variation in the population is preserved under Mendelian inheritance. This is an extremely important observation. It was realized, however, that in a population of finite size, and in particular of small size, random changes in gene frequencies will eventually cause one (of two alleles) to be lost. The model [1] was introduced to assess how long, on average, this loss would take. This matter is addressed again below.

Several exact results are known for the Markov chain process defined in [1]. Three of these are the following:

(i) The largest non-unit eigenvalue λ of the matrix $\{p_{ij}\}$ (the entries of which are given by [1]), which largely

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determines the rate at which genetic variation is lost, is $1 - 1/(2N)$. This means that

$$N = 1/\left[2(1-\lambda)^{-1}\right] \quad [2]$$

(ii) The probably α that two genes in an offspring generation are descended from the same gene in the parental generation is $1/(2N)$. This means that

$$N = (2\alpha)^{-1} \quad [3]$$

(iii) If the fraction of A_1 in some parental generation is x, then given this value, the variance of the (random) fraction y of A_1 in the offspring generation is $x(1-x)/2N$. This means that

$$N = x(1-x)/[2\text{Variance of y}] \quad [4]$$

Quite apart from these results, there is a very large literature giving more conclusions concerning a population evolving as prescribed by [1]. Many of the formulas deriving from these conclusions contain the population size N in them (as do the three listed above). One such formula is the following. It was stated above that under the model [1], eventually either the allele A_1 or the allele A_2 will become lost from the population. If the initial frequency of the allele A_1 is p, then to a very close approximation the (random) number of generations until this happens has a distribution with mean

$$-4N\{p\log p + (1-p)\log(1-p)\} \quad [5]$$

generations.

Models allowing complicating features such as the existence of two genders are very hard to analyze, and the entire purpose of introducing the concept of an effective population size is to use these various formulas but replacing N, wherever it appears in them, by an effective population size. For example, if in some more complex and realistic model allowing, for example two genders, the effective population size (of one type or another – which type will be discussed later) is N_e, common practice is to claim that the (random) number of generations until eventually either the allele A_1 or the allele A_2 will become lost from the population has a distribution with mean

$$-4N_e\{p\log p + (1-p)\log(1-p)\} \quad [6]$$

generations.

The theory justifying this step is not at all well developed, and the procedure just described, while perhaps reasonable, is not well supported theoretically.

For purely historical reasons, the Wright–Fisher model [1] above has assumed a 'gold standard' status. The concept of effective population size as it is discussed in the literature relates entirely to the gold-standard Wright–Fisher model. This fact is seldom noted or appreciated in the literature in which the effective population size is discussed.

The fact that the Wright–Fisher model is used as a gold standard is unfortunate, since a much more realistic model, due to Cannings (1974), would serve as a better gold standard. The Wright–Fisher model is a special case of the Cannings model, and the latter generalizes the Wright–Fisher model in an important way. In the Wright–Fisher model, the number of descendant genes from any parent gene has a binomial distribution with index $2N$ and parameter $1/2N$. Thus the mean number of offspring genes has mean 1 and variance $1 - 1/2N$. In the Cannings model, the number of descendant genes from any parent gene has mean 1 and arbitrary variance σ^2. The numbers of offspring genes from the various parental genes are assumed to be identically, but not independently, distributed (Independence is not possible since it is required that the total number of offspring genes is $2N$.). The full Cannings model is more general than is described here, but the above is sufficient for our purposes. Examples of this model will be given below.

Both the Wright–Fisher model and the Cannings model share one characteristic. This is that there is a concept of a parental and an offspring generation. Such models are called 'non-overlapping generation' models. Other models do not make this assumption. The most important of these is the Moran (1958) model. Here individuals die one by one, at random times, and at each death the dying individual is replaced by a newborn, deriving from a randomly chosen parent, thus maintaining the population size at a constant level. Effective population sizes in such 'birth-and-death' models differ from those in non-overlapping generations models, as discussed further below.

Definitions of the Effective Population Size

How is the effective population size defined? A second point, which is also seldom noted or appreciated in the literature in which the effective population size is discussed, is that there are various definitions of the effective population size. Suppose then that one considers a complicated evolutionary model involving, for example, the existence of two sexes or a geographically structured population. One might then construct a complicated Markov chain model generalizing [1], taking these complications into account. Suppose that in this model the largest non-unit eigenvalue of associated Markov chain matrix generalizing [1] is λ^*. One could then use [2] to define an eigenvalue effective population size $N_e^{(e)}$ as

$$N_e^{(e)} = 1/\left[2\left(1-\lambda^*\right)^{-1}\right] \quad [7]$$

Similarly, if in this more complicated model the probability that two genes in an offspring generation are descended from the same gene in the parental generation is α, one could use [3] to define an inbreeding effective population size $N_e^{(i)}$ by the formula

$$N_e^{(i)} = (2\alpha)^{-1} \quad [8]$$

Finally, one can define a variance effective population size $N_e^{(v)}$ through the variance of the (random) fraction (w) of A_1 in the offspring generation in model more complicated than the simple Wright–Fisher model [1]. Here we use [4] to define a variance effective population size $N_e^{(v)}$ by the formula

$$N_e^{(v)} = x(1-x)/[2\text{ variance of } w] \quad [9]$$

Further definitions of an effective population size are possible. Thus Sjödin *et al.* (2005) introduce the important

concept of an evolutionary effective population size. This is discussed further below.

Just as the central role playing by the Wright–Fisher model in defining the effective population size is not widely appreciated, so also is the fact that there are several definitions of effective population size not widely appreciated. One almost invariably sees the expression 'effective population size' without any qualifying adjective, or which concept of effective population size is most relevant to the situation at hand. These matters are also discussed further below.

Calculations of the Effective Population Size

We consider first the cases of two sexes. Suppose that in any generation there are N_1 diploid males and N_2 diploid females, with $N_1 + N_2 = N$. There are two possible alleles at some specific gene locus, A_1 and A_2, so that each individual is either A_1A_1, A_1A_2, or A_2A_2. We assume that the genetic makeup of any individual in the offspring generation, male or female, is found by randomly sampling one gene from the pool of $2N_1$ genes among the males in the parental generation and one gene from the pool of $2N_2$ genes among the females in the parental generation. All sampling is with replacement. Now there is a Markov chain of the vector (X_1, X_2), where X_1 is the number of A_1 genes among the males of any generation and X_2 is the number of A_1 genes among the females of any generation. This Markov chain is very complex. It can however be shown that the largest non-unit eigenvalue of its transition matrix is very close to $1 - (N_1 + N_2)(8N_1N_2)^{-1}$. Applying eqn [7] we find that to a very close approximation,

$$N_e^{(e)} = 4N_1N_2(N_1 + N_2)^{-1} \qquad [10]$$

Considering the possible sources of two genes taken at random in the offspring generation, it is found that the inbreeding effective population size is also very close to $4N_1N_2(N_1 + N_2)^{-1}$. On the other hand it is impossible even to define a variance effective population size, since there is no single Markovian random variable with a variance that can be used in eqn [9]. An advanced theory, using the concept of quasi-Markovian variables, allows a definition of a variance effective population size, which is found also to be close to $4N_1N_2(N_1 + N_2)^{-1}$.

The expression in [10] bears some discussion. Suppose that $N_1 = 1$ and N_2 is very large, so that there is only one male in the population and a large number of females. The effective population size is then very close to 4. Using this value in [6], it becomes clear that we can expect one or other of A_1 and A_2 to be quite quickly lost from the population. It is clear why this happens. Half the genes in any generation are derived from a single male parent, and the random genetic makeup of this one male ensures a rapid loss of one or other allele from the population.

Similar calculations can be made for a simple Wright–Fisher-type 'gender-less' geographically dispersed population. Here it is found that with even a small amount of migration from one geographical area to another, the eigenvalue effective population size is close to the actual population size. Calculations can also be made for a simple Wright–Fisher-type population whose size assumes cyclically the k values N_1, N_2, N_3, …, N_k, N_1, N_2, … One perhaps thinks in this cycle of an insect population having large summer and small winter sizes. Here the eigenvalue effective population size is close to the harmonic mean of the various sizes taken during any one cycle. This is usually much closer to the smallest size in the cycle than to the largest.

We turn now to the 'simple' Cannings model. By 'simple' we mean that the assumptions made in the simple Wright–Fisher model (on genders, no geography, unchanging population size, etc.) continue to hold. It can be shown for this model that

$$N_e^{(e)} = N_e^{(i)} = N_e^{(v)} = (N - 1/2)/\sigma^2 \qquad [11]$$

where (as above) σ^2 is variance in the number of 'offspring' genes from any parental gene.

It is worthwhile investigating some of the consequences of eqn [11]. Suppose for example that in a diploid population exactly k of the $2N$ parental generation genes are chosen to have 'offspring' genes, and that each offspring generation gene is chosen randomly, without replacement, from these k chosen parental genes. Any given parental gene has either no offspring genes (probability $1 - (k/2N)$) or some random number m of offspring genes, where m has a binomial distribution with index $2N$ and parameter $1/k$. It can be shown that in this case $\sigma^2 = (2N - 1)/k$. Inserting this value in [11] we find that all three effective population sizes are $k/2$. If for example only one individual is chosen to reproduce ($k = 2$) the effective population size becomes 1, as expected. Equation [6] then shows that we can expect that one allele is very soon lost from the population. For any small value of k the same conclusion is reached.

These calculations bear on the importance of the so-called neutral theory. In brief, this theory claims that many gene substitutions in the past did not occur because of selection but because of purely random changes in gene frequencies among selectively neutral genes. Calculations such as those above shows that random substitutions are more likely to arise in populations having a small effective population size than in those having a large effective population size.

More generally, for the Cannings model, eqn [6] should be replaced by

$$-(4N - 2)\{p \log p + (1 - p) \log (1 - p)\}/\sigma^2 \qquad [12]$$

The Cannings model can be generalized to the case where the diploid nature of the population is more explicitly taken into account (Apart from this we make all the simplifying assumptions described above.). Consider then a diploid population of N individuals, and define the diploid inbreeding effective population size $N_e^{(i,dip)}$ as the reciprocal of the probability that two genes taken at random from the offspring generation are both descended from the same diploid parental individual. It can be shown that

$$N_e^{(i,dip)} = (4N - 2)/(\sigma^2_{dip} + 2) \qquad [13]$$

where σ^2_{dip} is the variance of the number of offspring genes from any parent generation diploid individual.

In many cases the effective population size is less than the actual census size. Equation [13] provides an interesting

counter-example. Suppose that each diploid individual is allowed to pass on exactly two genes to the next generation. Then $\sigma^2_{\text{dip}}=0$ and $N_e^{(\text{i,dip})}=2N-1$, almost exactly twice the actual population size. In the simple Wright–Fisher model $\sigma^2_{\text{dip}}=2(N-1)/N$ and $N_e^{(\text{i,dip})}=N$, as expected. If two individuals are chosen to be the parents of all individuals in the offspring generation, with each of the chosen parents contributing exactly one gene to each offspring individual, then $\sigma^2_{\text{dip}}=2N-4$, and $N_e^{(\text{i,dip})}=(2N-1)/(N-1)$, or almost exactly 2, as expected.

The Time Factor

In order to discuss which version of effective population size is appropriate in any given circumstance the time factor has to be taken into account. Both the eigenvalue effective size and the variance effective size (when it can be calculated) look to the future, since they both relate to properties of an offspring generation given the parental generation. By contrast the inbreeding effective population size looks to the past. These facts should be taken into account when considering which version of effective population size is appropriate. When the population size remains constant from one generation to another, all three versions (again, when the variance effective size can be calculated) are either very similar or even identical. Interest in the effective population size concept arises nowadays often because of the need to infer past population properties from current genetic data. When the population size has fluctuated rapidly in the past, a concept of effective population size has been provided by Sjödin *et al.* (2005). These authors point out, however, that when (as with the human population) the population size has steadily increased for many generations, no useful definition of the effective population size has yet been found. It is therefore important to treat with skepticism statements such as 'Fifty thousand years ago the effective population size of the human population was about 25 000'. Such a statement probably means that it is assessed that at that time the breeding size of the human population was about 25 000, and has nothing to do with the concept of an effective population size as discussed above.

Comments about θ

One of the most important quantities in population genetics theory is the parameter θ. Very often it is simply stated that $\theta=4N_eu$ (here u is a mutation rate). There are at least three difficulties with such a statement. First, it is usually not stated which version of effective population size is implied by N_e. Second, as with the human population size in reference to its past history, there might not be any useful concept of an effective population size. Finally, even when these two problems do not arise, the formula $\theta=4N_eu$ implicitly assumes that the population evolved according to the (unrealistic) simple Wright–Fisher model. Other more realistic models, such as the Cannings model, have different formulas for the effective population size (in the Cannings model, the formula for θ is $\theta=4N_eu/\sigma^2$, where (as defined above) σ^2 is the variance of the number of descendant genes from any parent gene). The Moran model has a different formula again for θ, very close to $2N_eu$.

All the above remarks are intended to promote a much more critical use of the effective population size concept than appears all too often in the literature.

See also: Genetic Drift, Models of Random. Genetic Variation in Populations. Neutral Models of Genetic Drift and Mutation

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Endogenous Retroviruses and Coevolution

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Glossary

Endogenous viral element (EVE) A virally derived sequence in a host genome that originates from a germ line integration of a viral genome (or part thereof).
Enhancer A region of DNA that can bind proteins in order to increase the transcription of a gene. Enhancers and promoters are regulatory elements.
Evolutionary arms race An evolutionary conflict between two coevolving sides that adapt and counter adapt to each other.
Homologous recombination The exchange of nucleotide regions between similar genetic sequences.
Monophyletic lineage A group of species in a phylogeny that includes their common ancestor and all descendants.
Nonallelic homologous recombination Homologous recombination that occurs between stretches of DNA that are very similar, but are not alleles.
Nonsense mutation A mutation that results in a frameshift or the introduction of a premature stop codon.
Phylogeny A graphical representation of evolutionary relationships.
Fixation (population genetics) The point at which the population frequency of a genetic variant reaches 100%.
Promoter A region of DNA, which initiates the transcription of a gene.
Recombination hotspots Regions of a genome that are particularly prone to recombination.
Restriction factor A general term for host-encoded gene products of the innate immune system that partially or fully inhibit intracellular viral replication.
Retrotransposon Mobile genetic elements found in eukaryotic genomes that can amplify themselves via an RNA intermediate.
Retrovirus Viruses in the family 'Retroviridae' that possess RNA genomes that are reverse-transcribed into DNA.
Tumorigenic The capacity or potential to form tumors.
Virion The viral particle.
Xenotransplantation The transplantation of cells/tissue/organs from one species to another.

Introduction

Viruses are obligate intracellular parasites that replicate by hijacking their host's molecular machinery. Although the viral life cycle ordinarily takes place in somatic cells, germ line infections can sometimes occur and potentially lead to the inheritance of the virus. Such vertically transmitted viruses are called endogenous viral elements (EVEs; Katzourakis and Gifford, 2010) and they can occasionally reach fixation and persist in the host for millions of years. EVEs contain information about ancient infections that would otherwise not be observable, and for this reason they have been described – metaphorically – as a genomic 'fossil record.' The vast majority of EVEs originate from retroviruses because the life cycles of the latter involve obligate genome integration (**Figure 1**). This subset of EVEs arising from retroviruses is usually referred to separately as endogenous retroviruses (ERVs). The increasing availability of sequenced host genomes has revealed the vastness of the viral fossil record, and led to the emergence of paleovirology – a field dedicated to the study of EVEs. While non-retroviral EVEs have also recently been described, ERVs have contributed to studies of the evolutionary history of retroviruses for decades, and have facilitated investigations into the complex evolutionary relationship between retroviruses and their hosts (Feschotte and Gilbert, 2012; Weiss and Stoye, 2013).

The evolution of retroviruses and their hosts has been shaped by a series of adaptations and counter-adaptations. Adaptations of the host are driven by the selective pressure to restrict viral infections, which in turn inflict pressure on viruses to counter-adapt in order to evade host defenses (Daugherty and Malik, 2012). Such adaptive changes will recur in both coevolving partners, predominantly manifesting at the molecular interface between host immunity and viral countermeasures. The resulting evolutionary 'arms race' can also be observed at various timeframes: from host–virus interactions shortly after the first infection of a host cell, to adaptations in host immunity at deep evolutionary timescales. The detrimental effect of viral integration into the host can be assessed at short timescales, such as the course of a single infection or at the host population level over a few years or decades. One can also examine the host–virus relationship over evolutionary timescales using ERVs, or investigate the lasting effects of the arms race on antiviral genes (Duggal and Emerman, 2012a).

This article considers examples from the literature representing a cross section of studies on host–virus relationships as they relate to paleovirology. After a brief description of ERV classification, we discuss the process of ERV formation and the events thought to occur shortly after viral endogenisation. We then review a variety of different effects that ERVs can have on host biology, before considering how they can be useful tools in the study of both viral evolution and the evolution of host immunity. Finally, we examine how ERV research has begun to tackle broader questions about the evolutionary consequences of the virus–host arms race that will eventually lead to a better understanding of ERVs, viruses, and their respective pathogenicity.

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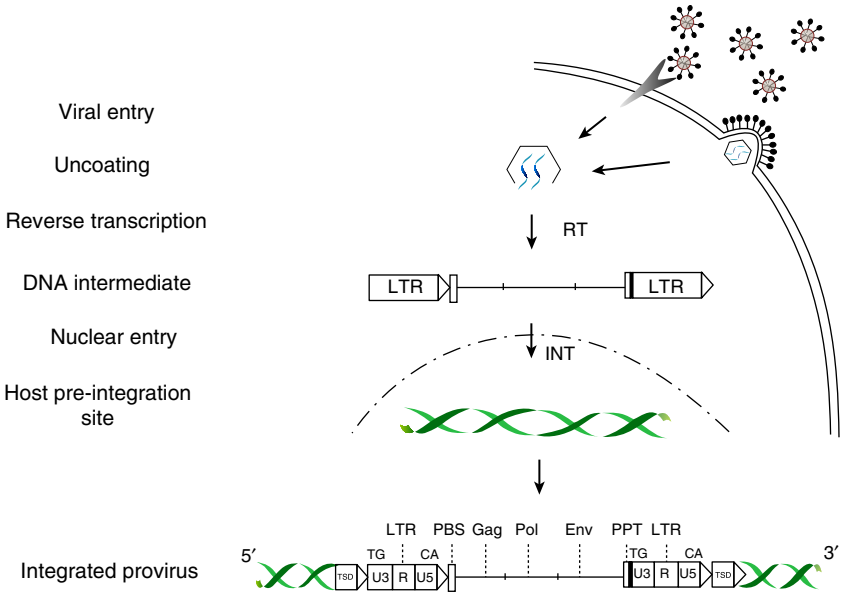

Figure 1 The process of integration. The diagram depicts a stylized cell infected by retroviruses. The virion achieves cell entry by directly fusing to the cell membrane or via receptor-mediated endocytosis. Once inside the cell, the RNA genome is released from the capsid and is converted to double stranded DNA by reverse transcriptase. This DNA intermediate then enters the nucleus where insertion into the host genome is catalyzed by integrase. This process results in the formation of characteristic 'target site duplications' either side of the provirus that are usually 4–6 nucleotides in length.

Classification and Identification of Endogenous Retroviruses

Historically, the classification of ERVs has been complicated by their relationship to both retrotransposons and exogenous retroviruses, both of which have established and distinct classification schemes. Efforts toward a unified system for ERV classification have encountered obstacles when studies that use one naming scheme are compared to studies that use another (Bannert and Kurth, 2006; Blomberg *et al.*, 2009). Early systems attempted to classify ERVs based on their primer-binding site (PBS). However, numerous ERVs use the same PBS, and as more novel families were characterized, it soon became clear that this scheme could not serve as a unified classification system. Contemporary classification of ERVs is based on reverse transcriptase phylogenies that include endogenous sequences and their exogenous relatives, but maintain widely accepted names based on older schemes. Current exogenous retrovirus taxonomy follows conventions set by the International Committee on Taxonomy of Viruses (ICTV). The family *Retroviridae* is subdivided into two subfamilies, the *Orthoretrovirinae* and *Spumaretrovirinae* with the genera *Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, and *Lentivirus* being members of *Orthoretrovirinae* and the genus *Spumavirus* of *Spumavirinae*. ERVs are classified based on relatedness to exogenous retroviruses; Gammaretroviruses are designated as Class I, while Class II and III ERVs are related to betaretroviruses and spuma-like retroviruses, respectively. Although this phylogenetically informed approach has proved capable of classifying highly degraded ERVs that are distantly related to exogenous retroviruses, the three classes have few distinguishing features (such as genomic, morphological, or functional), which limits the utility of the classification system. Recent discoveries place certain ERVs firmly within exogenous genera such as lentiviruses, while certain class I ERVs are in fact more closely related to the epsilonretroviruses than the gammaretroviruses (Katzourakis and Tristem, 2005). Furthermore, retroviruses undergo complex patterns of recombination, posing further limitations on efforts to classify them based on a single gene.

From Exogenous to Endogenous: How an Endogenous Retrovirus Is Formed?

Germ line Integration and Fixation

ERVs typically comprise 5–10% of vertebrate genomes (Lander *et al.*, 2001; Waterston *et al.*, 2002), and the history of these elements can be understood by considering the evolutionary forces at play, beginning with germ line invasion of the host by an exogenous circulating retrovirus. After heritable integration, an ERV will be subjected to genetic drift and its frequency in the population may change by chance due to random sampling. ERVs with a fitness cost will be negatively selected out of the population, and a small proportion of neutral (or nearly neutral) ERVs may eventually reach fixation. In rare instances where an ERV offers a fitness advantage, its population frequency will increase rapidly.

Intragenomic Proliferation

ERVs will proliferate in the host genome if a viable infectious particle can be produced, resulting in the reinfection of germ line cells. This will continue until they are either inactivated by nonsense mutations, or are removed as a result of recombination between their long terminal repeats (LTRs) (**Figure 2**; Stoye, 2001). ERVs that are incapable of producing virions (e.g., because of the inactivation of one of their genes)

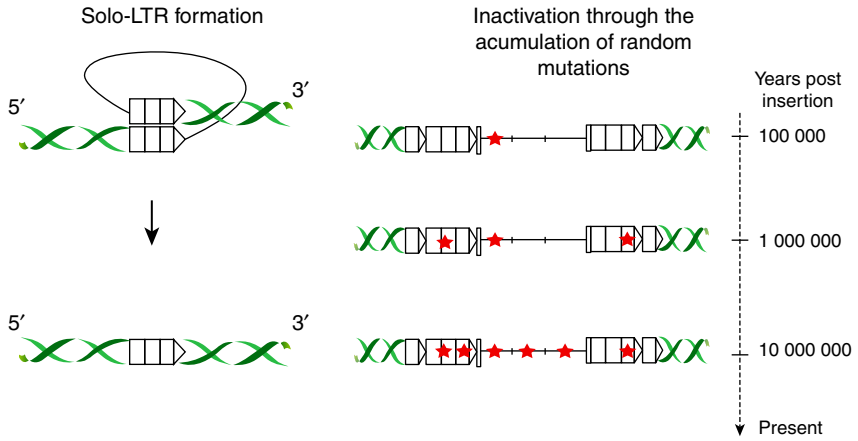

Figure 2 ERV inactivation. The two diagrams shown represent the main mechanisms of ERV deletion or inactivation. Because the long terminal repeats that flank retroviral genomes are identical at the time of integration, accidental recombinational deletion of the ERV can occur resulting in 'solo-LTR' formation. Alternatively, the accumulation of inactivating nonsense mutations over time will render an ERV incapable of producing viral particles or achieving retrotransposition.

can nonetheless proliferate by retrotransposition. Intracellular retrotransposition can occur in *cis* or *trans* where either an intact ERV or other viruses provide the enzymatic activity, respectively (Belshaw *et al.*, 2004). ERV proliferation will lead to the emergence of phylogenetically distinct groups called families that are defined as monophyletic lineages derived from distinct germ line invasions (Tristem, 2000). Once inactivated however, ERVs become subject to the host evolutionary rate that is much slower than that of the exogenous virus. Whereas the inactivation of a single ERV usually occurs within a few thousand years, the fossilization of an ERV family can presumably take several millions of years, and have substantially different evolutionary dynamics.

One way to determine the proliferation mechanism that dominated the early history of an ERV family is by interpreting the patterns of selection that can be detected in their sequences. For example, evidence of post-integration purifying selection in the *env* gene would suggest that the ERV family multiplied by reinfection, since the envelope is a necessary component of mature virions. For human ERV (HERV) families with less than 200 copies, almost all were found to have proliferated by reinfection (Belshaw *et al.*, 2005). However, loss of the *env* gene shifts the mode of ERV amplification from reinfection to retrotransposition, leading to far greater proliferation (Magiorkinis *et al.*, 2012). This is possibly because of the fitness costs to the host associated with reinfection or the expression of *env*, which in turn also determines the survival of the ERV (Magiorkinis *et al.*, 2012).

The Influence of Endogenous Retroviruses on Hosts

The Effect of Endogenous Retroviruses on Genome Architecture

ERV integrations affect the host in a number of ways that can be selectively neutral, detrimental or in rare cases advantageous to the host. We can assess the post-integration effects on genome architecture by considering the potential selective pressure they impose on their hosts based on the site of integration. For example, ERV insertions in genes or other functional regions are rapidly purged from the host population since they usually interrupt essential functions (Nellåker *et al.*, 2012). Integration is likely to be selectively neutral if it occurs in a noncoding region, assuming also that it does not interfere with transcription and can no longer produce infectious particles. However, multiple apparently harmless integrations can have non-neutral downstream consequences by providing recombination hotspots that can significantly alter genome architecture. This is because nonallelic homologous recombination (NAHR) between members of the same ERV family can result in genomic rearrangements such as duplication, deletion, translocation, or inversion (Feschotte and Gilbert, 2012).

In humans, the HERV-K family in particular has undergone extensive recombination since integration (at least 16% of full-length copies) (Hughes and Coffin, 2001). Moreover, some HERV-related genomic rearrangements have been associated with pathogenesis. For example, recent studies of the human genome have shown that structural variation may result from NAHR between HERV elements rich in recombination hotspot motifs (Campbell *et al.*, 2014). For instance, HERV-mediated NAHR has been reported to lead to the deletion of the *eyes absent 1* (EYA1) locus, which can cause branchio-oto-renal syndrome (Sanchez-Valle *et al.*, 2010). Another example is the deletion of the azoospermia factor gene (AZFA or AZF1). The consequent male infertility is the result of a nonreciprocal recombination event of two HERV-I loci on the Y chromosome (Kamp *et al.*, 2000; Sun *et al.*, 2000).

The Effect of Endogenous Retroviruses on Gene Expression

In addition to gene disruption, ERVs can also affect the genome's integrity by influencing their transcriptional regulation. Retroviral insertions near a host gene can modify its regulation by offering alternative promoters or enhancers during transcription. This is because retroviral LTRs contain a promoter for RNA polymerase II along with enhancers and transcription factor binding sites. In humans, for example,

salivary amylase expression was found to be associated with an ERV integration in a tissue-specific manner (Ting *et al.*, 1992). The domestication of an LTR has also been found to promote the expression of neuronal apoptosis inhibitory protein (Romanish *et al.*, 2007). If inserted into introns, ERVs may promote changes in gene transcription by introducing alternative termination sites or novel splice donor and acceptor sites. Such an example is the *slc15a2* locus in mice, where an ERV integration was shown to substantially decrease its transcription as a result of truncated transcripts (Li *et al.*, 2012).

Endogenous Retroviruses, Recombination and the Emergence of New Viral Types

The impact of ERVs extends beyond the direct effect of their integration on host genomes. Indirect consequences include the interaction of ERVs with exogenous viruses or other ERVs that can give rise to novel recombinant viral types. ERVs can be a source of emerging infection if recombination with exogenous viruses leads to the acquisition of novel pathogenicity (Sheets *et al.*, 1993). This can happen through the replacement of the *env* gene with one of different viral origin, and since *env* plays a key role in determining viral tropism, it can facilitate cross-species transmission of the virus. A typical example of *env* acquisition that has led to a new viral type is the feline leukemia virus type B (FeLV-B), which emerged after the endogenised FeLV sequences acquired an *env* gene by the exogenous circulating FeLV-A viruses (Overbaugh *et al.*, 1988; Stewart *et al.*, 1986).

Even more complex patterns of recombination that result from a series of sequence exchanges between viruses have also been described. The RD-114 virus is a product of recombination between the *gag-pol* region of the *Felis catus* endogenous retrovirus (gammaretrovirus) and the *env* locus of baboon endogenous retrovirus (BaeV; gammaretrovirus; van der Kuyl *et al.*, 1999). BaEV is in turn a chimeric virus formed by the *gag-pol* region of *Papio cynocephalus* endogenous retrovirus (PcEV; gammaretrovirus) and the *env* of a beta-like retrovirus. Furthermore, PcEV is the product of a recombination event between the *gag-pol* region of a betaretrovirus and the *env* region of a gammaretrovirus (van der Kuyl *et al.*, 1999). RD-114 is therefore the result of recombination between four distinct retroviruses from two different families. Members of the deltaretrovirus genus perhaps represent an entire clade of recombinant viruses in that their *env* gene appears to have been derived from gammaretroviruses (Manel *et al.*, 2005). Even though they are phylogenetically distant, gamma- and delta- *env* genes exhibit identical modular organization and they seem to utilize the same receptors in order to achieve cell entry (Manel *et al.*, 2005).

Recombination between two ERVs may also repair inactivating mutations and give rise to viral sequences with novel pathogenic characteristics, which in turn can lead to new disease phenotypes. Porcine endogenous retroviruses have been shown to spontaneously recombine, resulting in ERVs that are capable of infecting human cell lines, which has serious implications for pig-to-human xenotransplantation (Bartosch *et al.*, 2004). Furthermore, *in vivo* models have linked the emergence of recombinant viruses with potential pathologies, such as that of retrovirus-induced cancers (Young *et al.*, 2012). For example, it has been shown that infectious Murine Leukemia Viruses (MLVs) are produced by recombination between replication-incompetent ecotropic and non-ecotropic endogenous MLVs in antibody-deficient mice (Young *et al.*, 2012).

Occasional Beneficial Effects of ERVs on Hosts

ERVs can have a detrimental effect on their hosts by directly altering either genome architecture or gene expression, or indirectly by contributing to the emergence of new viral types through recombination. However, the flow of viral genomes into that of their hosts can also generate genetic novelty. Rather than being detrimental, ERV-mediated changes to expression can have a positive influence on the host. For example, the antiviral restriction factor APOBEC3G was found to be upregulated by an LTR insertion (Sanville *et al.*, 2010). As well as the beneficial co-option of LTRs, viral genes can also be co-opted by the host, and while this is extremely rare, such examples have been described in the literature. Domesticated ERV genes can perform important physiological functions – *syncytin*s being the most notable example. *Syncytins* are *env*-derived genes that contribute to the development of the placenta. Specifically, they facilitate the cell–cell fusion of the syncytial cell layer in the syncytiotrophoblast. Evidence shows that *syncytins* were the result of at least eight independent domestication events in at least six mammalian orders (Mi *et al.*, 2000; Lavialle *et al.*, 2013; Cornelis *et al.*, 2014).

As well as syncytins, a number of ERV genes have been captured as part of the evolutionary arms races and repurposed to fulfill an antiviral role. A number of these ERV-derived immunity (EDI) genes have been recognized and function by a range of mechanisms (Aswad and Katzourakis, 2012). Among them is the Friend-virus-susceptibility-1 (*Fv1*) restriction factor, which has been identified in mice and originates from the *gag* region of an endogenous retrovirus (Best *et al.*, 1996). *Fv1* restricts retroviral infections in the early stages of viral entry by interfering with the viral capsid (Best *et al.*, 1996). As well as *Fv1*, other EDIs that prevent viral infections originate from the *env* gene. For example, *FV4* is a defective *env* in mice which when expressed results in the occupation of cell-entry receptors, thereby preventing the binding of incoming viruses (Taylor *et al.*, 2001).

The impact of ERVs on their host can be studied based on the nature of the interaction, i.e., the direct effects of integration or the indirect consequences such as their influence on emerging infection. The outcomes of integration can also be categorized based on their selective effect, and both of these consequences of integration can be examined across the different evolutionary timescales at which they occur. For example, the consequences of a retroviral insertion into a host gene will take effect almost instantly, whereas the co-option of a viral gene is likely to take time before the selective pressure from a viral threat drives the emergence of a novel function. The timescale at which intragenomic proliferation occurs is more continuous, and is likely to be ongoing for millions of years, which in turn will influence the more discrete processes that occur at short and long timescales.

Endogenous Retroviruses as Tools in the Study of Host–Virus Coevolution

ERVs can also be used to study the evolutionary history of host–virus arms races by identifying their influence on both viruses and host immunity. Arms races are a distinct form of natural selection in that the selective pressure on viruses and hosts is highly recurrent (Daugherty and Malik, 2012). Moreover, because viruses evolve more rapidly than their hosts, they have an opportunity to quickly evade antiviral defenses in the cell. Stretched over evolutionary timescales, the evidence of this rapid adaptation will erode from the exogenous viral sequences. However, evidence of past evolutionary arms races can be detected in ERV sequences, as can the selective pressure past viruses may have imposed on restriction factors such as APOBEC, TRIM, SAMHD1, and Tetherin. In primates, APOBEC3G-derived mutations have been found in members of the HERV-K (HML2) family, indicating that the hypermutation-induced restriction of retroviruses by APOBEC3G had evolved long ago (Armitage *et al.*, 2008). This is consistent with the evidence of positive selection on APOBEC3G genes that has persisted for millions of years (Sawyer *et al.*, 2004).

Different host lineages will have been subjected to infection by different viruses at different times, and we can consider the variable effect this could have had on their respective restriction factors by examining the signatures of selection in their sequences. TRIM5α inhibits retroviral entry by recognizing the capsid protein and interfering with uncoating (Stremlau *et al.*, 2006). Selection analysis of *TRIM5* and other *TRIMs* such as *TRIM22* across multiple primate lineages revealed that positive selection has mainly acted on either *TRIM5* or *TRIM22* in a given lineage, but normally not on both (Sawyer *et al.*, 2007). A possible explanation for the mutually exclusive selection on *TRIM5* and *TRIM22* could be that they target different viral strains. This changing selection pressure can also result in the complete loss of a restriction factor. For instance, in carnivores, there were at least two independent *TRIM5* inactivation events in canines (gene disruption; Sawyer *et al.*, 2007) and felines (gene truncation; McEwan *et al.*, 2009), which is evidence for an unknown past viral threat that had been eradicated without replacement. These findings are consistent with the observation that dogs have an uncharacteristically low ERV copy number (Martínez Barrio *et al.*, 2011). Together, these studies suggest that the host repertoire of antiviral defense genes has a high turnover and is shaped by the specific viral threat(s) at a given time.

The inferences one can make about past viruses using only the information imprinted on host genes is indirect evidence of ancient host–virus arms races. Indeed, such approaches have been dubbed 'indirect paleovirology' (Patel *et al.*, 2011; Aswad and Katzourakis, 2012) and allow one to easily and cheaply investigate ancient virus–host arms races retrospectively. These ancient molecular battles can also be studied by creating chimeric viruses that are capable of infection and contain ERV sequences *in vitro*. For example, the capacity of APOBEC3-A, -B, -F, and -G to induce hypermutations in HERV-K have been demonstrated in cell cultures infected with artificially reconstructed viruses (Lee *et al.*, 2008). Similarly, functional envelopes of endogenous chimpanzee retroviruses 1 and 2 (CERV) were reconstructed to investigate the host range of this now extinct retrovirus, revealing the specific entry receptor it uses as CTR1 (Soll *et al.*, 2010). Interestingly, while no copies of CERV have been found in the human lineage, the resurrected CERV was nonetheless capable of infecting human cells, which raises questions about the barrier to endogenisation in humans. A different study that investigates the absence of CERV in humans determined that CERV was insensitive to TRIM5 inhibition, ruling out another explanation for its absence (Perez-Caballero *et al.*, 2008). *Tetherin* is another antiviral factor that was initially characterized as an inhibitor of HIV1, where it prevents release of newly formed virions by 'tethering' them to the cell surface (Neil *et al.*, 2008). It has been shown that *tetherin* has a broad viral activity, with the ability to restrict many enveloped viruses, including HERV-K (Neil, 2013). Interestingly, however, the most recently active family of HERVs (HERVK-HML2) were shown to inhibit *tetherin* activity *in vitro* (Lemaître *et al.*, 2014). Furthermore, this anti-*tetherin* activity was also found against the *tetherins* of other primates that also possess HERVK-HML2, suggesting that the property was advantageous to its proliferation in the lineage (Lemaître *et al.*, 2014).

Endogenous Retroviruses in the Study of Viruses and Virus–Host Interaction at Evolutionary Timescales

The study of ERVs goes beyond that of their causes and consequences; they can be used to understand the evolutionary history of viruses. ERVs are direct evidence of past infections that allow us to reach further back into the evolutionary history of viruses than is possible with contemporary data alone, thereby enabling us to characterize host–virus interactions at evolutionary timescales. Not only can this change an estimate of the age of a viral group, but it can also reveal previously unrecognized hosts. For example, foamy viruses are a group of complex retroviruses that infect a range of mammal hosts, and the identification of an endogenous foamy virus in the two-toed sloth (*Choloepus hoffmanni)* expanded their host range to include Xenarthrans. When combined with both biogeographic data and phylogenetic evidence of coevolution, this finding suggested that the close association between foamy viruses and their hosts had been maintained for over 100 million years (Katzourakis *et al.*, 2009). Foamy virus ERVs that confirm this timeframe were later identified in a number of other species, including the aye-aye (*Daubentonia madagascariensis*) and Cape golden mole (*Chrysochloris asiatica*) (Han and Worobey, 2012a; Katzourakis *et al.*, 2014).

Another clear example of what ERVs can reveal about deep virus evolution was demonstrated for lentiviruses, which include HIV and were thought to be only thousands of years old based on molecular clock dating, or at most a few million years old if inferring from co-speciation with their hosts (Holmes, 2003). However, the identification of an endogenous lentivirus (RELIK) in the genome of rabbits allowed us to confidently estimate a minimum age of $\sim$7 million years based on LTR-dating (Katzourakis *et al.*, 2007). This date was pushed back even further at least 12 million years with the discovery of orthologs in the European hare and weasel family (Keckesova *et al.*, 2009; Han and Worobey, 2012b). These studies were not only surprising in terms of how old we

thought lentiviruses were, but also revealed a much broader host range (at least in the past), which could have implications for the identification of reservoir species. Furthermore, analysis of RELIK revealed a much simpler genome that lacked contemporary features such as the HIV *vif* accessory gene (Katzourakis *et al.*, 2007) that defend the virus against host restriction factors (Larue *et al.*, 2010).

Investigating the lasting effect of antiviral factors on ERVs and demonstrating their efficacy *in vitro* has allowed us to understand the long-term dynamics of virus–host arms races, while studies of ancient foamy viruses and lentiviruses using ERVs have revealed their date of origin and other aspects of evolution. Furthermore, in addition to identifying previously unknown hosts, we can use ERVs to study the changes in host ranges in such large timescale studies of virus–host coevolution. In the case of foamy viruses, for example, discordance between the topology of host and viral phylogenies indicates at least two interspecies transfers in their evolutionary history (Katzourakis *et al.*, 2014). In gammaretroviruses, a major spillover has been revealed by the phylogenetic reconstruction of MLV-related viruses in vertebrates, indicating that certain mammalian viral strains emerged from a bird-to-mammal cross-species transmission (Martin *et al.*, 1999). Furthermore, phylogenetic analysis of endogenous betaretroviruses in bats indicates that interspecies transfer is particularly prevalent, and it has been suggested that this transmissibility could be the result of adapting to evade host defenses that also act as interspecies barriers (Hayward *et al.*, 2013).

Whether we investigate their pathological consequences, or use them to reveal ancient evolutionary history, ERVs enhance our understanding of viral biology and virus–host interaction that can in turn be applied to clinical intervention and epidemiological strategies. In addition to understanding their direct consequences on host genomes and effects on host immunity, we are beginning to build a broader picture of the extent to which ERVs have influenced host evolution. For example, the recent finding that the genomes of larger mammals harbor fewer ERVs raises questions about the mechanisms employed to control their endogenisation and proliferation (Katzourakis *et al.*, 2014). This observation has been linked to Peto's paradox, which is the observation that larger mammals do not exhibit higher rates of cancer despite a greater risk due to their size. It could be that the same mechanisms that control cancer emergence in larger hosts are also responsible for controlling ERVs, which themselves impose a tumorigenic risk (Katzourakis *et al.*, 2014). Answers to such fundamental questions are now being sought through the study of ERVs, which also continue to inform us about the evolutionary processes of viruses and their interaction with host immunity. In parallel to the continued effort to understand the influence of ERVs on host genomes, pathogenesis and broad evolutionary patterns, the study of non-retroviral endogenous viruses are rapidly opening new avenues of enquiry (Katzourakis, 2013; Feschotte and Gilbert, 2012; Aswad and Katzourakis, 2012). As more non-retroviral EVEs are described, we will be able to both investigate insights that emerged out of the study of retroviruses, as well as ask new questions that are unique to each non-retroviral group.

See also: Antagonistic Interspecific Coevolution. Coevolution, Introduction to. Cospeciation. Intraspecific Coevolutionary Arms Races. RNA Viruses, Evolution of. Transposable Elements, Population Genetics of

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Endophytic Microbes, Evolution and Diversification of

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Glossary

Antiherbivory Deterrence of consumption of plant by herbivores.
Ascomycota Phylum of fungi.
Ascospore Sexually produced spore of the Ascomycota.
Balansia Genus of fungal endophytes in warm-season grasses.
Biotrophic Feeding on living organisms.
Clavicipitaceae Family of Ascomycota.
Conidia An asexual reproductive spore produced by fungi.
Epichloë Genus of fungal endophytes in cool-season grasses.
Ergot alkaloids Toxic chemicals produced by fungi in the family Clavicipitaceae.
Fungivore Animal or insect that eats fungi.
Herbivore Animal or insect that eats plants.
Heterothallic Having multiple mating types.
Homopteran Insect in order Homoptera.
Intercellular Growing in spaces between cells.
Intracellular Entering into the cell itself.
L-form Intracellular form of a bacterium that loses cell walls.
Muller's ratchet Accumulation of deleterious mutations in asexual populations of organisms.
Mycorrhizae Fungi that grow on roots and assist plants to absorb soil nutrients.
Necrotrophic Feeding on dead organisms.
Perithecia Flask-shaped structure containing ascospores of fungus in Ascomycota.
Sclerotium Seed-like resistant structure produced by a fungus.
Spermatia Fungal reproductive cells comparable to sperms of animals.
Stromata Fungal structures on which reproductive structures or cells may form.

Introduction

In this article we examine the evolution of endophytic microbes of plants and some of the factors affecting evolution of endophytism. Endophytes are typically metabolically active microbes (usually bacteria, fungi, or algae) that enter into healthy tissues of plants without causing symptoms of disease (Stone *et al.*, 2000; Schulz and Boyle, 2006; Compant *et al.*, 2008; Cheplick and Faeth, 2009; Reinhold-Hurek and Hurek, 2011) (see **Figures 1** and **2**). The presence of endophytes in plant tissues cannot usually be detected without application of microscopic, culturing, or molecular methods of detection (White, 1987; Hardoim *et al.*, 2015). Mycorrhizae are often systemic root endophytes of plants, but in this article we will predominantly focus on the kinds of endophytes that may become systemic in aerial plant parts, including stems, leaves, flowers, and seeds (Rodriguez *et al.*, 2009).

Endophytic microbes have been shown to enhance the fitness, competitiveness, and sometimes growth of host plants (Clay, 1988; James *et al.*, 2002; Rudgers and Clay, 2008; Puente and Bashan, 2009; Janir *et al.*, 2010; Paungfoo-Lonhienne *et al.*, 2010; Beltran-Garcia *et al.*, 2014). In some cases endophytes have been shown to produce secondary metabolites that deter pathogens or herbivores of plants (White, 1987; Clarke *et al.*, 2006; Ongena and Jacques, 2008; Ambrose and Belanger, 2012; Gond *et al.*, 2014). Some endophytic microbes have been found to enhance tolerance of plants to abiotic stresses (Malinowski and Belesky, 2000; Redman *et al.*, 2002; Waller *et al.*, 2005; Kuldau and Bacon, 2008). The beneficial effects of endophytes on plants have increased interest in use of endophytic microbes to enhance agricultural production of crop plants.

In this article we will discuss features of some common types of endophytes and how these microbes evolved and adapted to plant hosts. Two important groups of endophytic

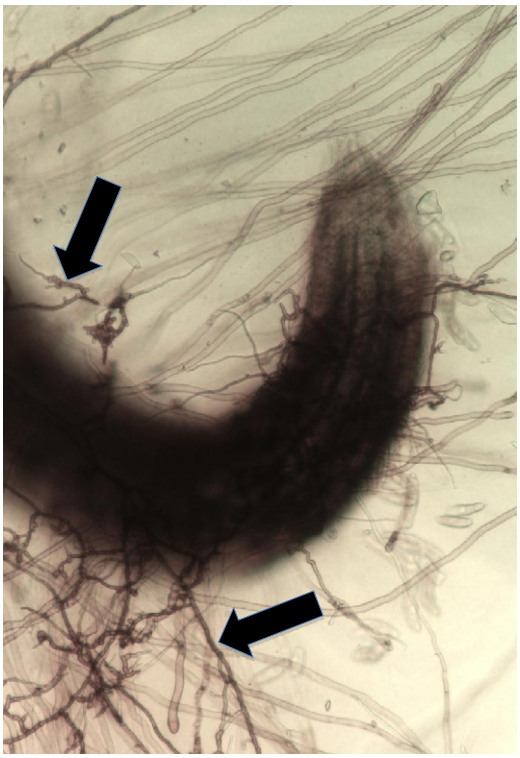

Figure 1 Endophytes in plant tissues. Endophytic hyphae (arrows) emerging from roots of *Phragmites australis* (100 ×).

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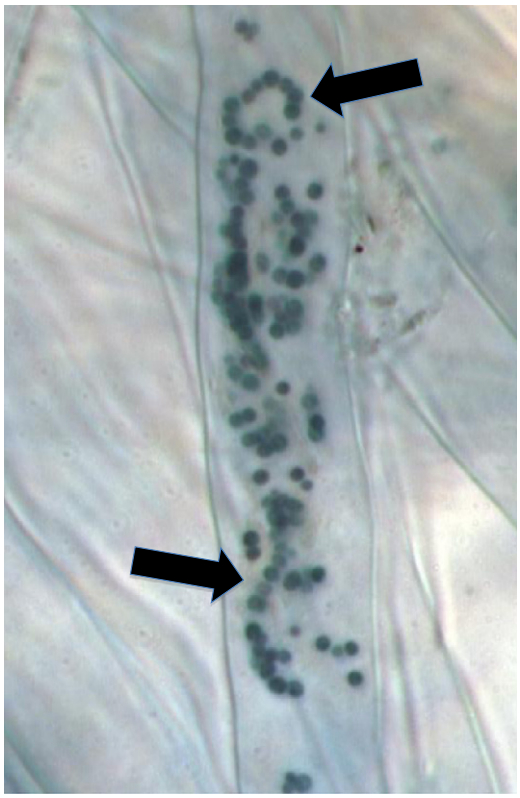

Figure 2 Endophytes in plant tissues. Endophytic bacterial L-forms (arrows) located within root hair cells of a candle cactus (*Subpilocereus repandus*) (1000 ×).

microbes include the class I and class II endophytic microbes (Rodriguez *et al.*, 2009). Class I endophytes are typically systemic in leaves, stems, and seeds, are obligately endophytic, and are often seed transmitted, entering into the embryo within seeds of host plants (Schardl *et al.*, 2004; Selosse and Schardl, 2007; Tadych *et al.*, 2014). The class II endophytes become systemic in plants but they are not obligately endophytic, and often they bear very close relationship to soil microbes or pathogens (Compant *et al.*, 2010; Johnston-Monje and Raizada, 2011). Class II endophytes do not show evidence of coevolution with any particular host and may move horizontally between hosts of diverse plant families. Class II endophytes may be seed transmitted but typically they are vectored on the surfaces of seeds rather than within embryos.

Class I Endophytes

The typical evolutionary path of class I endophytes can be illustrated by examination of the development of endophytism in the fungal family Clavicipitaceae (Ascomycota). In this family the ancestral state is insect pathogenic fungi like *Cordyceps* (Spatafora *et al.*, 2007). These species infect insects and use their mummified bodies to obtain the nutrients to produce reproductive structures (perithecia and ascospores). They generally do not obtain nutrients from plants. Some of these insect necrotrophs have adapted to obtain nutrients from scale insects that are themselves biotrophic parasites of plants (White *et al.*, 2003). Scale insects attach to plant tissues and use their stylets to penetrate the plant epidermis and tap into the nutrients in plant vascular tissues. Scale insect pathogens in the fungal genera *Hypocrella*, *Dussiella*, *Torrubiella*, *Hyperdermium*, and *Ascopolyporus* obtain nutrients from plants by consuming the scale insects and then using the nutrient flow from the plants to produce their own reproductive structures (Sullivan *et al.*, 2000; Bischoff *et al.*, 2004; Bischoff and White, 2004; Bischoff *et al.*, 2005). These fungal species are necrotrophic on insects and biotrophic on plants; however, they depend on the insect to create the stylet wound through which they obtain plant nutrients. The production of ergot alkaloids is notably high in these species that obtain nutrients from plants through parasitizing insects, whereas these alkaloids are generally not detectable in forms that feed exclusively on insect tissues (Bacon *et al.*, 1977; Torres *et al.*, 2008). It has been suggested that the abundant nutrients available in plant tissues enabled the fungi to invest in defensive secondary metabolites like ergot alkaloids; in addition, exposure on surfaces of plants make fungi vulnerable to predation, increasing the need for defensive compounds (Torres *et al.*, 2008).

In the family Clavicipitaceae, endophytism developed in species that adapted to live exclusively on nutrients available in plants. Among these species are those classified in the non-endophytic genus *Claviceps*. These species developed the ability to infect ovaries as a way to obtain plant nutrients (Bischoff *et al.*, 2004). *Claviceps* ascospores infect an ovary of a grass, suppress ovary development, and then produce a sclerotium using the nutrients that would have gone into the developing ovary. The resistant sclerotium later germinates to form a stalked stroma bearing perithecia and ascospores that may be ejected into the air to infect additional grass ovaries. *Claviceps* is restricted to ovule tissues, essentially replacing seeds.

Species of genera *Epichloë* and *Balansia* made the final step to endophytism by evolving meristem-infection mechanisms (White *et al.*, 1995; Tadych *et al.*, 2012, 2014). In *Epichloë*, the fungus produces conidia or ascospores that germinate on host tissues to form a mycelium that at first grows epiphytically onto the nutrient-rich grass shoot meristems, and then proliferates intercellularly within tissues of developing stems and leaves (Tadych *et al.*, 2012). As the plant meristem grows, the endophytic hyphae remain in the plant tissues as they differentiate and mature (White and Cole, 1986). Endophytes of genus *Balansia* adapted to warm-season (Panicoid) grasses, while species of genus *Epichloë* are endophytes of cool-season (Pooid) grasses (Bischoff *et al.*, 2004).

The *Epichloë* Life Cycle and Its Evolutionary Reduction

To understand factors affecting evolution of endophytism it is illustrative to examine the development and life history of *Epichloë*. *Epichloë*, classified as an endophyte because that is its predominant mode of living, forms two types of reproductive structures on grasses. The sexual reproductive structures form on fungal stromata that develop on plant inflorescence primordia (White and Bultman, 1987). A stromal mycelium forms as the inflorescence primordium expands, producing a stroma that embeds and permeates a living but modified inflorescence (White *et al.*, 1991; White and Bultman, 1987). Inflorescence

development is arrested and cuticular barrier layers do not form, permitting free flow of water from plant inflorescence to the mycelial stromal layers on its surface (White *et al.*, 1997). On the surface of the stromal mycelium a layer of spermatia are formed. *Epichloë* is heterothallic with two mating types; spermatia of one mating type must be transferred to a stroma of the opposite mating type to initiate sexual crossing and perithecial development on the stroma surface (White and Bultman, 1987). Symbiotic flies vector spermatia between stromata of the opposite mating type to initiate fertilization and perithecial formation (Bultman *et al.*, 1997).

Epichloë also forms asexual conidia on mycelium on surfaces of leaf blades (White *et al.*, 1996; Moy *et al.*, 2000). The epiphytic conidia of this stage are water dispersed and will not release from the conidiophores unless water is present (Tadych *et al.*, 2007). The conidia may flow off leaves to tillers or seedlings that grow in the vicinity of grass plants (Tadych *et al.*, 2012; Wiewióra *et al.*, 2015). A third means of reproduction of *Epichloë* endophytes is clonal, in which endophytic mycelium from maternal plants colonize developing ovaries and then embryos in seeds (White and Cole, 1986; Selosse and Schardl, 2007). This clonal mode of reproduction has been referred to as vertical dissemination; infection of neighboring plants is considered horizontal dispersal (White, 1988; Wiewióra *et al.*, 2015).

Water restriction is probably the main selective force that may cause reductions in the life cycles of *Epichloë* endophytes. This is due to the vulnerability of *Epichloë* reproductive stages to water restriction. Stromata lose water rapidly and in its absence stromata are selected against (White *et al.*, 1993; White and Camp, 1995). Grasses in very dry or rain-restricted areas will often contain *Epichloë* endophytes that grow very slowly in culture and are incapable of forming conidia, suggesting loss of capacity to reproduce by stromata or conidia. These totally sterile endophytes reproduce exclusively clonally through dissemination in seeds of the maternal plant (White and Cole, 1986; White and Morgan-Jones, 1987). Totally sterile endophytic strains represent the ultimate in reduction of the *Epichloë* endophyte life cycles because these strains cannot form stromata for sexual reproduction or disseminate horizontally via conidia.

Hybridization and Diversification

One important mechanism for diversification of endophytes such as *Epichloë* is through hybridization. Christopher Schardl and collaborators over several years have shown that many endophytes, especially those that do not show capacity to produce perithecia and ascospores, contain evidence of genome hybridization between distinct endophytes (Schardl *et al.*, 1994; Clay and Schardl, 2002; Moon *et al.*, 2002, 2004; Selosse and Schardl, 2007). Hybridization was proposed to be a way that asexual endophytes may increase their gene diversity and overall survival capacity, perhaps counteracting the accumulation of mutations resulting from loss of sexual reproduction and 'Muller's ratchet' (Selosse and Schardl, 2007).

Examples of Diversification into Different Plant Groups

Pseudocercosporella Trichachnicola

An endophytic fungus, *Pseudocercosporella trichachnicola*, was found to be widespread in the warm-season grass species *Trichachne insularis* (White *et al.*, 1990). *Pseudocercosporella* intercellularly colonizes leaves, culms, and seeds of its host through use of endophytic mycelium that appears to grow in meristematic tissues of leaves and tillers. The near relatives of *Pseudocercosporella* are plant pathogens. It thus seems reasonable that the ancestors of the *Pseudocercosporella* endophyte were pathogens. The *Pseudocercosporella* endophyte is comparable to the *Epichloë* endophytes in its life cycle, and also in that it likely coevolved with and is widespread in its host plant. As far as is known, this endophyte is also predominantly vertically transmitted in seeds in a way comparable to the highly reduced forms of *Epichloë* endophytes.

Morning Glory Symbionts

Some species of morning glories (Convolvulaceae) have been shown to contain systemic fungi in the genus *Periglandula* (Clavicipitaceae) in leaves, stems, and seeds (Steiner *et al.*, 2011). These epibionts have been found to produce high levels of ergot and other toxic alkaloids that are believed to be antiherbivory compounds. These fungi like class I endophytes, appear to be closely coevolved and obligate associates of their host plants. Before discovery of the morning glory symbionts it was generally believed that morning glories were the source of the toxic ergot alkaloids contained within their tissues. However, it is now clear that fungal symbionts are the source of the alkaloids that toxify morning glories.

Class II Endophytes

Class II endophytes are facultatively associated with hosts and there is little evidence that they coevolved with any particular host (Compant *et al.*, 2008; Rodriguez *et al.*, 2009). Examples of class II endophytes are those that are recruited from soils or from adjacent plants to systemically colonize hosts (Campisano *et al.*, 2014). The bacterial endophyte *Bacillus amyloliquefaciens*, widespread in soils and in plants (Gond *et al.*, 2014), is a good example of this type of endophyte. In recent studies we have encountered *B. amyloliquefaciens* as a systemic endophyte of vanilla orchids (White *et al.*, 2014a), grasses, and English Ivy; in these species the bacterium colonizes external and internal tissues of roots, stems, leaves, and seeds (Soares *et al.*, 2015). The bacterial endophyte can be spread both vertically on surfaces of seeds and horizontally by water splash to uninfected adjacent plants of diverse species. Unlike the class I endophytes, a strain of the bacterium from one host may be readily inoculated into a host of another plant species. One peculiar feature of many bacterial endophytes is that they may become wall-less 'L-forms' within shoot or root cells (**Figure 2**; White *et al.*, 2014b). In this endosymbiotic form the bacteria are thought to be able to evade host cell-defenses. The bacteria appear to masquerade as intracellular organelles, thus giving a clue as to how organelles may have evolved.

Fungal endophytes belonging to diverse genera (e.g., *Fusarium*, *Alternaria*, etc.) also belong to this class of endophyte. Evidence from studies of many plants suggests that plants may host communities of endophytes (Zambell and White, 2014). In a study of green brier (*Smilax* spp.) it was

found that stems were colonized by three dominant species of endophytes that tended to partition themselves in distinct zones within stems (Zambell and White, 2014). Some class II endophytes may also become pathogenic under certain circumstances. An example here may be seen in the *Diplodia mutila* endophyte of the tropical palm tree *Iriartea deltoidea* (Alvarez-Loayza *et al.*, 2011). *Diplodia mutila* occurs as a pathogen and perhaps endophyte in a wide array of plants. This endophyte is transmitted as a mycelial and conidial crust on surfaces of the palm seed. Seedlings bear the endophyte within leaves and stems. When these seedlings grow in the shaded understory of the forest, the fungus is mutualistic and protects seedlings from insect herbivory; however, when seedlings grow in full sunlight, the fungus reverts to a pathogenic necrotrophic phase and induces necrosis in leaves and stems of plants, often resulting in mortality of seedlings.

Parasitism–Mutualism Continuum

Diplodia mutila is a good example of the parasitism–mutualism continuum concept of Schulz and Boyle (2005) who postulated that beneficial effects (mutualism) of endophytic microbes are dependent on a specific set of conditions, while in some other conditions, the same organism may be a deleterious parasite of the host (Saikkonen *et al.*, 1998; Schulz *et al.*, 1999). Whether the endophyte persists as a widespread symbiont of the host may depend on its relative survival value to the host in its environment. Plants grown under ideal conditions may be expected to lose endophytic microbes; this has been observed in the case of plants grown in greenhouse culture (personal observation).

Host Role in Regulation of Endophytic Microbes

Fossil evidence has revealed that the earliest land plants were colonized by endophytic microbes (Taylor *et al.*, 2015), and modern plants are colonized by communities of microbes that enter into their tissues (Arnold *et al.*, 2003; Unterseher *et al.*, 2013; Zambell and White, 2014; White *et al.*, 2014b). The fact that some of the endophytic microbes also have the capacity to be pathogens makes it imperative that plants have mechanisms to control pathogenic behaviors of endophytes. It seems likely that many of the secondary metabolites produced by plants that have been thought to function in defense are actually produced to regulate endophytic microbes in plants (Ku *et al.*, 2013; Torres *et al.*, 2011; Mandal *et al.*, 2010; Tadych *et al.*, 2015). Such compounds may include those that suppress pathogenicity traits of microbes (Tadych *et al.*, 2015) such as rapid unchecked growth and production of reactive oxygen that would induce host defensive reactions. Tadych *et al.* (2015) demonstrated in cranberry fruits that the secondary metabolites quinic and benzoic acids reduced mycelial growth and suppressed reactive oxygen secretion of several fungi that colonize developing fruits. Investigators have shown that caffeic acid from mulberry leaves suppresses pathogenicity of the bacterial pathogen *Streptococcus faecalis*, a common microbe of the silkworm intestinal community (Koike *et al.*, 1979). When silkworms are removed from mulberry leaves and fed on an artificial diet, the *Streptococcus* pathogen inflicts disease and kills its silkworm host. These few examples are evidence that plants produce an array of compounds that evolved to regulate endophytic microbes. The full diversity of those compounds and their modes of action to regulate endophytic microbes remain to be discovered.

Two major types of systemic endophytes, classes I and II, differ from one another in the degree to which they have coevolved with hosts. Class I endophytes are closely coevolved with hosts and obligately endophytic, while class II endophytes are not closely coevolved with hosts and generally facultatively endophytic. Among the class I endophytes evolution of defensive secondary metabolites and availability of moisture are factors that played roles in evolutionary development and loss of sexual reproduction of the endophytes. Among the class II endophytes environmental factors, host suppression of pathogencity, and capacity of the endophytes to avoid elicitation of defensive responses of hosts may be important factors in evolution of endophytism.

See also: Coevolution, Introduction to. Ecological Fitting and Novel Species Interactions in Nature. Mycorrhizal Fungi, Evolution and Diversification of. Protist Diversification

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Endosymbiotic Theory

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Glossary

Aerobic organisms Aerobic organisms live in oxic environments. Obligate aerobes are strictly dependent on oxygen and need it to grow. Aerobes usually use oxygen as the terminal electron acceptor in energy metabolism.

Alveolates The alveolates are a group of unicellular eukaryotes, including dinoflagellates, apicomplexans, and ciliates.

Amoebozoa The Amoebozoa are one eukaryotic supergroup containing ameboid microorganisms.

Anaerobic organisms Anaerobic organisms do not require oxygen for growth. For obligate anaerobes oxygen is harmful, because various enzymes of anaerobes are readily inactivated by oxygen.

Apicomplexa Apicomplexa are a large group of obligate parasitic, unicellular eukaryotes belonging to the supergroup Chromalveolata.

Archaeplastida The Archaeplastida are one eukaryotic supergroup containing glaucophyta, rhodophyta, and chlorophyta (with land plants).

Autotrophy Autotrophic organisms are able to produce organic compounds (food/energy-source) from inorganic ones using light or chemical reactions.

Chlorarachniophytes Chlorarachniophytes are ameboid eukaryotes belonging to the rhizaria. They possess secondary (green) plastids with four membranes and a nucleomorph.

Chlorophytes The lineage of Archaeplastida having green primary plastids.

Endosymbiosis One cell living in stable symbiosis within another.

Facultative anaerobic organisms Facultative anaerobes are able to grow with or without oxygen.

Fermentation Enzymatic conversion of organic compounds (sugars) into acids, gases, or alcohol.

Functional redundancy (through endosymbiosis) The retention of divergent but homologous gene copies donated both by host and endosymbiont at organelle origin for the same functions (e.g., ribosomal proteins of chloroplasts, mitochondria, and the cytosol).

Glaucophytes A group of unicellular algae with plastids that still possess a rudimentary peptidoglycan wall and that together with the rhodophytes and the chlorophytes comprise the eukaryotic supergroup Archaeplastida.

Heterocyst Differentiated cells of some cyanobacteria that are specialized for nitrogen fixation.

Heterotrophy Heterotrophic organisms use reduced organic substances as their source of carbon.

Hydrogenosome Hydrogenosomes are anaerobic mitochondria that have a double membrane and synthesize ATP via hydrogen-producing fermentations. They lack cytochromes, a membrane-associated electron transport chain, and (except in rare cases) a genome. Hydrogenosomes arose several times independently in evolution and can be found in trichomonads, anaerobic ciliates, and some fungi.

Methanogen Methanogens are archaea that produce methane as a metabolic byproduct of core energy metabolism.

Mitosome Mitosomes are organelles of mitochondrial origin that do not produce ATP. They have retained components of FeS cluster assembly or sulfate activation.

Nucleomorph Found only in some groups of eukaryotic algae whose plastids stem from secondary endosymbiosis, the nucleomorph is the highly reduced nucleus of the eukaryotic endosymbiont, it is located inside the periplastidal compartment. The nucleomorph is lost in most algae with secondary plastids but still can be found in chlorarachniophytes and cryptophytes.

Opisthokonts Another name for the group consisting of animals and fungi.

Periplastidal compartment The periplastidal compartment can be found in plastids of secondary origin within the chlorarachniophytes and cryptophytes. It corresponds to the cytosol of the eukaryotic endosymbiont.

Phagocytosis Phagocytosis is the engulfment of cells or particles by living cells.

Primary plastids Designates plastids that stem from a symbiotic association of a cyanobacterium with a eukaryotic host.

Proteobacteria Proteobacteria are a major group of bacteria. They are Gram-negative.

Pseudogene Pseudogenes are DNA segments resulting from multiple mutations, which look like genes, but are dysfunctional.

Rhodophytes Red algae, a group of Archaeplastida.

Ribosome Ribosomes are cellular particles composed of proteins and rRNA, where proteins are synthesized. They can be found in the cytosol, in mitochondria, and in plastids. In algae with a nucleomorph, a fourth set of ribosomes occurs in the periplastidal compartment.

SAR An eukaryotic group of organisms including stramenopiles (heterokonts), alveolates, and rhizaria.

SCH An eukaryotic group including stramenopiles, cryptophytes, and hacrobia.

Secondary plastids Designates plastids that stem from secondary endosymbioses in which the product of the primary endosymbiosis (a green- or a red-algae) came to reside into a heterotrophic, eukaryotic host.

Symbiosis Living together. When symbiosis involves benefit for both partners, it is mutualism.

Syntrophy 'Eating together,' designates a kind of metabolic association in which one cell is dependent upon a metabolic endproduct of another. The metabolic endproduct is often molecular hydrogen.

Introduction

Endosymbiotic theory designates a class of hypotheses that view various organelles in eukaryotic cells as descendants of endosymbionts: cells that came to live inside another cell (a host). In its oldest and most familiar versions, endosymbiotic theory posits that mitochondria and plastids were once free-living cells: mitochondria (the powerhouses of eukaryotic cells) stemming from free-living proteobacteria and plastids (the chlorophyll-containing solar panels of plant cells) stemming from cyanobacteria. The Russian botanist Constantin Mereschkowsky is generally credited with the first formulation of endosymbiotic theory. He described plastids as reduced cyanobacteria that entered into a symbiosis with a heterotrophic host, which itself originated via a symbiosis between a heterotrophic host cell and a smaller endosymbiont that, in his view, gave rise to the nucleus (Mereschkowsky, 1905). Mereschkowsky's reasoning was remarkably modern with regard to the origin of plastids. He did not consider that mitochondria might also be of endosymbiotic origin (Mereschkowsky, 1910). That idea probably traces back to the French biologist Paul Portier (1918), who developed ideas about the relationship between bacteria and mitochondria. But Portier proposed that mitochondria could be cultured outside their host cells, and this precipitated considerable criticisms from peers (Archibald, 2014). The American biologist Ivan Wallin developed endosymbiotic theory further for mitochondria (Wallin, 1927). He was convinced that mitochondria are descendants of endosymbiotic bacteria, but he did not expound upon the ancestry of the host that acquired them (Wallin, 1927). Like Portier, he thought that the cultivation of mitochondria outside their host should be possible. Though initially quite popular in the early 1900s, endosymbiotic theories endured scathing criticism in a leading college textbook of the day (Wilson, 1928), whereupon they fell into disrepute for decades.

Endosymbiotic theory was revived in 1967 when Lynn Sagan (later named Margulis) argued that chloroplasts and mitochondria had descended from separate endosymbionts. Sagan envisaged as a host for the origin of mitochondria a heterotrophic anaerobic prokaryote, in whose cytoplasm an aerobic prokaryotic microbe had taken up residence. The resulting heterotrophic protozoan later engulfed a cyanobacterium, resulting in the origin of plastids (Sagan, 1967). However, germane to all of Margulis's versions of endosymbiotic theory, from 1967 onward, is the notion that the eukaryotic flagellum arose from a symbiotic spirochete (Margulis, 1970; Margulis *et al.*, 2006) – a view that never received reproducible experimental support and that remained outside the mainstream of developments surrounding endosymbiotic theory. Since Margulis's revival of the idea, more than 30 different versions of endosymbiotic theory, with varying degrees of detail, and with different areas of focus, have been put forward (reviewed in Martin *et al.*, 2015). Some versions introduce new ways to imagine the origin of mitochondria and chloroplasts, other versions suggest endosymbiotic origins for other cell compartments like peroxisomes or the nucleus, or aim to account for the origin of various eukaryotic traits. In the main, however, endosymbiotic theory is about the origin of chloroplasts and mitochondria.

Mitochondria

Early models for the origin of mitochondria have a primitive mitochondrion-lacking (amitochondriate) microbe as the hosts of an aerobic bacterium (De Duve, 1969). Following the discovery of archaebacteria (archaea), an archaeon was often viewed as the host that acquired the mitochondrion (Van Valen and Maiorana, 1980; Doolittle, 1980). The model of Vellai and Vida (1999) operates with a prokaryotic host, as does the sulfur cycling theory of Searcy (1992). López-García and Moreira (2006) proposed a three-partner endosymbiosis between a fermenting, heterotrophic, hydrogen-producing ancestral myxobacterium (delta-proteobacterium) that serves as the host, a strictly anaerobic, methanogenic archaeon that becomes the nucleus, and an alpha-proteobacterium that was then surrounded by the syntrophic couple and became the mitochondrial ancestor. The model presented by Martijn and Ettema (2013), like that put forward by Yutin *et al.* (2009), suggests a phagocytosing archaeal host, which engulfed an alpha-proteobacterium.

Most models for the origin of mitochondria posit that the mitochondrial endosymbiont was an aerobic bacterium, if they take a stance on its physiology at all. But various anaerobic forms of mitochondria like hydrogenosomes also occur among eukaryotes (Müller *et al.*, 2012) and these also need to be accounted for under endosymbiotic theory. One variant of endosymbiotic theory, called the hydrogen hypothesis, accounts for these anaerobic mitochondria. It posits a symbiotic association of an anaerobic, strictly hydrogen-dependent and autotrophic archaebacterium as the host with a facultatively anaerobic, heterotrophic bacterium as the endosymbiont, with specialization and differential loss leading to aerobic and anaerobic forms of mitochondria (Martin and Müller, 1998). Today, some models for the origin of mitochondria entail the assumption that the host that acquired the mitochondrial symbiont was already a eukaryote, others operate on the premise that the host was a prokaryote and that the origin of eukaryote cell complexity came later (reviewed in Martin *et al.*, 2015). Present data tend to favor the view that the host was a prokaryote, specifically an archaeon in most current views (Lane and Martin, 2010; Williams *et al.*, 2013; Bolte *et al.*, 2015; Raymann *et al.*, 2015; Spang *et al.*, 2015). The endosymbiotic origin of mitochondria in an archaeal host is illustrated in **Figure 1**.

Plastids

All models for the origin of chloroplasts propose that the host was already a eukaryote. The nature of the symbiotic association between host and plastid symbiont varies across models. Today, plastids are involved in photosynthesis, carbon fixation, amino acid biosynthesis, lipid and cofactor biosynthesis as well as nitrogen metabolism. This gives rise to several hypotheses about the physiological context for the establishment of the plastids. In Mereschkowsky's version of endosymbiotic theory, the production of carbohydrates for the host was the key contribution by the cyanobacterial endosymbiont right from the start (Mereschkowsky, 1905). Another reason for the establishment of the symbiosis could

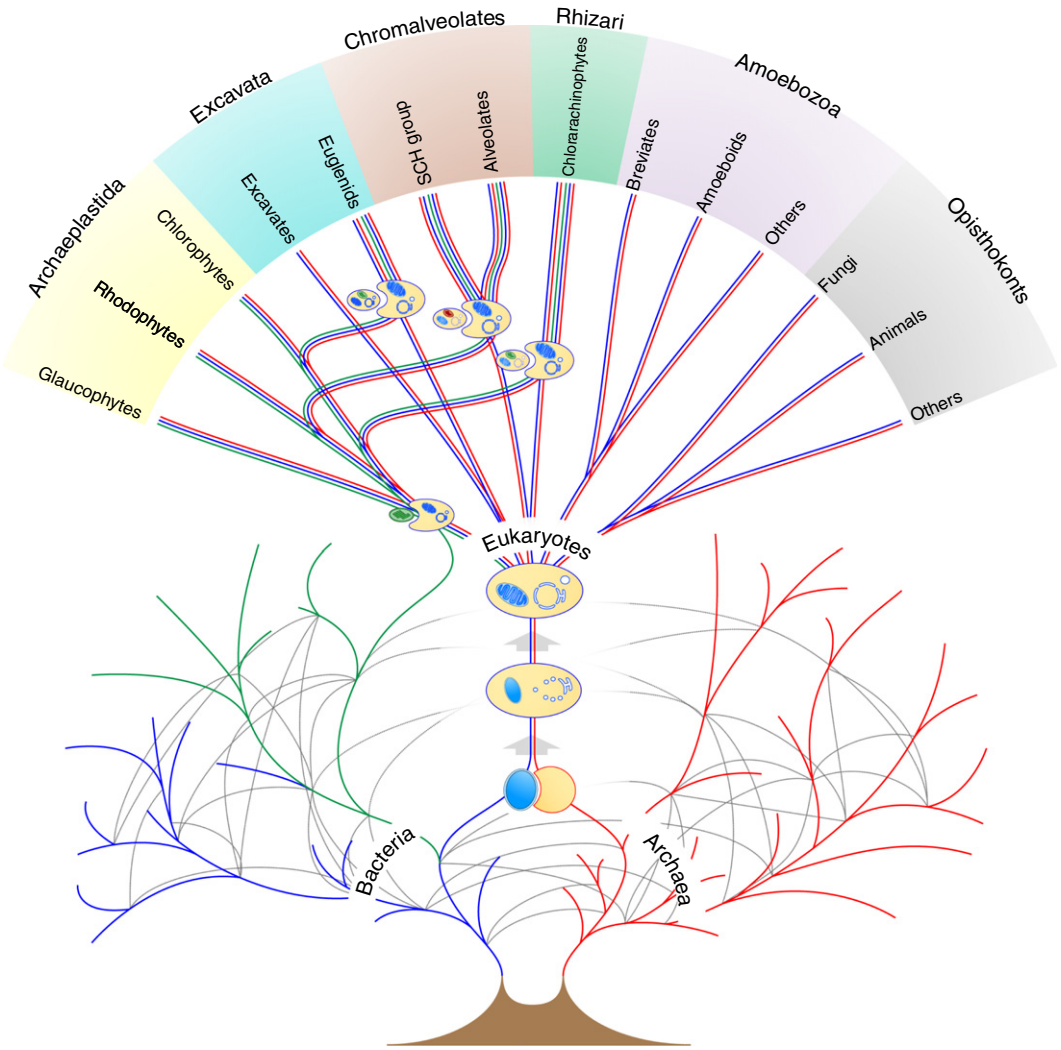

Figure 1 A schematic representation of the origin of the three domains of life and their relationships: The bacteria are shown in blue while the archaea are shown in red. Each colored line serves to show the number of genomes involved in shaping that lineage while also indicating phylogenetic relationships between the various groups. Life arose from alkaline hydrothermal vents as the two bacterial kingdoms the bacteria and the archaea. Prokaryotic evolution is rampant with Lateral Gene Transfers (LGT) – shown in dotted gray lines between the various groups – that shape the various prokaryotic lineages. A symbiotic association of an archaea and bacteria gave rise to eukaryotes where genome evolution is dominated by endosymbiotic events rather than LGT. The taxonomic groups shown correspond to those recognized by Adl *et al.* (2005). The term SCH-group is introduced here to designate Stramenopiles, Cryptomoads, and Haptophytes, whose plastids appear to share a single common origin (Zimorski *et al.*, 2014; Gould *et al.*, 2015).

have been the low concentration of oxygen in the air at the time of endosymbiosis, such that the oxygen produced by the symbiont subversed the host's mitochondrial respiration (Martin and Müller, 1998). However, if we look at modern symbioses involving cyanobacteria, they mostly involve cyanobacterial nitrogen fixation (Kneip *et al.*, 2007; Ran *et al.*, 2010). Today's plastids do not fix nitrogen. Possibly they have lost this attribute (and the associated genes) as a consequence of the evolution of the nitrogen cycle (there is more nitrate in the environment today because of environmental O_2). This aspect of endosymbiotic theory for plastid origin (nitrogen fixation) is not directly supported by data, but is congruent with recent analyses that today's filamentous, heterocyst-forming and nitrogen fixating cyanobacteria (sections IV and V) are most similar, from the genomic perspective, to the plastid ancestor (Deusch *et al.*, 2008; Dagan *et al.*, 2013).

In recent literature, a proposal for the origin of plastids involving chlamydia continues to surface. It posits that a chlamydial endosymbiont was involved as a kind of metabolic helper in the origin of plastids (Ball *et al.*, 2013), a kind of preplastidial infection that the cyanobacterial symbiosis somehow cured. That suggestion, though published in prominent journals, is problematic, because the observations upon which it is founded (phylogenetic trees) are subject to simpler alternative interpretations that do not require the participation of any symbionts other than a cyanobacterium at the origin of plastids (Deschamps, 2014; Zimorski *et al.*, 2014;

Domman *et al.*, 2015). Moreover, the chlamydia story has the problem that if one infers the existence of a symbiont from a few gene trees (which is how the chlamydia symbiosis narrative operates), then for every unexpected branch that we observe in phylogenetic trees we would have to infer a new endosymbiont, and that kind of reasoning in endosymbiotic theory simply does not work (Ku *et al.*, 2015).

A member of the rhizaria, the ameba *Paulinella chromatophora*, harbors within its cytoplasm cyanobacteria of the genus *Synechococcus* (Marin *et al.*, 2005; Yoon *et al.*, 2006). These endosymbiotic cyanobacteria have been known for over 100 years and are called chromatophores (Mereschkowsky, 1905; Melkonian and Mollenhauer, 2005). Their genome is reduced compared to their closest free-living relatives, but is larger than typical plastid genomes (Nowack *et al.*, 2008). Some authors refer to this classical cyanobacterial endosymbiont as a 'photosynthetic organelle' (Marin *et al.*, 2005, Nowack and Grossman, 2012).

The timing in Earth history of mitochondrial and plastid origin cannot be pinpointed, but plausible ranges are often cited that stem from fossil evidence. The oldest eukaryotic microfossils are about 1.8 billion years old (Knoll, 2014). Because mitochondria arose only once in eukaryote evolution (Lane and Martin, 2010), this age can also be seen as a minimum age for mitochondria. The origin of plastids has been estimated at about 1.5 billion years ago (Parfrey *et al.*, 2011), the minimum age for plastids is 1.2 billion years ago because a fossil red alga called *Bangiomorpha* (Butterfield, 2000) is found in rocks of that age. The origin of plastids is sketched in **Figure 1**.

Secondary Endosymbiosis

Mitochondria and chloroplasts can take on a diversity of form and function across different eukaryotic groups. But they have one attribute in common: chloroplasts and mitochondria are always surrounded by two membranes. This is a telling character that betrays their endosymbiotic origin. The two membranes surrounding chloroplasts and mitochondria correspond, in terms of homologies, to the plasma membrane and lipopolysaccharide layer of the Gram-negative bacteria from which the organelles arose (Lister *et al.*, 2005; Schleiff and Becker, 2011). Yet there are photosynthetic eukaryotes whose plastids are surrounded by three or even four membranes (Gould *et al.*, 2008; Stoebe and Maier, 2002; Van Dooren *et al.*, 2001), such organelles are often called 'complex plastids.' The plastids of euglenids and dinoflagellates are surrounded by three membranes. The plastids of the chlorarachniophytes, the cryptophytes, the diatoms, the brown algae, and relatives are surrounded by four membranes.

How did these algae obtain their plastids? Under endosymbiotic theory, these additional membranes are explained as a result of secondary symbiosis. That is, the plastids of these algal groups stem from secondary endosymbiosis: symbioses between a eukaryotic host and a eukaryotic alga. In contrast to the single endosymbiotic origin of primary plastids from cyanobacteria, these secondary symbioses have occurred at least three times independently in evolution: once in the euglenid lineage, once in the chlorarachniophyte lineage, and (at least) once in the 'chromalveolate' lineage (for a discussion of what chromalveolates are, see Zimorski *et al.* (2014) and Gould *et al.* (2015)). The euglenid and chlorarachniophyte lineages acquired their plastids from green algae, the chromalveolate lineages acquired their plastids from a red alga (or red algae).

The uncertainty about the number of secondary endosymbiotic events in the red algae has to do with the conflicting data from molecular phylogenies (gene trees). The gene trees that would address the question of how many secondary endosymbiosis took place among the chromalveolates conflict with one another, giving rise to many suggestions for independent origins of the red secondary plastids. Considerations relating to protein import into red secondary plastids argue for a single secondary endosymbiotic event at the origin of this group (Zimorski *et al.*, 2014; Gould *et al.*, 2015). The additional two membranes surrounding red secondary plastids are most easily interpreted as the inner and outer leaves of the endoplasmic reticulum (ER) of the host that acquired the red algal endosymbiont (Zimorski *et al.*, 2014; Gould *et al.*, 2015). The workings of secondary endosymbiosis in algal evolution are shown in **Figure 1**.

The number and nature of secondary hosts involving the origin of red secondary plastids remain unclear – some red secondary plastids are been suggested to be of tertiary or even quaternary endosymbiotic origin (Stiller *et al.*, 2014). Lineages with secondary red plastids include the nucleomorph-bearing cryptophytes, the haptophytes, the diatoms (stramenopiles), some dinoflagellates, the chromerids, and the perkensids and some apicomplexans (McFadden, 2014), which secondary lost their photosynthetic ability – all lineages of the SCH-group and alveolates. Within these lineages the dinoflagellates are the only organisms with three-membrane bound plastids. Considerations relating to protein import suggest that it was the second outermost membrane (corresponding to the host's distal ER leaf) that was lost in the dinoflagellates (Zimorski *et al.*, 2014; Gould *et al.*, 2015), all other secondary plastids derived from red algae are surrounded by four membranes. In the context of membrane homologies in endosymbiotic theory, there have been several suggestions that the nucleus was once an endosymbiont (reviewed in Martin, 2005). However, such theories often state that the nucleus is surrounded by two membranes (or a double membrane), which is incorrect: the nucleus is surrounded by one folded membrane that is contiguous with the ER (Martin, 1999).

The Rationale Behind Mitochondrial Ubiquity

Because plastids arose more recently in evolution than mitochondria, we know a bit more about the host of plastids than we do about the host of mitochondria. It is a particularly curious aspect of endosymbiotic theory that ideas about the bacterial ancestry of mitochondria developed historically long before concepts about the host for the origin of mitochondria appeared. Ideas about the nature of the mitochondrial host came as a necessary afterthought in the wake of the more pressing debate about whether endosymbiosis for organelle origins was a good idea or not. In the early 1970s and well into the 1990s it was customary to view the mitochondrial host as a mitochondrion-lacking eukaryote – a cell that had mastered the evolutionary transition from being a prokaryote to one that had

a nucleus, a cell cycle, and all the other myriad attributes that separate eukaryotes from prokaryotes (for a long list of such attributes see Cavalier-Smith, 2002). In that view, summarized succinctly by Doolittle (1998), the mitochondrial host became eukaryotic more or less by point mutation, and eukaryotes that were then known to lack mitochondria were most simply seen as descendants of that host.

It turned out, however, that all of the eukaryotes that then appeared to lack mitochondria actually had mitochondria after all, albeit sometimes in highly reduced forms (Tovar *et al.*, 1999, 2003; Williams *et al.*, 2002). That placed the origin of mitochondria at the very base of eukaryote evolution (Embley and Martin, 2006). All the while it should have been evident that the host for the origin of mitochondria was related to archaea (or was an archaeon outright), because the eukaryotic cytosol harbors archaeal ribosomes (Esser *et al.*, 2004). Improvements in phylogenetic methods have gradually brought forth a picture in which the host for the origin of mitochondria branched within the archaea (Cox *et al.*, 2008; Williams *et al.*, 2013; Spang *et al.*, 2015), not as a sister to the archaea, as the older rRNA tree of life implied (Pace, 2006). That suggests that the mitochondrion was acquired by an archaeon (a prokaryote), as some of the endosymbiotic models had suggested. As a consequence, the origin of eukaryotic-specific traits might have come in the wake of mitochondrial symbiosis.

In line with that view, the nucleus could have arisen in the wake of mitochondrial endosymbiosis, the proliferation of (rapidly self spliced) group II introns and their transformation into (slowly spliced) spliceosomal introns may have caused the need for a nuclear membrane to separate splicing from translation (Martin and Koonin, 2006). Also in agreement with the view that eukaryote complexity emerged in the wake of the mitochondrial endosymbiosis is the comparatively recent recognition that the many evolutionary inventions that separate eukaryotes from prokaryotes did not come for free, they came at an energetic price. The configuration of bioenergetic membranes that mitochondria conferred upon the ancestor of the eukaryotic lineage allowed it to do the evolutionary inventing required to forge the eukaryotic lineage (Lane and Martin, 2010). Such bioenergetic considerations would readily explain why mitochondria are ubiquitous among eukaryotes (they were required for eukaryote origin) and why no prokaryote on its own ever made the leap to eukaryote-like complexity: without a mitochondrial endosymbiont, it lacked the energy per gene to do so (Lane and Martin, 2010; Lane, 2014). Thus, the ubiquity of mitochondria among eukaryotes, including among anaerobic eukaryotes (Müller *et al.*, 2012), is perhaps best seen as evidence that endosymbiosis really was important, not just in terms of making eukaryotes more efficient at what they do, but bringing them into existence in the first place.

Endosymbiotic Gene Transfer

One of the important aspects of endosymbiosis is that it can, and does, lead to gene transfer from organelles to the nucleus (Martin *et al.*, 1998; Martin and Herrmann, 1998; Timmis *et al.*, 2004). Ninety years ago, even Wallin sensed that somehow the process of endosymbiosis should be connected to a transfer of genetic material from the organelle to the host. He wrote: "It appears logical, however, that under certain circumstances, [...] bacterial organisms may develop an absolute symbiosis with a higher organism and in some way or another impress a new character on the factors of heredity. The simplest and most readily conceivable mechanism by which the alteration takes place would be the addition of new genes to the chromosomes from the bacterial symbiont" (Wallin, 1925; p. 144). That is a fairly modern formulation of a process that is now called endosymbiotic gene transfer (Martin *et al.*, 1993). About 15–18% of the genes in a higher plant's nuclear genome come from the cyanobacterial antecedent of plastids (Martin *et al.*, 2002; Deusch *et al.*, 2008), and in eukaryotes that lack plastids, such as yeast, the vast majority of genes having prokaryotic homologues come from bacteria, not archaea (Esser *et al.*, 2004; Cotton and McInerney, 2010; Thiergart *et al.*, 2012). The simplest interpretation is that these bacterial genes in nonphotosynthetic eukaryotic lineages come from the mitochondrial ancestor (Pisani *et al.*, 2007; McInerney *et al.*, 2014).

The process of endosymbiotic gene transfer entails the integration of bulk chunks of organellar chromosomes, or in some cases even a whole organelle genome spanning more than 100 kb (Huang *et al.*, 2005). The evidence that this has happened can be seen at the computer by comparing organelle genomes to nuclear genomes (Hazkani-Covo and Covo, 2008) and in laboratory experiments where organelles are transformed with constructs that only become active in the nucleus (Huang *et al.*, 2003, 2004). The mechanism of DNA insertion entails nonhomologous end joining and most eukaryotic genomes are replete with such recent organelle insertions (Hazkani-Covo and Covo, 2008). One might wonder how organelle DNA gets to the nucleus in the first place so that it can recombine. The most likely mechanism is simply stress induced organelle lysis, and there is some evidence for this in plants (Lane, 2011; Wang *et al.*, 2012). Importantly, organelle lysis means that there has to be more than one organelle copy in the cell, one to lyse and one for progeny, and this is the crux of the 'limited window' hypothesis (Barbrook *et al.*, 2006).

There is another important aspect to gene transfer to the nucleus. Both at the origin of mitochondria and at the origin of plastids, host, and symbiont possessed a large number of genes for homologous functions. Such genes would include ribosome biogenesis, amino acid biosynthesis, nucleotide biosynthesis, core carbon and energy metabolism, cofactor biosynthesis, and the like. Chloroplasts and mitochondria have both retained their own ribosomes, for example, and divergent members of homologous gene families for ribosomal proteins as one example, but other examples have been well studied, including core carbohydrate metabolism. This phenomenon is called 'functional redundancy through endosymbiosis' (Martin and Schnarrenberger, 1997). It generates highly divergent copies of genes homologous to prokaryotes even though they reside on eukaryotic chromosomes.

Protein Import

The origin of organellar protein import machineries played an important role in the evolution of mitochondria (Dolezal *et al.*, 2006) and plastids (Schleiff and Becker, 2011), because

it allowed the genetic integration of host and endosymbiont while allowing the endosymbiont to maintain its biochemical identity. In the early phases of organelle evolution, before the invention of the protein import apparatus that allowed plastids and mitochondria to import proteins from the cytosol, the transferred genes either became pseudogenes or became expressed as cytosolic proteins. In this way, endosymbionts can easily transfer whole pathways from the organelle to the cytosol. The transfer of whole pathways from the cytosol to an organelle is also possible, but the mechanisms are different (Martin, 2010).

With the advent of organelle protein import, however, transferred genes had the opportunity to obtain the necessary expression and targeting signals to be targeted back to the organelle from which the nuclear gene was acquired. This process has resulted in an expansion of the eukaryotic nuclear gene repertoire and in reductive genome evolution in the organelle. While it has long been known that the genes retained most tenaciously by plastids and mitochondria encode for proteins involved in the electron transport chain of the bioenergetic organelle or for the ribosome required for their synthesis (Allen, 2003, 2015), only recently was it recognized that even within the ribosome, the same core of proteins has been retained independently by plastids and mitochondria, probably owing to constraints imposed by the process of ribosome assembly (Maier *et al.*, 2013).

Conclusion

Endosymbiotic theory explains why some organelles of eukaryotic cells are so similar to prokaryotic cells. It is a fairly powerful theory in that it can explain a number of disparate observations within a single unifying framework. Mutation theory, population genetics and selection can explain many aspects of evolutionary divergence among cells, but they cannot explain how mitochondria, chloroplasts, and complex plastids arose; for those major events in evolutionary cell biology, endosymbiotic theory is the only explanatory tool available. It works quite well, but it works best when used sparingly and in close conjunction with neighboring disciplines like microbial physiology and genetics.

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See also: Mitochondrial and Nuclear Genome Coevolution. Symbiogenesis, History of. Symbiosis, Introduction to

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EDITOR IN CHIEF

RICHARD M. KLIMAN
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EDITOR IN CHIEF

Richard M. Kliman, PhD, is Professor of Biological Sciences at Cedar Crest College in Allentown, Pennsylvania. He received his BA from Colby College in biology and music. His graduate work at Wesleyan University focused on quantitative genetics of circadian rhythms and photoperiodism in the Djungarian hamster, *Phodopus sungorus*. As a postdoctoral fellow at Rutgers University and Harvard University, he studied molecular evolution and population genetics. Prior to Cedar Crest College, he taught at Radford University in Virginia and Kean University in New Jersey. He has also served as a program director in the Division of Environmental Biology at the US National Science Foundation (NSF).

Kliman's research interests center on questions in molecular evolution, including the evolution of codon usage bias in a variety of organisms; speciation and natural history; and ecology and conservation. Much of this work has relied on population genetics/genomics and bioinformatics approaches. He has also collaborated with Cedar Crest colleague John Cigliano on an Earthwatch-supported "before-after-control-impact" study on the effects of a new marine reserve in Belize on queen conch populations. His research in evolutionary and ecological genetics has been supported by the US National Institutes of Health and by Conservation International.

Kliman has served on the editorial boards of *Genetica* and *The Journal of Molecular Evolution*. He has been deeply involved in evolution education, helping to coordinate "Undergraduate Diversity at SSE/SSB," an NSF-supported program to bring a diverse group of undergraduates to the annual Evolution research conference. He was a lead editor of population/quantitative genetics and evolutionary genetics for *Nature Education/Scitable* at its inception. He is a member of the Education and Outreach Committee of the Society for the Study of Evolution, and editor of the society's peer-reviewed educational resource, the *EvoEd Digital Library*.

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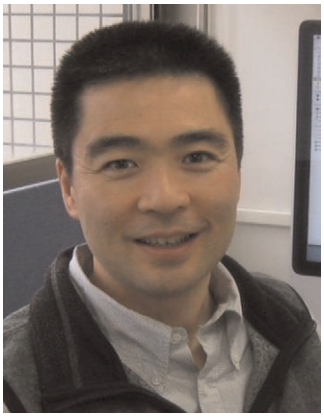


Hiroshi Akashi is a Professor of Evolutionary Genetics at the National Institute of Genetics, Japan. He worked with Marty Kreitman for his PhD in Ecology & Evolutionary Biology from the University of Chicago (1996) and with John Gillespie as a postdoctoral fellow at UC Davis. He has been a faculty member at the University of Kansas (1998–2000), Penn State University (2000–2008), and NIG (2009–present). Akashi's research focuses on inferring causes of genome evolution, especially weak selection, from within and between species sequence variation. His studies of codon usage employed population genetic methods to detect natural selection acting at its limit of efficacy and identified a phenotypic basis of natural selection (translational accuracy) from sequence comparisons in *Drosophila*. Extensions of this work revealed constraints related to biosynthesis that act globally on compositional properties of microbial proteins. The interplay of weak evolutionary forces appears to shift frequently among closely-related species and current interests include tests of adaptive changes in protein/DNA composition.

Tim Coulson's primary interest is in creating better links between the fields of ecology and evolution. He does this by developing theory, parameterising models for field and laboratory systems, making predictions from these models, and, where possible, testing these predictions with experiments. He works on a range of systems, from bulb mites within the laboratory, to guppies living in streams in Trinidad, to wolves in Yellowstone. His motivation to do this comes from observations that ecological and evolutionary change can be observed occurring on similar time scales, yet ecological theory typically ignores evolutionary processes and vice versa.

Tim was awarded his PhD in plant ecology from Imperial College, London, in 1994. He moved on to research genotype-by-environment interactions as Natural Environment Research Council (NERC)-funded post-doc at the Institute of Zoology in London. He remained at the Institute on a fellowship where he developed models to investigate the economic and life history consequences of a range of population management strategies. In 2000 he moved to the University of Cambridge, where he briefly lecturered in the Zoology department. In 2004 he moved back to Imperial College London as a senior lecturer where he started developing models that allow the simultaneous investigation of the dynamics of life history, populations, and quantitative characters. In 2007 he became Professor of Population Biology at Imperial College London. He left Imperial in 2013 to take up his current position as Professor of Zoology at the University of Oxford. He is also a Professorial fellow of Jesus College, Oxford.

Andrew Forbes

Rosemary Gillespie is a Professor at the University of California, Berkeley, where she also holds the Schlinger Chair in Systematics. She is Past President of the *International Biogeography Society* and Trustee and Fellow of the *California Academy of Sciences*, and serves as Associate Editor for *Molecular Ecology*. Gillespie was born and educated in Scotland, receiving her BSc in Zoology from Edinburgh University in 1980. She came to the US to conduct graduate work on the behavioral ecology of spiders at the University of Tennessee. After her PhD she spent several months at the University of South in Tennessee, and then started work at the University of Hawaii in 1987, initially as a postdoc, and then in 1992 as Assistant Professor in Zoology and Researcher in the Hawaiian Evolutionary Biology Program. It was during her first year in Hawaii that she discovered an adaptive radiation of *Tetragnatha* spiders. She left Hawaii in 1999 to join the faculty at the University of California in Berkeley, where she continues her research focus on the islands of the Pacific, Hawaii in particular, using islands of known age and isolation to assess the combined temporal and spatial dimension of biogeography and determine patterns of diversification, adaptive radiation, and associated community assembly.

David Guttman received his PhD from Stony Brook University in 1994 working with Daniel Dykhuizen on questions related to the role and importance of recombination in structuring genetic diversity in bacterial populations. He followed this with a postdoc in molecular evolution with Brian and Deborah Charlesworth at the University of Chicago, and a second postdoc at the University of Chicago with Jean Greenberg to gain experience in the fields of molecular plant pathology and plant-microbe interactions. He started his faculty position at the University of Toronto in 2000, and is currently a Professor in the Department of Cell & Systems Biology (CSB). He is also the Associate Chair for Research in CSB, founder and Director of the University of Toronto Centre for the Analysis of Genome Evolution & Function, and Canada Research Chair in Comparative Genomics. He has served as the Chair of the American Society for Microbiology, Division R (Evolutionary and Genomic Microbiology), and was the *PLoS Pathogens* Section Editor for Bacterial Evolution & Genomics.

Dr. Guttman runs a highly diverse research program generally focused on bacterial evolutionary genomics, with three major foci: (1) the evolution of host specificity and virulence in plant pathogenic bacteria; (2) microbial comparative genomics; and (3) studies of the human and plant-associated microbiome. He is best known for elucidating and linking evolutionary and mechanistic processes that determine the course and fate of bacterial infections, and characterizing the impact of genetic variation on the balance between disease and immunity.

Norman A. Johnson, the section editor for Applied Evolution, is an evolutionary geneticist and author. He received his PhD from the University of Rochester in 1992 and did postdoctoral research at the University of Chicago. His research interests have generally focused on aspects of speciation, specifically those related to the genetics and evolution of hybrid incompatibility: sterility, inviability, or other reduction of fitness in hybrids between species. Dr. Johnson, an adjunct professor in the Biology Department at the University of Massachusetts at Amherst, has taught classes there, as well as at Hampshire College, the University of Texas at Arlington, and the University of Chicago.

Dr. Johnson also has a long-standing commitment toward improving the communication of science in general and evolutionary biology in particular to other scientists, educators, and the public at large. He is the author of *Darwinian Detectives: Revealing the Natural History of Genes and Genomes* (Oxford University Press: 2007), a book geared to general audiences that shows how biologists use DNA sequence data to make inferences about evolutionary processes. He also was the lead organizer for a working group on communicating human evolution at the National Evolutionary Synthesis Center (NESCent).

Laura Kubatko received a PhD in Biostatistics from The Ohio State University (OSU) in 1999. After seven years on the faculty at the University of New Mexico, she returned to OSU in the Fall of 2006, and is now Professor of Statistics and of Evolution, Ecology, and Organismal Biology at OSU. Laura served as an Associate Director of the Mathematical Biosciences Institute at OSU from 2013–2015. At OSU, she is a Faculty Affiliate of the Initiative in Population Research, and a Faculty Affiliate in Translational Data Analytics (TDA@OSU). She holds appointments as an Affiliate Faculty Member at the Battelle Center for Mathematical Medicine at Nationwide Children's Hospital in Columbus and as an Adjunct Research Scientist at Lovelace Respiratory Research Institute in Albuquerque, NM. Laura's research interests are in statistical genetics, with a focus on the development of statistical methods for inferring phylogenies from molecular data. Her recent work in this area concentrates on bridging the gap between traditional phylogenetic techniques and methodology used in population genetics analyses, primarily through the application of coalescent theory to species-level phylogenetic inference. She develops and distributes several software packages for phylogenetic inference, and has been an active member of the *Society of Systematic Biologists*. She has served as an Associate Editor for the journal *Systematic Biology* since 2007.

Amy Litt has been studying plant evolution and diversity since her PhD on floral structure and evolution in the neotropical plant family Vochysiaceae, known for its beautiful but unusual flowers many of which have only one petal and one stamen. While completing her PhD in plant systematics and morphology in the joint City University of New York/New York Botanical Garden Plant Sciences program under Scott Mori and Dennis Stevenson, she became interested in the molecular basis of plant diversity. She did her post-doc in the developmental genetics lab of Vivian Irish at Yale University on the evolution of a family of transcription factors involved in flower development, and she continues to study the functional evolution of this gene family currently. After one year on the faculty of University of Alabama, she moved back to The New York Botanical Garden as Director of Plant Genomics, where she developed her research program studying the evolution of plant form along two paths: studying evolutionary changes in genes to see how those changes affected flower and fruit form; and identifying the genes that underlie differences in form among closely related species. Dr. Litt also served as a program director in Plant, Fungal, and Microbial Development and Evolutionary Development at the National Science Foundation. She recently moved to the University of California at Riverside, where she continues to study the genetic basis of plant diversity.

Maria E. Orive is a professor of evolutionary genetics in the Department of Ecology and Evolutionary Biology at the University of Kansas. Her research in theoretical population genetics aims to develop mathematical models that provide a conceptual framework for exploring important questions in evolutionary biology and analytical tools for demographic and genetic data. Her work has considered levels of selection and mutation in organisms that reproduce both sexually and asexually, the relationship of population structure and life-history attributes to gene flow and genetic diversity, and models of within- and between-host pathogen and symbiont population dynamics. Orive received her BS from Stanford University and her PhD from the University of California at Berkeley. After spending two years as a postdoctoral researcher in genetics at the University of Georgia, she was an NSF-NATO Postdoctoral Fellow at the University of Edinburgh. Her research has been funded by multiple grants from NSF and NIH. In 2007–2008, she was the Carl and Lily Pforzheimer Foundation Fellow at the Radcliffe Institute for Advanced Study (Harvard University), and has served as the University Faculty Ombudsman for the University of Kansas since 2007.

Daniel Ortiz-Barrientos is an Associate Professor in evolutionary genetics in the School of Biological Sciences at The University of Queensland, Brisbane, Australia. During his scientific career he has investigated the ecological and genetic basis of speciation both in plants and animals. His current research program explores the early stages of speciation, the molecular basis of parallel speciation, and the interplay between recombination and natural selection during the origin of new species. His research funds come from The Australian Research Council. He is married to Antonia Posada, and is the father of three energetic and beautiful kids.

Claudia Russo was born in Leeds, England, but has lived in Rio de Janeiro, Brazil since she was two years old.

Claudia has an academic major in Ecology from Universidade Federal do Rio de Janeiro completed in 1989, and finished her Master's thesis in 1991 on population genetics of two actiniid species of sea anemones with different reproductive strategies, under the supervision of Associate Professor Antonio Mateo Sole-Cava. Her PhD dissertation was on the diversification of drosophilids and on the use of a known phylogenetic tree to estimate the reliability of tree building methods. The dissertation was completed in 1995 under the supervision of the Evan Pugh Professor Masatoshi Nei who recently received the prestigious Thomas Hunt Morgan Medal. Her graduate degrees were obtained as a student at the Genetics Program from the Universidade Federal do Rio de Janeiro and as a visiting scholar at the Pennsylvania State University (1992–1995).

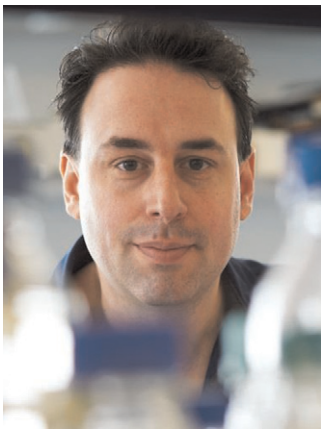
Claudia is currently the Head of the Genetics Department at the Federal University of Rio de Janeiro, having been a member since 1997. Claudia has supervised 13 Master's dissertations, eight PhD theses and seven post-docs, of which eight are now Assistant Professors at universities in Brazil and abroad. She has published 42 academic papers that have been cited over 1,200 times. Her *h-index* is 14. Since 2012, Claudia has been a member of the editorial board, and an associate editor of the *Molecular Biology and Evolution* journal. Since 2012 she has been a council member for the Pan American Association of Computational Interdisciplinary Sciences and since 2009 for the Brazilian Association for the Advancement of Science.

Claudia's general academic interests are on key aspects of animal phylogenetics, including their diversification patterns in time and space. She has worked with various metazoans groups but more prominently on marine sponges, sea anemones, arthropods, passerine birds, and mammals. Claudia has also published on the use of known phylogenetic trees to estimate the efficiency of phylogenetic methods in recovering and rooting those trees. More recently, she has developed some interesting *hands-on* educational tools for evolutionary biology practices in the classroom.

Karen E. Sears is an evolutionary developmental biologist whose primary research goal is to determine how developmental variation within a species produces congenital malformations in humans, and among species generates new evolutionary forms in mammals. Dr. Sears earned her PhD from the University of Chicago, did postdoctoral research at in the Howard Hughes Medical Institute (HHMI) lab of Dr. Lee Niswander, and joined the faculty of the University of Illinois at Urbana-Champaign. At Illinois she holds positions as an Associate Professor in the Department of Animal Biology, a Faculty Member in the Institute of Genomic Biology, and an Affiliate of the Program in Ecology, Evolution and Conservation Biology and the Department of Cell and Developmental Biology. She is also the President of the Pan American Society for Evolutionary Developmental Biology. She has authored or co-authored over 35 publications including first-authored publications in *Nature*, *Proceedings of the National Academy of Sciences*, and *Evolution*. She has served as a principal investigator on multiple, nationally-funded research projects, and presented invited seminars at more than 30 institutions and symposia. She is routinely ranked among the top 10% of Illinois professors for her teaching, and was a featured scientist in the PBS/HHMI documentary "*Your Inner Fish*."

Vassiliki "Betty" Smocovitis is Professor of the History of Science in the Department of Biology and in the Department of History at the University of Florida. Her areas of expertise include the history of evolutionary biology, genetics and systematics and the history of botany. She is best known for her contributions to understanding the historical event known as the "evolutionary synthesis" and in gaining greater understanding of the origins of the discipline of evolutionary biology. She has published extensively on both the intellectual and social aspects of the history of evolutionary biology including a history of the Society for the Study of Evolution, a history of the Darwin Centennial of 1959, and the integration of botany, genetics, and anthropology into the evolutionary synthesis. She was the contributor to the *Oxford Bibliographies* entry on Charles Darwin at over 25,000 words and the entry on the modern synthesis. She is the author of *Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology* (Princeton: Princeton University Press, 1996).

Nina Wedell is a professor of evolutionary biology with research interests focused on the evolutionary ecology of sex. She has worked extensively on various aspects of sexual selection and sexual conflict, in particular on the role of selfish genetic elements in reproductive biology. Nina is the Academic lead for the Behaviour research group at the University of Exeter.



Jason Wolf is Professor of Evolutionary Genetics in the Department of Biology & Biochemistry and The Milner Centre for Evolution at the University of Bath. His research is unified with a special focus given to understanding the influence that frequently ignored or under-appreciated sources of genetic variation have on the genotype-phenotype relationship and how this, in turn, influences evolutionary processes. He integrates theoretical, computational and empirical quantitative and population genetic techniques to achieve this goal. He is particularly interested in understanding the evolutionary consequences of various types of interactions, including gene interactions (epistasis), parent-offspring interactions and social interactions. He received a PhD from the University of Kentucky, after which he was a postdoctoral researcher at Indiana University and a US National Science Foundation Postdoctoral Fellow at Washington University School of Medicine. Prior to moving to the University of Bath he held positions at the University of Tennessee and the University of Manchester. He won the Dobzhansky Prize from the Society for the Study of Evolution, a Young Investigator's Prize from the American Society of Naturalists and the Scientific Medal from the Zoological Society of London.

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Alphabetical Contents List

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

All articles within the encyclopedia have an extensive list of cross-references which appear at the end of each article, for example:

Adaptive Mutation Controversy

> *See also*: Directional Selection and Adaptation. Microbial Experimental Evolution. Mutation, Population Genetic Models of. Natural Selection, Introduction to. Recombination in Bacterial Populations

3. Subject Classification List

A subject classification list has been developed by the editors to group articles under broad topics. This index is useful for making broad connections between articles and locating the relevant article for a topic that is covered in more than one article.

4. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

5. Contributors

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Population Genetics

Quantitative Genetics

Sex, Recombination, and Mating Systems

Speciation and Hybridization

PREFACE

The *Encyclopedia of Evolutionary Biology* was developed to provide an authoritative overview of the current state of evolutionary biology. It was an ambitious goal, especially given that the field did not pause for the two and a half years needed to complete the project. The encyclopedia's 15 section editors collaborated to ensure that content gaps were kept to a minimum, and their efforts show. When the project was completed, we had compiled 256 entries, covering a broad range of topics selected by the editors to ensure a comprehensive resource. It was a privilege to read every one of these entries, and I was truly humbled by the collective efforts of hundreds of authors to communicate the excitement and sophistication of a field of study that touches on every conceivable topic in biology today.

There are many ways to envision an encyclopedia of evolution, and we had to choose an approach that would lead to a cohesive resource. Readers will note that, in the more organismal-focused entries (edited by David Guttman, Amy Litt, and Claudia Russo), there is an emphasis on *diversification* of life. We did not set out to provide an overview of the diversity of life, as such a goal would be untenable; rather, we focused on the evolutionary processes and key events responsible for diversity. Numerous entries deal with speciation, life history evolution, evolutionary biogeography, and coevolution. These entries (edited by Daniel Ortiz-Barrientos, Tim Coulson, Rosemary Gillespie, and Andrew Forbes) bring to light how the evolution and diversification of life is intimately entwined with ecology. Of course, there is extensive coverage of population genetics, quantitative genetics, evolutionary developmental biology, the evolution of sex and mating systems, molecular/genome evolution, and phylogenetic analysis (edited by Maria Orive, Jason Wolf, Karen Sears, Nina Wedell, Hiroshi Akashi, and Laura Kubatko), all fundamental to our understanding of evolutionary processes. And as thematic bookends, several entries (edited by Betty Smocovitis and Norman Johnson) cover the history of evolutionary biology and applications of evolutionary biology.

Readers of the encyclopedia will find that entries are generally pitched at a somewhat advanced level, although with great effort by authors to make entries as accessible as possible to a broad audience. Encyclopedias, like living organisms, are compromises. If all entries could be readily understood in their entirety by first-year university students, this encyclopedia would be of limited value to experts. At the other extreme, if entries were extremely technical – and our authors were undoubtedly capable of producing such entries – the encyclopedia might be inaccessible to students. While there is, by necessity, variation among entries in this regard, we settled on a general target: the majority of an entry should be accessible to a motivated, advanced undergraduate. Readers are, of course, directed to additional resources, with authors providing bibliographies and lists of further reading.

As with any undertaking of this scale, there are many individuals who should be recognized for their roles in the development of this encyclopedia. Special thanks go to Norman Johnson for early discussions that helped us develop the general structure of the encyclopedia. The dedicated and distinguished team of section editors deserves the credit for drafting the table of contents, recruiting authors, and working extensively with authors to ensure the highest quality product. It should go without saying that the high quality of this encyclopedia ultimately reflects the efforts of the editors and authors. Finally, the project management and development teams at Academic Press were always ready to assist, and while it is not possible to name everyone who contributed to the effort, I am particularly indebted to Simon Holt, Will Bowden-Green, Paula Davies, and Justin Taylor.

Richard Kliman
Editor in Chief

FOREWORD

What is life, how did it originate, and what accounts for its great diversity? These are fundamental scientific questions that have and will always be the source of endless fascination and wonderment. Charles Darwin and Alfred Russel Wallace provided an answer to the latter question through the grand idea of evolution and the process of natural selection. Darwin also speculated on the where question of the origin of life by hypothesizing it originated long ago in a warm lagoon. Most importantly, however, Darwin shattered the notion that the natural world is static and replaced it with a biology that is dynamic and continually changing. Species are not fixed, typological entities. Rather, they are related by common descent in a great tree of life. Analogous to tracing one's ancestors back in time in a pedigree, one can climb down the tree along its branches and boughs that connect species in a hierarchy of phylogenic relationships until reaching the base of its trunk and the common ancestor of us all. One can also climb up the tree and quickly realize that evolution produces a seemingly endless array of new forms (and sometimes extinction). Thus, as the tree of life grows, populations are continuously evolving and diverging from one another, creating novel varieties and races (showing slight differences) that eventually evolve into new species (separated by distinct gaps). And natural selection – the differential survival of individuals in populations possessing heritable traits favorable for their survival and reproduction – is the primary materialistic process causing evolutionary change and the origin of new species.

The *Encyclopedia of Evolutionary Biology* chronicles our current state of understanding of the dynamics of evolution and its product, Darwin's great tree of life. A diversity of seminal topics are covered including overviews of the history of the field, the origin of life, the history of life (including the phylogentic methods used to reconstruct life's history), the myriad ways and means (including mechanisms other than natural selection) that evolution is affected, and the important roles that conflict versus cooperation, and mergers and acquisitions, occurring within across varying levels of biological organization, play in the narrative of life. In so doing, the *Encyclopedia* highlights the grandeur in Darwin's view of life. We are not separate, but rather a twig along a branch of life, a twig that has evolved the ability to comprehend the existence of and our connectedness to the tree, and climb around its branches to see what has been and think about what may come. It is a wonder of life that it can look at and understand the meaning of itself.

But Darwin's grand view has even larger ramifications, going beyond providing a materialist basis for organismal change and putting us in our place. The reality of evolution also answers the question of what life is. If pressed to define life, most of us would reply with a list of the things that living organisms do. For example, living organisms metabolize, grow, develop, move, behave, mutate (are variable), and reproduce with inheritance. One can investigate these different characteristics of life separately and discern the mechanistic basis for the different processes that constitute life – the "how" of life. And such studies represent the basis for many fields of the life sciences. However, these are only the components of life and, in isolation, produce a static view of the natural world. Rather, the seminal insight is that populations of living beings possessing these characteristics have the emergent property that they evolve. Darwin's "*On the Origin of Species*" therefore not only describes how populations evolve, and as a logical extension how new species form, but also conveys the essence of what life itself is – evolution. Thus, as Theodosius Dobzhansky famously stated "nothing makes sense except in the light of evolution." The Encyclopedia wonderfully brings this view of life to light, providing the reader with the breadth of knowledge and overview of the current state of the field of evolution needed to appreciate and participate in the next major ongoing synthesis in our understanding of life, the so-called "Omics Revolution."

The study of evolution is in an accelerated phase of discovery brought about by major technical advances in our ability to DNA sequence whole genomes (genomics), and to generate profiles of mRNA transcription (transcriptomics), protein levels and enzymatic activity (proteomics), and metabolic products (metabolomics) at varying stages in the life cycle and development of organisms. This "Omics Revolution" may not change foundational evolutionary principals, per se. Our understanding of evolution has been heightened by a series of such advances in the past, including the "Modern Synthesis" when Mendelian genetics was wedded to Darwinian thinking and the "Molecular Revolution" in which genetic technology increasingly allowed allele frequencies in natural populations to be analyzed. The Omics Revolution is an extension of these pervious advances, but one in which the workings of whole organismal systems and the composition of entire communities can be gleaned at once.

Perhaps, the most important discoveries in Omics will come from linking an understanding of the process occurring at the cellular and microevolutionary level with large scale patterns and trends at the macroevolutionary scale. Previously, processes occurring within and interactions occurring among cells could be studied in at least some detail. Omics is providing an opportunity to fully understand how all of these processes interact simultaneously to result in the development and functioning of integrated, multicellular systems of life. At the other end of the spectrum, fossils attest to the evolution of new life forms through time and the creation of great and observable morphological diversity. Genomic sequencing is providing a powerful means to help accurately place these fossils within the framework of a fully resolved molecular phylogenic tree to better understand the history of life, including major trends, themes, and variation in the tempo and mode of evolutionary change. But it is the middle of the micro and macro at the branching points in the tree of life that Omics may prove most insightful. Now it is possible to not only DNA sequence large numbers of individuals within populations, but to equate these genetic differences within and between populations to morphological, physiological, and behavioral phenotypes, and discern the developmental

and physiological mechanisms by which these genetic changes produce organismal variation, diversity, and reproductive isolation – the stuff of evolution and speciation itself. Thus, we will be able to not only understand the everyday processes responsible for how the tree of life grows, but be able to translate this into a mechanistic appreciation of how these processes result in new branches on the tree forming and others dying out, giving shape to the history of being on our planet.

The field of evolution is currently inundated with a mass of Omics data and the bottleneck is the development of bioinformatic analytical tools to edit, analyze, and interpret the results. However, it is clear that many new insights are on the horizon, and even if they do not affect the root principles of evolution, soon a deep connection of the how and why of life will emerge to help forge a truly integrative evolutionary biology: The *Encyclopedia of Evolutionary Biology* is an excellent guide to prepare readers to assimilate these new findings, keeping bioinformatics grounded in the bio and providing a valuable source for seeing the tree through the forest to understanding the grand synthesis of life that is flowering.

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†Deceased.

E

Epigenetic Inheritance

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Glossary

CpG sites A cytosine base followed by a guanine base in a DNA sequence. The p is for the phosphate linking the C and G.

DMR Part of a DNA molecule that is methylated differently (usually) according to its parental origin. DMRs are critical in effecting the differential expression of paternally and maternally derived gene copies that characterizes genomic imprinting. Also known as differentially methylated domains (DMDs); some DMRs function as imprinting control region (ICRs).

DNA methylation The addition of a methyl (CH_3) group to a cytosine base in place of a hydrogen atom. This biochemical change usually occurs at CpG sites; when methylation modifies clusters of CpG sites, known as CpG islands, which are often associated with gene promoters, the expression of the gene can be significantly reduced or even silenced. Special enzymes are required for *de novo* methylation and separate enzymes are responsible for its maintenance.

Genomic imprinting The differential expression of genes according to their parental origin. In the archetypal cases, which are confined to mammals and angiosperms, only one copy (say, the maternal copy) is expressed in certain tissues for some period of development.

Histones Proteins that help in packing DNA into chromatin. Their modification changes the shape of the chromatin and hence access other molecules have to the DNA. Thus, histone modifications affect the expression of the genes coded in that section of DNA.

Metabolic disease (or syndrome) Several linked conditions – high blood pressure, elevated blood-sugar levels, excess body fat, especially around the waist, and abnormal cholesterol levels – that increase the risk of heart disease, stroke, and diabetes, often later in life.

Epigenetic Modification

Epigenetic inheritance constitutes the processes by which epigenetic modifications of the genome are passed on during cell divisions, either within an individual (usually via mitosis), which we call 'persistence,' or between generations (usually via meiosis), a property we view as 'inheritance.' (Some molecular biologists use 'inheritance' for both phenomena, but they differ in fundamental ways, and so here we use different words to describe them.) Epigenetic modifications can alter the way in which genes are expressed, but they do so without altering the DNA sequence; instead a variety of mechanisms, such as methylation of CpG sites (a cytosine base followed by a guanine, which are linked by a phosphate), non-coding RNAs (such as micro-RNAs) or modifications of histones that result in chromatin remodeling, change the way in which the gene is transcribed and hence how it is expressed (Box 3 in Heard and Martienssen (2014) outlines a number of mechanisms). Critically, these marks are reversible, and hence epigenetic modification allows gene expression to switch easily between different states.

Epigenetic modifications arise from a variety of stimuli, from the cell's immediate surroundings to the individual's external environment. For example, mouse pups inheriting an *agouti* viable yellow allele (A^{vy}) from their father have darker fur when their mothers are fed a diet rich in methyl donors, such as folate and vitamin B_{12}. These offspring are exposed *in utero* to these methyl donors, which promote the methylation of their normally dominant A^{vy} allele and reduce its expression (Cropley *et al.*, 2006; Waterland *et al.*, 2007).

Genomic imprinting, whereby gene expression at a small number of mammalian and angiosperm loci (and perhaps those in other taxa, but the evidence remains equivocal) depends on the sex of the parent that passed on the gene, is also often mediated by cytosine methylation. For instance, the mouse *H19* gene is expressed only from the maternally inherited copy; a region upstream of the paternally inherited copy known as the differentially methylated domain (DMD), differentially methylated region (DMR), or imprinting control region (ICR) is heavily methylated, suppressing the expression of this copy of *H19* (Gabory *et al.*, 2010).

Another epigenetic phenomenon, paramutation, is an interaction between homologous alleles at a single locus in which one allele (described as 'paramutagenic') induces a heritable change in the expression of the other (which is said to have 'paramutated'). Importantly, in the following generation, the paramutated allele usually becomes paramutagenic itself. Paramutation was originally described in maize, but more recently has been found to occur in nematodes, *Drosophila* and mammals (Erhard and Hollick, 2011; de Vanssay *et al.*, 2012; Adams and Meehan, 2013). Small interfering RNA has been suggested as one possible molecular mechanism of this phenomenon (Erhard and Hollick, 2011), but methylation may also be important in mammals (Adams and Meehan, 2013).

Finally, some epigenetic changes are a secondary consequence of a genetic change elsewhere in the genome: insertion of a transposable element in the genome of *Cucumis* (melon) has led to heritable methylation and altered expression of a nearby sex-determination gene, *CmWIP1* (Martin *et al.*, 2009). Examples such as this last one blur the distinction between genetic and epigenetic inheritance (see Becker and Weigel, 2012).

Inheritance

Although many epigenetic modifications within a cell are likely to be short-lived, some are described as heritable. Confusingly, in addition to the normal use of the term to mean that offspring of a given individual also possesses the epigenetic modification in question, in this context, heritable has also been used to mean that daughter cells within the same organism have the same modification as the parental cell. Nevertheless, these two phenomena differ in many important respects and should be differentiated. Hence, in order to make this distinction clear and to avoid any confusion, we will refer to these two meanings as (transgenerational) inheritance and (within-generational) persistence, respectively.

Persistence is essential if the epigenetic mark is to have some lasting effect in a growing organism. Accurate transmission of an epigenetic modification during development of an organ in an individual means that gene expression will be modified in the same way throughout that organ. Such fidelity is critical if the stimulus inducing the epigenetic change is transient or if it can be perceived by the organism only during a short developmental window. In mammals, the persistence of methylation marks during mitosis is governed by a family of enzymes known as DNA methyltransferases (DNMTs). Dnmt1, for example, maintains methylation state during DNA replication, methylating an unmethylated DNA strand when the other strand is already methylated.

The potential for the stable somatic persistence of epigenetic marks has fundamental biological implications. It is one mechanism by which gene expression can be differentiated among different cell types in an organism but remain co-ordinated within a particular tissue. In addition, epigenetic modification is likely to be a proximal mechanism for the phenomenon of phenotypic plasticity, in which organisms with the same genetic constitution respond in different (and usually appropriate) ways to different environments. Herman *et al.* (2014) explore the evolutionary expectations we might have for the degree of stability of epigenetic modifications. For example, the sites of modification must be able to respond to appropriate cues (especially early in development) but simultaneously ignore spurious cues; moreover, epigenetic states should be resistant to degradation over time if the gene expression they are modifying is important.

Transgenerational epigenetic inheritance also has profound biological consequences (Richards *et al.*, 2012). If epigenetic modifications can be transmitted across generations, then far more than genes are being passed on from one generation to the next (Laland *et al.*, 2014). The extent to which epigenetic marks are transgenerationally transmitted has been seen as controversial (see Haig, 2007; Waterland *et al.*, 2007; Wray *et al.*, 2014), but a large and growing number of unequivocal cases can now be found in the scientific literature (see below). Foremost among these straightforward cases is genomic imprinting in mammals, where differential marking (usually, but not always, methylation) during gametogenesis results in different expression levels of the maternally and paternally derived gene copies in the offspring. Imprinted genes often occur in clusters within which expression is co-ordinated (Morison *et al.*, 2005) and so one set of marks can affect the expression of several genes. The marks at ICRs are critically different from other methylation marks, however. Soon after fertilization, first in the male pronucleus and later over the first few cell cycles in the female pronucleus, most methylation marks across the whole genome are erased (Sanz *et al.*, 2010). Imprinted genes, however, are resistant to this zygotic demethylation and maintain their parentally induced marks until they are reset, according to the sex of the developing embryo, in the primordial germ cells (which go on to produce the next generation).

In addition to imprinting many maternal (and, indeed, paternal) effects are mediated by epigenetic modifications. For example, unfertilized mammalian eggs contain maternal messenger RNAs that subsequently interact with the zygote's genome (Daxinger and Whitelaw, 2012). Maternal behaviors can also be transmitted across generations: differences among mouse dams in how they groom their pups are inherited non-genomically and affect how the pups respond to stress (Francis *et al.*, 1999). Interestingly, these behavioral differences appear to be the result of methylation differences induced by their own mother's grooming; thus the grooming behavior is re-created every generation (Weaver *et al.*, 2004). Such parental regulation of offspring methylation states provides a mechanism for stable non-genomic inheritance over many generations.

Controversies about the importance of transgenerational epigenetic inheritance fall into two categories. First, differentiating between direct and inherited effects can be difficult, in practice. For example, in many mammalian species, the cells that, when eventually fertilized, will grow into pregnant female's grand-offspring are present in the proto-ovarial tissues of the fetus growing within her uterus. Thus exposure of a pregnant female to an environmental stress may also expose the next two generations as well, and these direct effects will mimic genuine transgenerational inheritance (Heard and

Martienssen, 2014). These superficially similar outcomes are significantly different from an evolutionary viewpoint, however: the former can be viewed as one aspect of the standard way in which genes and environment interact to produce the phenotype; the latter requires an expanded view of inheritance (Grossniklaus *et al.*, 2013).

Second, an apparent lack of mechanism for accurate transmission of non-genomic information has led some to argue that the phenomenon is not of major significance (see Campos *et al.*, 2014 for a review of these arguments). The genome-wide demethylation that occurs during mammalian embryogenesis, for example, seems to rule out the archetypal epigenetic mark, methylation, as being of widespread importance (i.e., beyond the exception of genomic imprinting) in mammals (Daxinger and Whitelaw, 2012). Nevertheless, there is an increasing number of well-documented cases of transgenerational epigenetic inheritance (Jablonka and Raz, 2009), from plants (Scoville *et al.*, 2011; Herman and Sultan, 2011; Holeski *et al.*, 2012) to mammals (Lim and Brunet, 2013), *Drosophila* (Seong *et al.*, 2011), and nematodes (Rankin, 2015), and there is general agreement that it is likely to be common as well as critical to our understanding of inheritance and development (Grossniklaus *et al.*, 2013).

Evolutionary Implications

If epigenetic modifications underlie many plastic responses (as seems likely), then persistence of these non-genetic marks (within an organism) is fundamental to how development works. Plasticity plays a crucial role in evolution, because it endows a developing organism with the ability to respond in an appropriate way to various environmental conditions. In addition, numerous properties of the inheritance of epigenetic marks underlying plastic responses, such as whether the signal should attenuate over time, may be the target of selection (Herman *et al.*, 2014).

Of even greater interest to evolutionary biologists, and the reason for much of the controversy on the topic, are the evolutionary consequences of transgenerational epigenetic inheritance. Such inheritance is often described as 'Lamarckian' (Jablonka and Lamb, 2005; Haig, 2007), because it appears to provide a mechanism for 'the inheritance of acquired characters.' Nevertheless, modern observations of transgenerational epigenetic inheritance differ fundamentally from old-school Lamarckian inheritance; in the latter, environmental influences somehow become permanently encoded in the genome and are thenceforth transmitted as in standard genetics (Bonduriansky, 2012).

Certainly, even the uncontentious and well-understood case of genomic imprinting has potential effects at the population level and hence for evolution. Expression of an imprinted gene is effectively haploid in those tissues in which just one copy is transcribed and hence the unexpressed allele is at least partially hidden from selection in that individual. This masking may affect the outcome of natural selection. For example, the frequency of individuals affected by a deleterious allele at an imprinted locus is likely to be approximately twice that of those affected by a similarly harmful recessive mutation at a non-imprinting gene (Spencer and Williams, 1995). Attempts to improve agricultural outcomes through artificial selection on a quantitative trait affected by imprinting are also altered: the standard breeder's equation (which predicts the change in a population's mean in response to selection) is no longer accurate, often overestimating the response (Santure and Spencer, 2011).

The population-level effects of transgenerational inheritance of environmentally induced epigenetic marks have been dubbed 'population epigenetics' by analogy with the purely genetic models of population genetics. Very simple population-epigenetic models of the action of natural selection behave very differently from population-genetic models of selection (e.g., Geoghegan and Spencer, 2012). Most basically, the induction of fresh epigenetic marks each generation means that epigenetic variation is never depleted; in many population-genetic models, by contrast, selection quickly eliminates genetic variation. A critical parameter in such models, somewhat analogous to the mutation rate in population genetics, is the probability with which an environmental cue induces an epigenetic change. In models of paramutation, a similar role is played by the 'paramutation rate,' the chance that the paramutable allele will be paramutated by the partnering paramutagenic allele in a heterozygous individual (Geoghegan and Spencer, 2013).

More general attempts to include non-genetic inheritance (including epigenetic inheritance, but also various other parental cues, such as hormonal signals) into evolutionary theory confirm that epigenetic inheritance is of fundamental importance, especially when it interacts with standard genetic inheritance. In a review of theoretical studies of evolution in changing environments, Bonduriansky *et al.* (2012) found that such forms of inheritance can increase the rate of evolution and even alter its direction. Interestingly, maternal inheritance has similar consequences (Kirkpatrick and Lande, 1989). Moreover, careful empirical investigations are likely to find further natural examples: mathematical analyses imply that non-genetic inheritance should evolve easily in fluctuating environments, depending on the values of critical parameters such as the temporal autocorrelation in environmental state and the accuracy of environmental cues (Leimar and McNamara, 2015). Transgenerational epigenetic inheritance allows the organism to integrate more sources of information (including, obviously, genetic information) in its development, which may give it a selective advantage.

The interaction between genetic and epigenetic inheritance also has the potential to explain previously puzzling observations, such as the 'lek paradox,' the maintenance of female choice in species in which males do not provide their offspring with care or direct benefits. Standard genetic theory had suggested that a population's genetic variation in male quality would rapidly be depleted, which would then lead to the elimination of what is presumably a costly female choice. Mathematical models predict that if males also pass on environmentally induced fitness benefits to their offspring, then costly female choice will remain (Bonduriansky and Day, 2012). This finding is an instance of how such interactions may resolve Lewontin's (1974) 'paradox of variation,' in which theoretical models fail to predict the ubiquity of phenotypic variation in natural populations of many organisms (Sultan, 2015).

Future Questions

The potential for major evolutionary implications of the various sorts of epigenetic effects has generated intense research interest, albeit tempered with a cautious skepticism, largely about mechanism (Grossniklaus *et al.*, 2013). Indeed, some authorities remain unconvinced. For example, writing about adaptive epigenetic inheritance, Heard and Martienssen (2014) say 'concrete evidence from model systems is still lacking.' Ideally, we would have unequivocal cases of transgenerational epigenetic inheritance, preferably in a laboratory species, with clear proximate molecular mechanisms, in which different environmental cues induce measurable phenotypic responses.

Assuming such examples can be discovered, we would then use them to answer some fundamental questions: What is it about these environmentally induced epigenetic changes that allow them to resist the normal molecular erasure processes such as demethlyation? How many generations do the marks persist? Do they fade over several generations or do they have the potential to last indefinitely (or until the next environmental cue)? If they fade gradually, does their effect fade in parallel or is there some threshold above which their phenotypic effect remains and below which it is absent? What is the effect of repeated environmental induction? Does such repetition reinforce the mark(s)? Can transgenerationally inherited epigenetic changes be reversed? If so how? Nadeau (2009) and Holeski *et al.* (2012) have extensive lists of questions like these.

Such questions are not simply matters of academic curiosity. Epigenetic changes have numerous health implications. For example, environmental insults are well known to induce epigenetic changes that increase susceptibility to disease (especially cancer). If such changes are heritable, then offspring, perhaps for several generations, will also have increased susceptibility, even if the original environmental insult is absent (Nilsson and Skinner, 2015). Gluckman and Hanson (2006) have argued that a mismatch between our modern high-energy diets and our evolutionary past has led to the current high rates of metabolic disease. Moreover, they contend that dietary insults can also induce heritable epigenetic changes that predispose our children to these same diseases. Hence, understanding the epigenetic basis of such disease may lead to a solution to the problem.

See also: Epigenetics and Genome Evolution. Genotype-by-Environment Interaction. Maternal Effects. Waddington's Epigenetic Landscape, History of

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Epigenetics and Genome Evolution

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Glossary

DNase hypersensitivity assay DNase is a nuclease that cleaves DNA at phosphodiester linkages. When genomic DNA is subject to DNase, regions that are not tightly bound by nucleosomes are cleaved out. These regions include active enhancers and promoters, as well as nucleosome reposition regions. On the other hand, regions tightly bound by nucleosomes are protected from DNA cleavage.

H3K4me3 Tri-methylation of the lysine 4 residue of the histone 3.

H3K9me3 Tri-methylation of the lysine 9 residue of the histone 3.

Polyploidy When cells and organisms contain more than two sets of paired chromosomes (diploid: $2n$). Polyploidy is especially common in plants. For example, cotton is tetraploid ($4n$) and sugarcane is octaploid ($8n$).

PR/SET domain PR stands for positive regulatory domain, and SET is a domain initially characterized in the *Drosophila* protein (Su)var3–9, *Enhancer-of-zeste* (hence called 'SET'). SET domain consists of 130 to 140 amino acids that are evolutionarily well conserved. The SET domain of some proteins encodes histone methyltransferase activity, especially in case of the PRDM9. The PR domain is 20–30% similar to the SET domain and found in many histone lysine methyltransferases.

SNP It stands for single nucleotide polymorphism. DNA sequence variation where two or more nucleotides are found at the same position in different individuals.

Introduction

The term 'epigenetics' was initially coined to describe how genetic background influences the progress of development (Waddington, 1942; Haig, 2004). However, since its original introduction, the term epigenetics has been used in multiple different biological contexts (e.g., Burggren and Crews, 2014; Holliday, 2006; Jablonka and Lamb, 1998). It is fair to say that epigenetics means different aspects of biology to different individuals. It is thus useful to explicitly define epigenetics for the scope of this article. Here we will take a mechanistic, reductionistic approach and define epigenetics from the biochemical perspective (**Figure 1**). We focus on chemical modifications of genomic DNA template and histone cores of nucleosomes which affect how genomic DNA is packaged in each cell. The packaging of genomic DNA is essential for cells: all of the many cells in complex organisms need to package their genomic DNA in an extremely sophisticated way for accurate transmissions across cell generations. At the same time, local DNA regions that are required for the phenotype of the specific cells should be readily accessible to cellular machineries. As such, epigenetic modifications are critical for cellular processes, in particular gene expression.

The first epigenetic modification we will discuss is DNA methylation (**Figure 1**), especially the addition of the methyl ($-CH_3$) group to the 5th carbon of the cytosine base. This is the most common mode of DNA modification observed in animal and plant genomes (methylation of the adenine base also exists in bacteria, but will not be discussed in this article). In animal genomes, DNA methylation typically occurs at Cs followed by Gs, or 'CpGs,' although low level of non-CpG methylation are also observed. In plants, DNA methylation in CpG, CpHpH, and CpHpG (H refers to A, C, or T) all occur in substantial frequencies (Lister *et al.*, 2008). Recently, another type of modification that is biochemically related to DNA methylation is gaining attention: the hydroxymethylation of the 5th carbon of the cytosines. Hydroxymethylation occurs by the family of 'ten-eleven-translocation' (TET) oxygenases from methylated cytosine templates (Branco *et al.*, 2012; Williams *et al.*, 2011). Hydroxymethylation is also observed in non-mammalian animal genomes (e.g., Bracht *et al.*, 2012; Cingolani *et al.*, 2013). However, the functional role of hydroxymethylation on regulation and evolution are currently not as well understood as that of DNA methylation.

The second major component of epigenetic mechanisms we will discuss is modifications of specific amino acids in histone cores of the nucleosomes (**Figure 1**). Nucleosomes are the fundamental building blocks of chromatin, consisting of eight histone proteins (histone octamers). These histone proteins contain N- and C-terminal 'tails' that often bear specific chemical modifications, or 'marks.' The distinctive chemical modifications of histone tails, including methylation, acetylation, ubiquitination, and phosphorylation, are now appreciated as key regulatory signals in cellular environment (Bernstein *et al.*, 2007; Strahl and Allis, 2000). DNA methylation and histone modifications are deeply implicated with development and diseases, and also with regulatory mechanisms such as gene expression, imprinting, and recombination (e.g., Bernstein *et al.*, 2007; Strahl and Allis, 2000; Bird, 2007).

Epigenetics and Evolution: Compatible Concepts?

Epigenetic mechanisms are intensely studied in many fields of biology, most prominently in development, disease (especially cancer), and regulation. On the other hand, historically, epigenetics has received much less attention from evolutionary biologists. Intuitively, regulation of epigenetic programming should be under strong selection for its integrity, given the critical significance of epigenetics on development and regulation. A potential genetic change that can cause epigenetic

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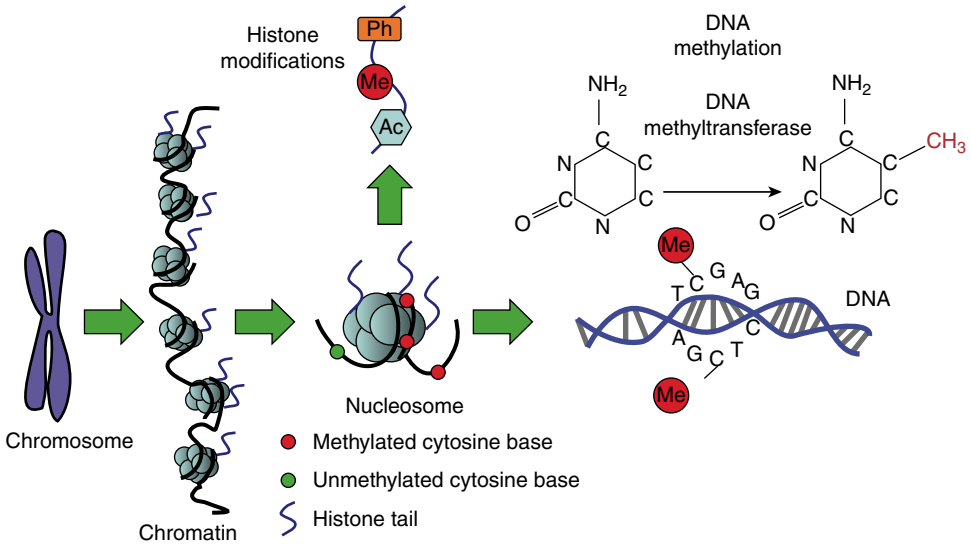

Figure 1 Epigenetic modifications including histone tail modifications and DNA methylation. The basic structure of chromosomes consists of genomic DNA wrapped around histone octamer nucleosomes. Histone proteins have N- and C-terminal 'tails' which are often marked by specific chemical modifications. Genomic DNA is also often modified by the addition of methyl group (CH_3) by dedicated enzymes called 'DNA methyltransferases.' In most animal genomes DNA methylation preferentially targets C followed by G, or 'CpGs.' Modified from Relton, C.L., Davey Smith, G., 2010. Epigenetic epidemiology of common complex disease: Prospects for prediction, prevention, and treatment. PLoS Medicine 7 (10), e1000356.

shifts should be subject to natural selection as long as the epigenetic shifts generate evolutionarily visible phenotypes. One candidate for such a phenotype is gene expression, which is often tightly regulated by epigenetic mechanisms.

However, a prevailing perspective on epigenetics has kept evolutionary biologists at bay. Until recently, it was widely viewed that epigenetic patterns are highly variable within an organism, and even between the same cell types depending upon specific biological conditions, because they are largely determined by environmental signals rather than the genetic code. However, emerging data suggest an imperative revision of this view. Like many other fields of biology, epigenetics is experiencing a transformative explosion of data, thanks to advances of next-generation sequencing technology. These new data begin to reveal that, in the human genome, only minor fractions of positions exhibit epigenetic variation across cell types for DNA methylation (Ziller *et al.*, 2013; Zeng *et al.*, 2014). Consequently, the extent of cellular epigenetic reprogramming is much more substantial than previously envisioned. Moreover, targeted and genome-wide studies continue to present evidence that epigenetic variations have genetic bases, at least within some genomic contexts (e.g., Heyn *et al.*, 2013; Lienert *et al.*, 2011; Eichten *et al.*, 2013).

On the practical side, the lack of comparative data on epigenetic variation between species was also a limiting factor for evolutionary biologists. However, data on genome-wide epigenetic modifications from diverse group of taxa are accumulating, in some cases from matched cell types (e.g., Molaro *et al.*, 2011; Shulha *et al.*, 2012). Large-scale population data on epigenetic variability are also becoming available (e.g., Schmitz *et al.*, 2013). These data, together with the increased understanding of the extent of epigenetic variation across cell types and developmental stages (Lister *et al.*, 2013; Ziller *et al.*, 2013), provide a ripe opportunity for evolutionary biologists to explore the evolutionary dynamics and significance of epigenetic modifications.

It should be noted that epigenetic modifications at some genomic regions appear as truly variable across cell types (Ziller *et al.*, 2013), and potentially determined by environmental signals (Schmitz *et al.*, 2013; Stroud *et al.*, 2013). It is possible that epigenetic patterns of some genomic sites are under strong genetic control while those of other positions are more labile and susceptible to environmental effects. Comparative studies of epigenetic variation have the potential to illuminate such yet unknown aspect of genome organization and regulation.

Classical Role of DNA Methylation on Mutation and Sequence Composition

One important evolutionary consequence of epigenetics is the impact of DNA methylation on genomic mutations. It has been long known that methylated cytosines are highly subject to rapid spontaneous deamination, which turns methylated cytosines to thymines (e.g., Coulondre *et al.*, 1978). Consequently, DNA methylation increases rates of C to T (or G to A) transition mutations.

The effect of this process is best understood in animal genomes, and recognized as an important evolutionary force affecting mutation rates, mutation spectra, and genome sequence composition. For example, in primate genomes, transitions due to DNA methylation are over an order of magnitude more prevalent than those that arise due to other mechanisms such as DNA replication errors (Elango *et al.*, 2008). In fact, transitions at CpG sites account for a quarter of all point mutations that separate the genomes of humans

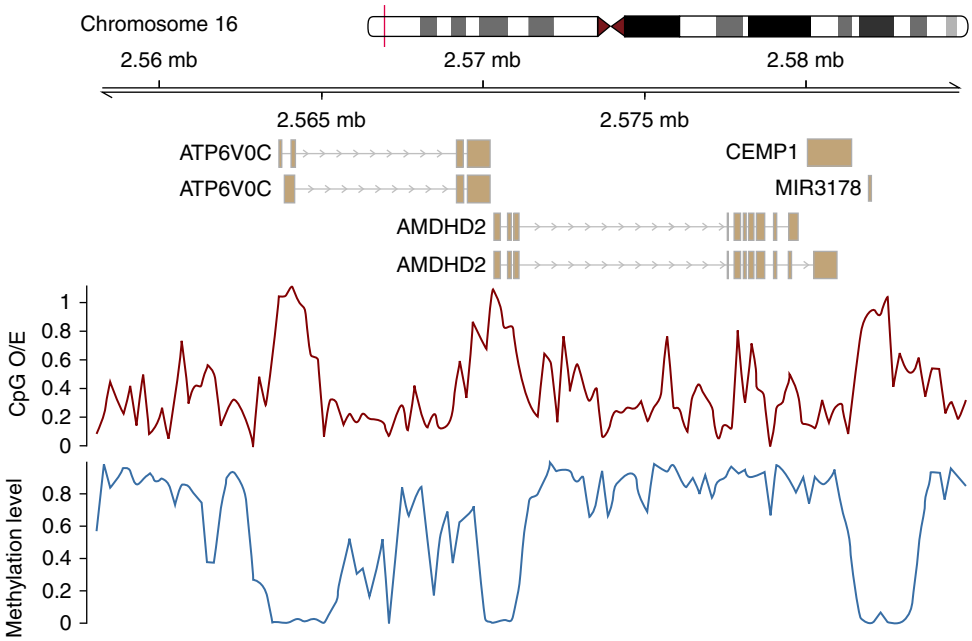

Figure 2 Depletion of CpG dinucleotides is negatively correlated with the actual DNA methylation levels. CpG O/E, which is a metric calculated as the observed CpG counts divided by the expected CpG counts (Bird and Taggart, 1980; Yi and Goodisman, 2009) reflects the level of CpG depletion. Here a 200 kb region of the human chromosome 16 is depicted: CpG O/E are generally low with some peaks, particularly near transcription start sites and exons. Correspondingly, DNA methylation level is generally high with conspicuous dips. Lowly methylated regions tend to overlap with high CpG O/E regions and near transcription start sites and genes. Note that ATP6V0C and AMDHD2 are encoded on the sense strand, while CEMP1 and MIR3178 are encoded on the complementary strand. DNA methylation data are from the brain of a 25-year-old male. Reproduced from Lister, R., Mukamel, E.A., Nery, J.R., *et al.*, 2013. Global epigenomic reconfiguration during mammalian brain development. Science 341 (6146), 1237905.

and chimpanzees (The Chimpanzee Genome Sequencing and Analysis Consortium, 2005). Mutations that originate due to DNA methylation are also less prone to the generation time effect (Hwang and Green, 2004; Kim *et al.*, 2006).

Due to the increase of CpG to TpG (or CpA) transition, DNA methylation effectively reduces the CpG content of affected regions, when compared to the expected values calculated from the observed frequencies of G and C nucleotides (Yi and Goodisman, 2009). In fact, almost four decades ago, based upon the observed CpG contents of small genomic fragments, it was proposed that mammalian genomes are heavily methylated, and invertebrate genomes are sparsely methylated (Bird and Taggart, 1980). These predictions largely hold true. For example, in the human genome, CpG dinucleotides exist at only approximately 1/5th of the expected frequency (**Figure 2**). Some genomic regions, however, maintain high CpG contents, and are often devoid of DNA methylation (**Figure 2**). Such regions tend to occur near genes, especially near the transcription start sites (**Figure 2**). Regions particularly enriched in CpGs and exhibiting reduced DNA methylation are referred to as 'CpG islands' (Illingworth and Bird, 2009). DNA methylation of CpG-rich regulatory regions near genes (such as promoters) is often associated with silencing of gene expression (e.g., Jones, 2012).

The degree of CpG depletion is widely used to infer historical DNA methylation events in diverse animal (e.g., Elango *et al.*, 2009; Sarda *et al.*, 2012) and plant genomes (Takuno and Gaut, 2011). A particularly useful feature of the CpG contents analysis is that it can detect regional differences in DNA methylation, even in genomes where DNA methylation is rare and highly localized. For example, genomes of many hymenopteran insects are sparsely methylated, and most of their DNA methylation is targeted to a subset of transcription units (often referred to as 'gene bodies' (e.g., Lyko *et al.*, 2010; Zemach *et al.*, 2010; Gavery and Roberts, 2013)). Unlike DNA methylation of regulatory regions, DNA methylation of gene bodies are often associated with increased gene expression and reduced gene expression variability (Zemach *et al.*, 2010; Huh *et al.*, 2013). Interestingly, the overall CpG contents of these hymenopteran insect genomes are often highly increased beyond the expected value (e.g., Honey Bee Genome Project, 2006; Werren *et al.*, 2010) for reasons that are not clearly resolved yet (however, a recent study suggests recombination-associated processes, see Wallberg *et al.*, 2015). Nevertheless, CpG content analyses can clearly separate methylated and unmethylated gene bodies (**Figure 3**).

Potential Mutational Effects of Histone Tail Modifications and Chromatin Structures

Studies have also begun to examine the effects of histone tail modifications on sequence composition and mutation rates. In the case of cancer cells, there is strong evidence that specific modifications of histone tails affect mutation rates and spectra (Schuster-Böckler and Lehner, 2012). In particular, the H3K9me3 modification, which is associated with heterochromatin and repression, accounts for a large number of

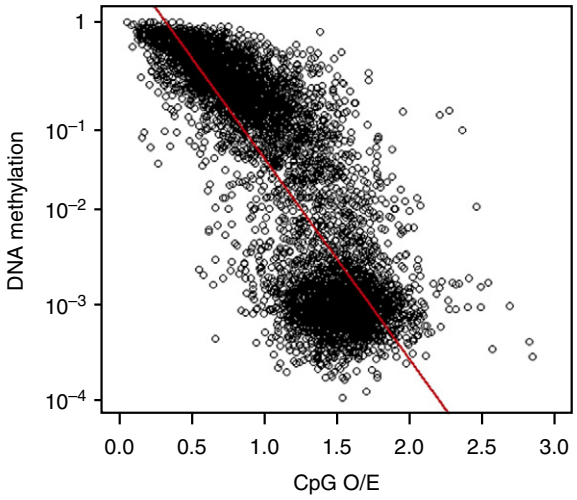

Figure 3 The relationship between CpG depletion (on the *x*-axis) and the experimentally measured methylation levels (*y*-axis) in the honey bee genes. Highly methylated genes and sparsely methylated genes are found in two distinctive clusters, and CpG O/E is very well correlated with the actual DNA methylation levels. The red line is the linear regression between the two variables. Data from Sarda, S., Zeng, J., Hunt, B.G., Yi, S.V., 2012. The evolution of invertebrate gene body methylation. Molecular Biology and Evolution 29, 1907–1916.

mutations in several cancer cell lines. The relationship between H3K9me3 modification and mutations was statistically independent of other sequence-associated features that are known to affect mutation rates (Schuster-Böckler and Lehner, 2012). Whether this finding extends to evolutionary timescale and germline mutations is currently unknown.

It is worth noting that there is also some evidence that large-scale chromatin structures affect evolutionary rates. Prendergast *et al.* (2007) demonstrated that regions characterized by more 'closed' chromatin structures in the human genome tend to harbor more nucleotide substitutions. This trend was consistent across various genomic regions (intergenic regions, ancestral repeats, and coding sequences) and across different phylogenetic distances (human SNPs, human–chimpanzee, and human–mouse pair comparisons), indicating that mutation rates themselves may be affected by chromatin structures. A caveat of this study was the fact that the chromatin structures were derived from lymphoblastoid cell lines, rather than germ cells. There is also a somewhat opposing observation that genomic regions that are particularly 'open' in pluripotent cells, measured by DNase hypersensitivity assays, are known to accumulate more mutations (Thurman *et al.*, 2012). Future studies are necessary to determine how large-scale chromatin structures and histone modifications influence evolutionary rates and genome sequence composition.

Emerging Studies of Epigenetics on Molecular Evolution

Recent advances in genome-wide epigenetic profiling have allowed researchers to examine aspects of molecular evolution other than mutation rates that are potentially affected by epigenetic modifications. We first discuss two areas of research that are particularly promising.

Recombination Hotspots and Epigenetic Variation

A fantastic example of the interplay between evolution and epigenetics is the evolution of recombination hotspots. Recombination hotspots are genomic regions (typically in ~kb ranges) that experience intensely high levels of recombination compared to the genomic background (Jeffreys *et al.*, 2001). Recombination hotspots are numerous in mammalian genomes (Myers *et al.*, 2005), yet extremely transient over evolutionary timescales. For example, few recombination hotspots are shared between human and chimpanzee genomes (Auton *et al.*, 2012). Why, and how do such critical genomic hallmarks evolve so fast?

A major piece of this puzzle came to light when it was discovered that a gene called PRDM9 is an important regulator of recombination hotspots in the human and mouse genomes (Baudat *et al.*, 2010; Berg *et al.*, 2010; Myers *et al.*, 2010; Parvanov *et al.*, 2010). The molecular structure of PRDM9 indicates that it is capable of interacting with genomic DNA as well as performing histone tail modification: it contains both a zinc-finger domain that binds genomic DNA, and a PR/SET domain capable of histone tri-methylation (Hayashi *et al.*, 2005). Interestingly, the DNA-binding zinc-finger domain evolves extremely rapidly in mammalian genomes, often accompanied by sequence signatures of positive selection (Oliver *et al.*, 2009; Buard *et al.*, 2014; Schwartz *et al.*, 2014). The rapid evolution of the DNA-binding domain of PRDM9 can explain why the locations of recombination hotspots change rapidly between species, as the locations of the majority of human and mouse recombination hotspots are controlled by PRDM9 itself (Ségurel *et al.*, 2011). However, the nature of the evolutionary force underlying the rapid evolution of zinc-finger domains themselves is not fully resolved: it may be directly due to its effect on recombination, or due to some other functions of PRDM9 (Ségurel *et al.*, 2011).

The evidence so far supports that PRDM9 performs 'epigenetic' functions. In addition to its ability to generate H3K4me3 marks (Hayashi *et al.*, 2005), modifying the DNA-binding zinc-finger domain of PRDM9 redistributes recombination hotspots as well as of H3K4me3 marks (Grey *et al.*, 2011). However, apart from its function as an epigenetic modifier, precisely how PRDM9 determines recombination hotspots is not known. For example, in the human genome, there are many tens of thousands of sequence motifs that PRDM9 can potentially bind, yet only a subset of them function as recombination hotspots (Ségurel *et al.*, 2011). Likewise, there are many other proteins capable of generating H3K4me3 marks, without influencing recombination events. In addition, even though H3K4me3 marks are typically associated with active gene promoters, mammalian recombination hotspots generally avoid genic regions and targeted to non-genic, repetitive regions (McVean, 2010). It is possible that additional chromatin marks may be necessary for the determination of recombination hotspot locations (Zeng and Yi, 2014).

Furthermore, PRDM9 is directly linked to hybrid sterility in mouse. In fact, it remains as the only known 'speciation gene' in mammals up to date (Mihola *et al.*, 2009). This role is most likely to be mediated by the epigenetic functions of the PRDM9. For instance, a modification of the PR/SET domain itself causes sterility (Hayashi *et al.*, 2005). It is notable that there exists a large body of evidence connecting epigenetic incompatibilities and molecular mechanisms of speciation in a variety of taxa (e.g., Brown and O'Neill, 2010). The study of PRDM9, with its distinctive roles on recombination and speciation, promises to be a unique system to elucidate the details of a crosstalk between epigenetics and evolution.

Evolutionary Trade-Off between TE Methylation and Gene Expression

Another research area where the functional effects of epigenetic modification can be put into specific evolutionary contexts is the effect of transposable element (TE) methylation on expression of nearby genes in plant genomes (Hollister and Gaut, 2009; Diez *et al.*, 2014). Plant genomes are moderately methylated compared to the heavily methylated mammalian genomes and sparsely methylated hymenopteran genomes. DNA methylations in plants are often targeted to transposable elements and gene bodies. Transposable elements are autonomous selfish genetic elements that can propagate by inserting themselves to different positions of the genome. Host genomes have utilized epigenetic modifications such as DNA methylation to suppress the mobilization of TEs. Consequently, DNA methylation of TEs should be 'beneficial' to the host genome. However, DNA methylation of some TEs can 'spread' or 'leak' to nearby regions (Ahmed *et al.*, 2011; Eichten *et al.*, 2012). One consequence of such a leaky TE methylation is that expression of nearby genes can be directly affected (Ahmed *et al.*, 2011; Lippman *et al.*, 2004; Martin *et al.*, 2009; Eichten *et al.*, 2012). Specifically, methylation of TEs can silence the expression of neighboring genes, which can be deleterious for host plants.

Formalizing these potential evolutionary conflicts, Hollister and Gaut (2009) proposed the 'evolutionary trade-off' model. They posited that genomic distribution of TEs could be affected by the balance of the beneficial effect of TE silencing and the deleterious effects of TE methylation on the expression of nearby genes. One prediction of this model is that heavily methylated, older TEs should be preferentially removed from genic regions via natural selection (Hollister and Gaut, 2009). This prediction has been met for several species examined so far (Diez *et al.*, 2014). In addition, methylated TEs that occur in genic regions exhibit stronger signals of natural selection than non-methylated TEs or non-genic TEs, rendering additional support to the evolutionary trade-off model (Hollister and Gaut, 2009).

It is further proposed that the evolutionary trade-off model could explain non-random patterns of gene expression in plant hybrids or polyploids. Transcriptional reprogramming in hybrids or polyploids is largely driven by epigenetic changes (Rapp and Wendel, 2005). Freeling *et al.* (2012) proposed that expression of specific alleles in hybrids or allopolypoids could be influenced by the differences in the TE distribution of the parental genomes. According to the evolutionary trade-off model, if one parental allele of a gene harbors strongly methylated TE nearby, its expression in the hybrid/polyploidy would be reduced (Freeling *et al.*, 2012). Changes in the epigenetic profiles of the hybrids/polyploids can further cause preferential loss of alleles of specific parental origin (Freeling *et al.*, 2012). Even though more studies are necessary to comprehensively evaluate these hypotheses, it is clear that the evolutionary trade-off model presents an attractive evolutionary framework to examine how natural selection on gene expression mediated by epigenetic patterns affect genome evolution and hybridization events (Diez *et al.*, 2014).

Concluding Remarks

Even though the term epigenetics is used by different groups of researchers with different foci, on the mechanistic level epigenetic modifications can be classified into only a few major molecular processes. Here we discussed two of the best-understood processes: DNA methylation and histone tail modification. Apart from the important role of DNA methylation on mutational processes, how epigenetic mechanisms themselves evolve, and how epigenetics shapes evolution, remain largely unresolved. Auspiciously, functional epigenetics research is gaining dramatic momentum at the moment, fueled by the technical advances of next-generation sequencing methods. As our understanding on the molecular processes of epigenetics improves, so will our knowledge of the relationship between epigenetics and evolution. It is an opportune time for evolutionary epigenetics: several solid examples where key evolutionary processes are associated with epigenetic modifications exist (as discussed above). Population-level and comparative epigenetic data have begun to appear and likely to rapidly accumulate. Disentangling genetic versus environmental effects on the establishment and maintenance of epigenetic modifications may become feasible soon. Consequently, we will be able to illuminate genetic and evolutionary bases of epigenetic programming, which may reveal new aspects of genome evolution.

See also: Epigenetic Inheritance. Mutation and Genome Evolution. Waddington's Epigenetic Landscape, History of

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Evo-Devo: Regulatory and Protein-Coding Evolution in Plant Diversification

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Glossary

Alternative splicing The inclusion or exclusion of exons from messenger RNA (mRNA) resulting in a single gene that codes for multiple proteins.
Chromatin Complex of eukaryotic DNA, protein, and RNA that packages chromosomal DNA around histones for increased cellular control.
Darwinian evolution Charles Darwin's theory that all organisms are derived from a common ancestor whose descendants diversified through natural selection of small, inherited variations.
Epialleles Genes that are identical at the sequence level but differ in their extent of methylation.
Heterochrony Change in the timing or rate of developmental processes, such as gene expression, growth rate, and differentiation, which commonly lead to changes in character size and shape.
Heterometry Changes in the size or amount of a developmental process.
Heterotopy Changes in the position of a developmental process, such as reiteration of gene expression at different locations to form serial homologs (e.g., leaves or flowers).
Homeologs Duplicate genes in an allopolyploid plant genome derived from the maternal and paternal parent.
Macroevolution Evolution that occurs above the level of species through similar (see microevolution) or distinct (e.g., mass extinction and species selection) mechanisms as microevolution.
Macromutation (saltation) A mutation that causes a profound change in an organism, originally conceived by Richard Goldschmidt to explain the divide between microevolution and macroevolution.
Microevolution Change in allele frequencies over time within a population.
Modules Quasi-independent parts of an organism that are tightly regulated, but develop somewhat independently of each other.
Neo-functionalization The evolution of a novel function for one paralog derived from a gene duplication event.
Noncoding RNA Functional RNA that is not translated into a protein.
Orthologs Related genes in different species that evolved from a common ancestral gene through speciation.
Paralogs Related genes derived from duplication events within a genome.
Pleiotropy The influence of a gene on two or more phenotypic traits.
Sub-functionalization The partitioning of ancestral function between descendent duplicate genes.
Toolkit genes Small subset of highly functionally conserved genes whose products control the body plan, and can affect morphological evolution depending on when and where they are deployed.

An Historical Perspective on Plant Evo-Devo

The evolution of development (evo-devo) is a field of biology that attempts to uncover the biophysical, ontogenetic, and molecular genetic changes that have resulted in the Earth's vast array of organismal diversity. Plant evo-devo can be traced back to the pioneering work of comparative morphologists of the eighteenth century, whose observations of similarities between body plans/organs of distinct species during early development provided key evidence for Darwin's theory of evolution, i.e., descent with modification (Friedman and Diggle, 2011). However, it was not until the 1970s that the modern age of evo-devo was really born, fueled primarily by the synthesis of developmental genetics, molecular phylogenetics, and both micro- and macro-evolutionary theory (Gould, 1977).

Among the major discoveries that followed the birth of modern evo-devo, a few were to have profound effects on our understanding of plant evolution. These include the fact that plants share a conserved (deeply homologous) genetic toolkit that is subject to the laws of natural selection and genetic drift (Coen and Meyerowitz, 1991), and that changes in where and when a gene is expressed have major consequences for plant development and its evolution (Hoekstra and Coyne, 2007; Carroll, 2008; Lemmons *et al.*, 2014). The following sections discuss the role of genetic and epigenetic (heritable non-genetic) variation in the evolution of plant form and function, and summarize our understanding of how genome structure/behavior can both constrain and promote developmental evolution.

Gene Expression, Development, and Phenotypic Evolution

In the 1950s Watson, Crick, and colleagues elucidated the structure of DNA, a type of nucleic acid found in all cells that encodes the heritable information required for protein synthesis (Franklin and Gosling, 1953; Watson and Crick, 1953a, b). These findings finally provided the mechanistic basis for faithful cellular replication. However, they also begged the question as to how, if all cells of multicellular organisms have the same DNA, tissues can differentiate to form distinct structures. The answer to this question turned out to lie partially with the environmental control of gene expression, which can be regulated at both the transcriptional and

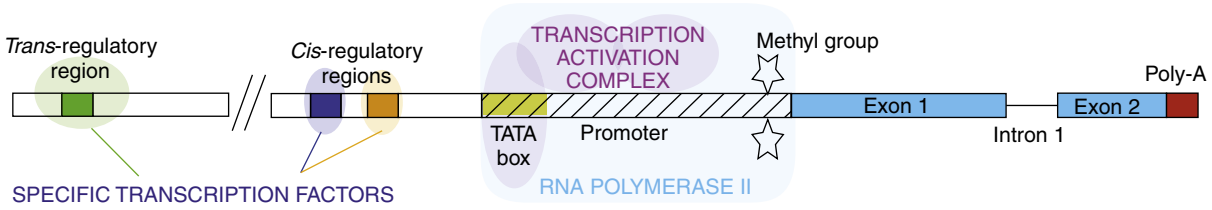

Figure 1 Simplified representation of a eukaryotic gene showing the role of epigenetic DNA methylation (stars), and both generic (purple) and specific transcription factors in the binding of RNA polymerase II to initiate transcription. The TATA box (yellow) is a common regulatory element that facilitates binding of the transcription activation complex within the promoter.

posttranscriptional level. In this context 'environmental' can refer to anything from cell position and lineage effects to the stresses applied by neighboring tissues or abiotic forces (Rodríguez-Mega *et al.*, 2015). In the last several decades, evo-devo studies have found increasing evidence that changes in the position (heterotopy), amount (heterometry), and timing (heterochrony) of gene expression have influenced the evolution of plant development. Nevertheless, it is only recently that the diversity of proximate mechanisms underlying shifts in gene expression has started to be uncovered.

Transcriptional Control and the Evolution of Gene Expression

The transcription of eukaryotic messenger RNA (mRNA) from DNA is a complex process involving numerous interacting proteins (transcription factors) that guide RNA polymerase II to the 5′ promoter regions of protein-coding genes (**Figure 1**). Although general transcription factors are essential for the transcription of all coding genes, so-called specific transcription factors bind to DNA motifs within (*cis*-regulatory elements) or distant to (*trans*-regulatory elements) the promoter of just a few coding genes, acting to either promote or repress their transcription (Phillips, 2008). A good example of specific transcription factors in plants are MADS-box proteins that bind to specific regulatory motifs often associated with downstream genes involved in flowering and flower development (Theissen *et al.*, 2000).

Evolutionary shifts in the binding efficiency and spatiotemporal expression of specific transcription factors can lead to different expression profiles of their downstream targets (Badis *et al.*, 2009). A compelling example of a heterometric change comes from an examination of binding preferences for the transcriptional regulator LEAFY (LFY) across land plants. Specifically, amino acid differences in LFY proteins across the plant kingdom have been demonstrated to affect DNA-binding specificities (Sayou *et al.*, 2014), which combined with quantitative changes in protein expression are hypothesized to explain clade-specific roles for these proteins in cell division and flower development (Tanahashi *et al.*, 2005).

In addition to evolution of upstream transcription factors, modifications to the number, position, and sequence of *cis*- and *trans*-regulatory elements of specific genes can have major impacts on gene expression, and thus the developmental functions of translated proteins. In the Brassicaceae (cabbage family) species *Capsella grandiflora*, insertions and/or single nucleotide polymorphisms in the promoter region of *REDUCED COMPLEXITY* (*RCO*) have resulted in a major heterometric reduction in gene expression, causing a concomitant reduction in leaf dissection compared to its close relative *Capsella rubella* (Sicard *et al.*, 2014). A similar alteration (this time caused by insertion of a retrotransposon) in *cis*-regulatory sequences of the maize (*Zea mays* spp. *mays*) gene *TEOSINTE BRANCHED 1* (*TB1*) resulted in a shift from the highly branched pre-domesticated body plan of the maize progenitor te1osinte (*Zea mays* spp. *parviglumis*) to the unbranched domesticated body plan of maize (Doebley *et al.*, 1997; Studer *et al.*, 2011). Multiple studies have thus demonstrated that changes to both transcription factor proteins and the regulatory sequences they bind to are important components shaping the evolution of plant form and function.

Posttranscriptional Control and the Evolution of Gene Expression

It is becoming increasingly clear that posttranscriptional processes, including mRNA degradation by noncoding small interfering RNAs (siRNAs) and long noncoding RNAs (lncRNAs), and differential splicing of mRNA introns, have major impacts on patterns of gene expression, and thus might be important for the evolution of plant form. In maize, mutations that cause overexpression of the microRNA miR156 result in plants with features reminiscent of their progenitor teosinte, including increased root formation and vegetative branching (Chuck *et al.*, 2007). Thus, since miR156 targets several known transcription factor mRNAs for degradation, it is hypothesized that maize domestication, and perhaps phenotypic evolution of other grasses, occurred partly through the evolution of miR156 function (Chuck *et al.*, 2007). A more recent example of posttranscriptional mechanisms affecting the evolution of plant form is in the elaboration of novel splice forms of the *Solanum* (e.g., potato, tomato, and eggplant) gene *BRANCHED1a* (*BRC1a*) (Nicolas *et al.*, 2015). The ancestral unspliced form of *BRC1a* functions as a nuclear transcription factor to repress stem branching, whereas the alternative splice form in *Solanum* becomes localized to the cytoplasm and has no transcriptional activity. The ratio of splice forms is dependent upon both internal and external environmental conditions, including hormone levels and light quality. Thus, the evolution of alternative splicing in this gene allows flexibility in *Solanum* branching, possibly as an adaptation to occasional shading.

Epigenetic Regulation of Gene Expression

Narrow sense epigenetics refers to stable heritable changes in gene expression that occur in the absence of DNA sequence

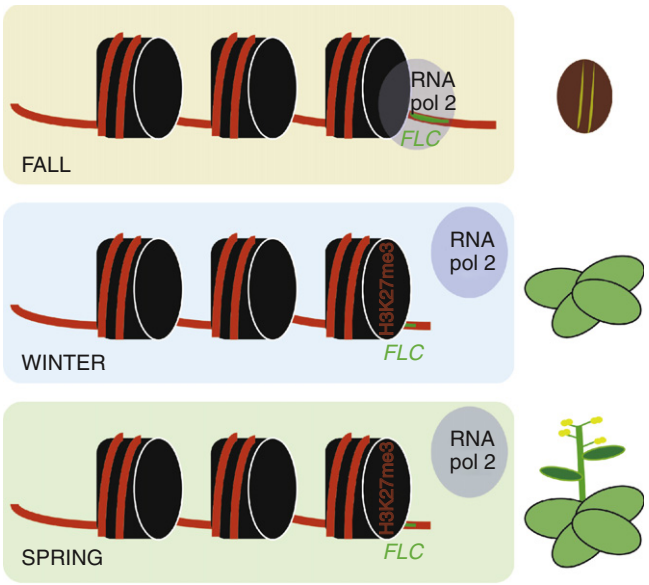

Figure 2 Temperature regulation of the *Arabidopsis* flowering repressor gene *FLOWERING LOCUS C* (*FLC*) through DNA methylation. In 'winter' ecotypes of *Arabidopsis*, seeds are planted in the fall, during which time warm temperatures promote transcription of *FLC* by reducing DNA methylation, thus allowing RNA polymerase II to bind to the promoter. During winter, cold vernalizing temperatures promote the transcription of long noncoding RNAs (lncRNAs) that guide histone 3 lysine 27 trimethylation (H3K27me3) to *FLC*, repressing *FLC* transcription. The epigenetic silencing of *FLC* is maintained through mitotic divisions even when temperatures increase in the spring, triggering flowering and the production of flowers (yellow) and seeds.

variation (Iwasaki and Paszkowski, 2014). The most common mechanisms underlying epigenetic inheritance are DNA methylation, histone modifications, and the synthesis of noncoding RNAs (Rodríguez-Mega *et al.*, 2015), all of which can affect the structure of chromatin, and consequently the ability of RNA polymerase to bind to promoters for transcriptional activation (**Figure 2**). Environmental stresses, such as cold temperatures and pathogen attack, are known to have major impacts on gene expression by triggering the transcription of siRNAs and lncRNAs that mediate DNA methylation (Matzke and Mosher, 2014). Furthermore, epigenetic changes can be stably inherited through several generations, and 'epialleles' generated through the process of natural selection (Richards, 2008).

Several plant species growing in seasonally cold regions of the world are able to use winter chilling (vernalization) as a cue to ready them for rapid spring flowering. Although the cold-sensing mechanism is not well understood, cold-mediated epigenetic modifications to flowering time loci have been implicated in evolution of vernalization responsiveness in the distantly related model species *Arabidopsis* and cereal grass barley (*Hordeum vulgare*) (Heo and Sung, 2011; Oliver *et al.*, 2009). In *Arabidopsis*, the flowering repressor gene *FLOWERING LOCUS C* (*FLC*) is transcriptionally active in individuals grown at warm fall temperatures, but during cold winters the evolutionally conserved polycomb complex PRC2 is recruited to the *FLC* locus, causing increased H3K4me3 DNA methylation (Bastow *et al.*, 2004) (**Figure 2**). Localization of PRC2 to *FLC* can partially be explained by the cold-mediated transcription of the lncRNA *ColdAIR*, which physically interacts with PRC2 and is located within the first intron of *FLC* (Heo and Sung, 2011). Following vernalization, *FLC* methylation (and hence repression) is stably maintained through mitotic divisions, only to be 'reset' during meiosis (Iwasaki and Paszkowski, 2014). This is one of many examples of a derived epigenetic mechanism that has allowed plants to adapt to harsh environmental conditions that might otherwise be too detrimental for growth and development.

Protein-Coding Changes and Developmental Evolution

Eukaryotic genes usually have multiple *cis*- and *trans*-regulatory elements, allowing their protein products to be deployed in a number of developmental contexts. This multi-usage of proteins is termed genetic pleiotropy and is an important factor in explaining why protein-coding regions of genes might be strongly conserved over long evolutionary timescales (Carroll, 2008). However, despite long-standing debates over the prevalence of regulatory versus protein-coding changes in the evolution of plant development (e.g., Doebley and Lukens, 1998), there are multiple examples where amino acid transitions have led to phenotypic evolution. Two important mechanisms that can lead to escape from genetic pleiotropy, and hence make protein-coding changes less deleterious to the developing organism, are modularity and gene duplication.

Modularity Can Promote Both *Cis*-Regulatory and Protein-Coding Evolution

An important feature of toolkit genes is their high level of modularity (Carroll, 2008). In other words, many regulatory genes such as transcription factors are composed of independent

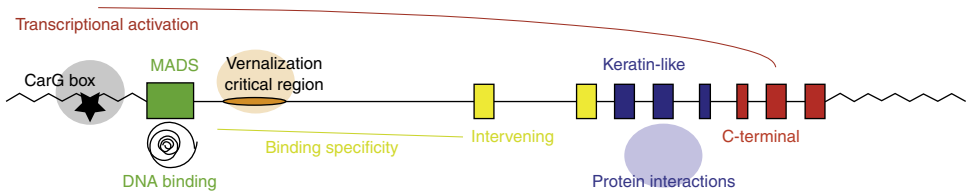

Figure 3 Typical modular structure of the diploid wheat (*Triticum monococcum*) MIKC MADS-box transcription factor *VERNALIZATION 1* (*VRN1*) that might facilitate evolution through discrete *cis*-regulatory and protein-coding changes. The coding region can be separated into four distinct functional domains separated by introns (thin lines): MADS (green) involved in DNA-binding, Intervening (yellow) affecting DNA-binding specificity, Keratin-like (blue) allowing protein–protein interactions, and C-terminal (red) often involved in transcriptional activation and/or forming multi-protein complexes. Important *cis*-regulatory regions, such as the MADS-box binding CarG box and the vernalization critical region, are found upstream of the promoter and within the large first intron. Deletions in these regulatory sequences are implicated in the loss of vernalization responsiveness in some cereal grasses.

units or regions, each of which has a discrete effect on protein function and/or development. An exemplar for modular genes in plants is the MIKC subfamily of MADS-box transcription factors that have evolved novel functions through changes to different protein-coding modules (**Figure 3**). MIKC MADS-box genes are composed of four functional domains: the MADS-box domain involved in DNA-binding, the Intervening domain that influences the specificity of DNA-binding dimers, the Keratin-like domain involved in protein–protein interactions, and the C-terminal domain that is often involved in transcriptional activation and forming multimeric protein complexes (Gramzow and Theissen, 2010). In the genus *Medicago* (e.g., alfalfa) an amino acid change in the C-terminal domain of the MADS-box gene *SHATTERPROOF* (*SHP*) was recently shown to affect protein–protein binding specificities, and is correlated with an important change in fruit morphology that led to the evolution of a novel seed dispersal strategy (Fourquin *et al.*, 2013). Thus, although the biochemical function (i.e., a nuclear-localized transcription factor) has not evolved within *Medicago*, its developmental function has.

In addition to modularity within the coding region of developmental genes, a large number of regulatory elements upstream of the promoter, and within introns and the 5′- or 3′-untranslated regions of genes, can cause modularity in gene expression. Indeed, following up on the previous example, MADS-box genes have a huge number of *cis*-regulatory binding sites that can be bound by their protein products (i.e., autoregulated) or other transcription factors, miRNAs and siRNAs involved in epigenetic modifications (Gramzow and Theissen, 2010). In the vernalization-regulated MADS-box gene *VERNALIZATION 1* (*VRN1*) of cereal grasses, multiple regulatory elements have been found within the promoter and first intron that have different effects on developmental gene function (Yan *et al.*, 2003; Hemming *et al.*, 2009; Alonso-Peral *et al.*, 2011; Zhang *et al.*, 2012) (**Figure 3**). Discrete changes in the regulation of *VRN1* can have important fitness consequences through the control of flowering time, depending on the duration, intensity, and predictability of winter within different geographic regions.

Gene Duplications Can Reduce Genetic Pleiotropy and Foster Phenotypic Novelty

Gene duplication events in plants are a common occurrence. Single genes can be replicated through the action of transposable elements, slippage during DNA replication, or unequal crossing during meiosis, whereas whole genomes can be duplicated via accidents in meiosis (autopolyploidization) or hybridization between distinct taxa (allopolyploidization). The most common fate for duplicate genes is the functional conservation of one paralog and non-functionalization of the other (Rensing, 2014) (**Figure 4(a)**). Non-functionalization can occur extremely rapidly through epigenetic silencing of paralogs or homeologs (genes duplicated through allopolyploidy), possibly as an adaptation to combat gene dosage effects, or gradually over evolutionary time due to relaxed selection (Woodhouse *et al.*, 2014). Less often, both functional paralogs can be maintained in the genome, either through the process of sub-functionalization (where the descendent genes partition the functions of their parent) (**Figure 4(b)**), or neo-functionalization (where one gene evolves a new function) (Ohno, 1970) (**Figure 4(c)**).

Sub- and neo-functionalization of duplicate genes can occur through mutations in either protein coding or *cis*-regulatory regions of genes (**Figure 4**). Although sub-functionalization does not promote phenotypic change *per se*, it can substantially reduce genetic pleiotropy by constraining the impact of mutations to only a subset of developmental functions carried out by the parental (pre-duplication) gene. In this case, mutations in the coding regions of sub-functionalized genes can lead to evolutionary 'tinkering' of phenotypes (Carroll *et al.*, 2001) rather than 'hopeful monsters' caused by so-called macromutations (Goldschmidt, 1940). For example, in snapdragon (*Antirrhinum majus*) a single amino acid change in combination with the rewiring of interacting protein expression has been sufficient to convert the MADS-box gene *FARINELLI* (*FAR*) from a regulator of stamen and carpel develop (as is seen in its paralog *PLENA* (*PLE*)) to a regulator of stamen development alone (Airoldi *et al.*, 2010).

There are numerous examples of neo-functionalized duplicate genes that have driven major developmental innovations in plants, including shoot apical meristems and flowers (Rensing, 2014). In the fewer instances where the underlying mutation for neo-functionalization has been determined, some are due to regulatory changes, some to protein-coding changes, and others both. For example, a single amino acid substitution in the duplicated domestication gene *TEOSINTE GLUME ARCHITECTURE 1* (*TGA1*) was responsible for converting the solidly encased kernels of teosinte to naked kernels

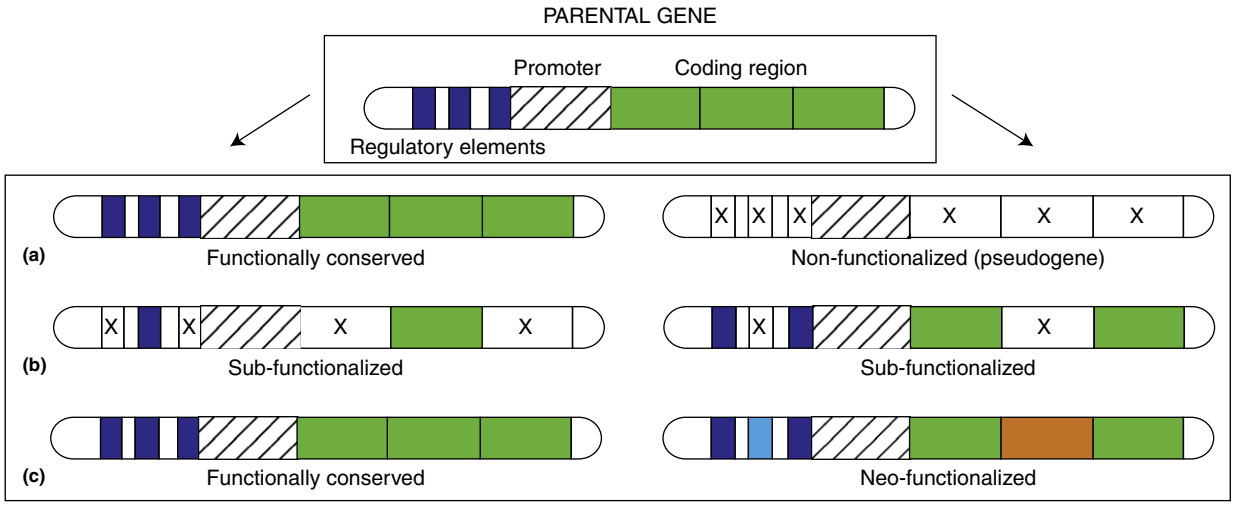

Figure 4 Potential fates of duplicated genes. (a) One paralog becomes a non-functional pseudogene by acquiring deleterious mutations while the other retains the ancestral functions. (b) Both genes acquire deleterious mutations in different *cis*-regulatory and protein-coding modules, thus partitioning the ancestral gene function through sub-functionalization. (c) One paralog acquires a novel advantageous mutation (orange) that can lead to evolutionary innovation in plant form, whereas the other paralog retains the ancestral functions.

in maize (Wang *et al.*, 2005, 2015). Thus, protein-coding changes can affect discrete phenotypes in plants, and are perhaps more important to the evolution of development than was once thought.

Conclusions and Future Directions in Plant Evo-Devo

Several decades of work have demonstrated the importance of both changes in *cis*-regulatory elements and their interacting regulatory proteins to the evolution of organismal development (Carroll, 2008). However, there are also several examples of mutations within nonregulatory proteins (e.g., enzymes and structural proteins) – such as changes in substrate specificity of pigment pathway enzymes that cause shifts in flower color (Smith *et al.*, 2012) – that have been important for morphological evolution. Together these data thus suggest a lack of inherent constraint on what types of developmental genes or genic regions are subject to natural selection or genetic drift. Rather, the modularity of genes, and their relative position within one or more developmental gene regulatory networks (GRN), might be more important determinants driving diversification.

Since the advent of affordable comparative/functional genomic and complex system modeling platforms, our ability to predict regions within GRNs that have affected plant developmental evolution has steadily increased. Like regulatory genes, GRNs are modular, consisting of highly interconnected units (modules) that are more loosely connected to other units (Rodríguez-Mega *et al.*, 2015). Transcription factors that regulate the expression of many to few genes in GRNs are referred to as hub and peripheral proteins, respectively, and it is proposed that peripheral GRNs and peripheral proteins are more likely to evolve than integral GRNs and hubs due to the smaller impact of genetic pleiotropy. Supporting this hypothesis, differential expression of the peripheral *BLADE-ON-PETIOLE* (*BOP*) GRN module across tomatoes correlates well with leaf complexity (Ichihashi *et al.*, 2014). Although many more studies are needed to test the effects of gene position on the evolvability of GRN elements, large-scale network studies, combined with functional analyses in both model and non-model species (e.g., Bortesi and Fischer, 2015), are paving the way for a comprehensive understanding of the commonalities and differences underlying evolution of distinct plant traits.

See also: Modularity and Integration. Modularity and Integration in Evo-Devo. Novel Structures in Plants, Developmental Evolution of

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Evolution and Agriculture I. The Evolution of Domestication

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Glossary

Commensal An organism engaged in commensalism. Commensal organisms in the human environment include house sparrows, mice, and some agricultural weeds such as darnel.

Commensalism An interaction between species in which one species benefits, but the other is unaffected.

Domestication The adaptation of an organism to a host (usually human) organism environment that occurs in response to a mutualistic relationship in which the host assumes a high degree of control over the life cycle of the organism in the interests of procuring a resource such as food or service.

Domestication syndrome A group of traits that is typically associated with the adaptations of domestication. In the case of plants these include a reduced ability to disperse seeds, loss of dormancy, changes in architecture, and seasonality of flowering. In animals traits include increased docility, changes to reproduction pattern and output, altered coat color, floppy ears, neotony, a reduction in size, and other changes in body proportions.

The incorporation of plants and animals into human ecologies and their subsequent domestication was one of the most significant evolutionary transitions in the history of the human species. The associated division of labor and intensification of settlement patterns precipitated the social evolution of complex societies replacing the previous hunter-gatherer existence that accounts for 95% of human history (McDougall *et al.*, 2005). Since Darwin first used domestication as an illustration of how natural selection might work (Darwin, 1859, 1868), it has increasingly become recognized as a complex suite of evolutionary trajectories that display parallelisms and convergences (Larson *et al.*, 2014).

Spatiotemporal Rise of Domestication

Hominin lineages have interacted with plants and animals by disturbing the environment and providing new niches since the Pliocene epoch (Allaby *et al.*, 2015), but it was not until the late Pleistocene and early to mid-Holocene epochs that a profusion of domestications occurred around the world over a relatively short period of time. Currently, there are up to 20 regional centers of domestication recognized around the world in which the process is thought to have occurred independently (**Figure 1**). Domestications broadly cluster into two time periods, a late Pleistocene/early Holocene group and a mid-Holocene group (Larson *et al.*, 2014). The older group is mostly comprised of the southwest Asian domesticates that originated in and around the Fertile Crescent region over a time period of 12 000–8000 years ago. These include wheat, barley, cattle, sheep, and pigs that make up the bulk of western agriculture today. Very few crops were domesticated in this time period outside of this region, exceptions being species of squash in Meso-America and South America and the pig in East Asia. There is evidence of pre-domestication exploitation of numerous wild species at this time that later became domesticated in South Asia, East Asia, New Guinea, Africa, and southern areas of the Americas. The mid-Holocene group of domesticates includes a large range of plants and animals from diverse areas around the world over a time period of around 8000–3000 years ago. Only a handful of species were domesticated after this period, including the duck, turkey, and guinea fowl. A notable exception to the general temporal trend is that of the dog, which represents the earliest known instance of domestication and predates these clusters by as much as 20 000 years (Larson *et al.*, 2012; Skoglund *et al.*, 2015).

The Domestication Syndrome

A diverse range of plants and animals have become domesticated and while few generalizations can accurately be applied universally, domestication can broadly be described as a selection process for the adaptation to the human agroecological niche. A number of traits recur frequently between organisms in this adaptation that have collectively become known as domestication syndrome traits (Hammer, 1984; Harlan, 1992). In plants, these traits include reductions in seed dispersal without human intervention, physical and chemical defenses, side-shoots and seed dormancy, combined with increased seed size and more synchronous germination and maturation. In animals, these traits include an increased docility, changes to reproduction pattern and output, altered coat color, floppy ears, neotony, a reduction in size, and other changes in body proportions. Any one species may only contain a selection of such traits, and the primacy of traits may differ between species.

In the case of plants the genetic mechanisms underpinning domestication syndrome traits are well understood in many cases (Fuller and Allaby, 2009; Olsen and Wendel, 2013). Given the similarities between the various plant groups around the world in their respective syndrome traits, it has been postulated that parallel mutations in equivalent genes between species could be responsible (Paterson *et al.*, 1995). However, while there is considerable convergence at the phenotypic level, at the genetic level the general rule has been that each plant species lineage has been found to have different specific mechanisms underlying traits such as loss of

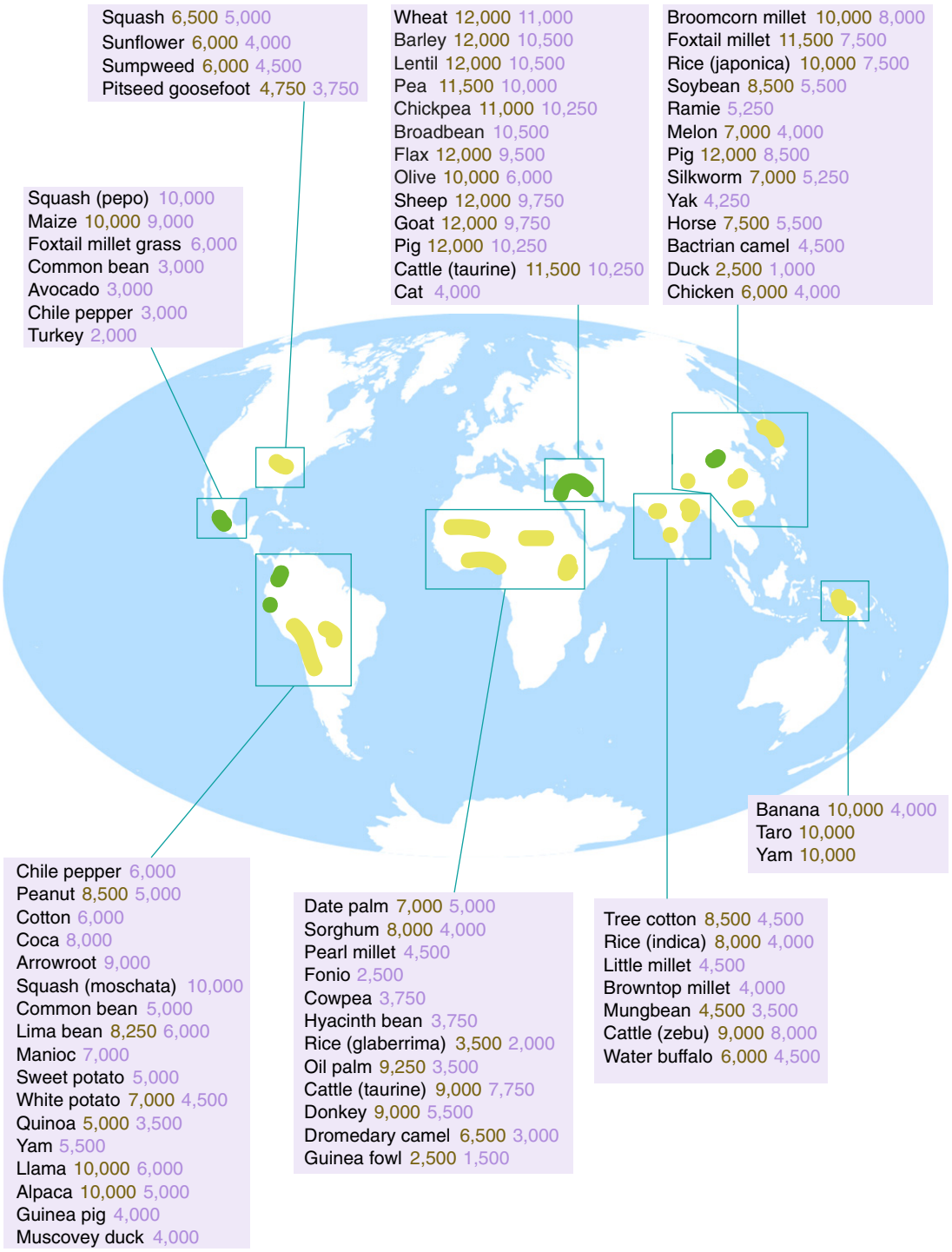

Figure 1 Regions of domestication around the world. Areas of early domestication are shown in green, areas of later domestication are shown in yellow. Dates for species listed for regions indicate first known exploitation or pre-domestication management before domestication (brown), and first known indications of domesticated forms (purple). Reproduced from Larson, G., Piperno, D., Allaby, R.G., *et al.*, 2014. Current perspectives and the future of domestication studies. Proceedings of the National Academy of Sciences of the United States of America 111, 6139–6146.

shattering (Li and Gill, 2006), although there have been exceptions to this (Lin *et al.*, 2012). The complexity of the underlying genetic control of traits varies from very simple with one or two genes being involved in the case of seed shattering, dormancy, and architecture (Abbo *et al.*, 2014; Vollbrecht *et al.*, 2005), to highly polygenic control for traits such as seed size (Gupta *et al.*, 2006). In the case of plants the overwhelming majority of domestication syndrome loci have been identified to be regulatory in nature (Purugganan and Fuller, 2009; Meyer and Purugganan, 2013), consequently it

can be inferred that most mutations have been epistatic in their effect. Far less is known about the genetic control of domestication syndrome traits in animals (Larson and Fuller, 2014). A clear parallelism is the modification of coat color in the same gene (MCR1) in many different species. However, it seems to be the case that the animal domestication syndrome is more typically controlled by many genes of small effect with very few instances of single genes underlying traits (Larson and Fuller, 2014; Carneiro *et al.*, 2014). It has been speculated that most of the domestication syndrome traits in animals could be accounted for by a reduction in the production and spread of cranial neural crest cells resulting in reduced facial features, floppy ears, coat patterning, and tameness, although this remains unproven (Wilkins *et al.*, 2014). Attempts to identify the number of loci involved in the domestication process by identifying genomic regions of low genetic diversity in the domesticated species relative to the wild progenitor species generally suggest a similar story in plants and animals with around 30–100 regions typically involved (Axelsson *et al.*, 2013; Carneiro *et al.*, 2014; Chapman *et al.*, 2008; Peleg *et al.*, 2011; Wright *et al.*, 2005).

Domestication Evolution Trajectories: Pace, Mode and Mechanism

Debate on this topic includes the role of unconscious versus conscious-based selection (Abbo *et al.*, 2014), whether domestication was concentrated in time to events (single or multiple) or whether more protracted and complex processes underlie (Allaby *et al.*, 2008; Brown *et al.*, 2009), and the motivations of hunter-gatherer societies of the Mesolithic to take up agriculture (Larson *et al.*, 2014). It is likely that no one specific explanation of mechanism and process can be universally applied to all domesticated species; however, there is general agreement that the factors of a changing climate, human demography, and changing social systems through time were involved (Larson *et al.*, 2014; Belfer-Cohen and Goring-Morris, 2011). Traditionally, the rise of domestication has been associated with small groups of innovators from localized areas, who rapidly acquired domesticated forms through changing practices in the early Holocene. It is also well established that it is possible to domesticate wild progenitors of domesticated plants (Hillman and Davies, 1990) and animals (Trut *et al.*, 2009) rapidly, but the archeological record indicates a more complex mix of processes. It has become apparent that at least in the case of cereals such as wheat and rice the process was slow with selection strengths akin to that found with natural selection (Purugganan and Fuller, 2011). Similarly, in the case of dogs, the rise of the domesticated phenotype occurred tens of thousands of years after wolves entered into a mutualistic partnership with humans (Skoglund *et al.*, 2015). In the case of cereals archeological evidence also suggests a long period of pre-domestication cultivation into the Pleistocene prior to domestication (Weiss *et al.*, 2006; Willcox and Stordeur, 2012). Reviews of the archeological evidence of the Near East also challenge the notion domestication rising from a single group in a single area, but rather from diffuse activity over a wide area in space and time (Fuller *et al.*, 2011; Willcox, 2005). Recognition of the slow nature of the process for some plants and animals challenges the notion of rapid domestication events, and therefore the mechanistic processes underlying. Most scholars are agreed that domestication was for the most part an unconscious process in contrast to Darwin's original vision of domestication being a case of conscious skilled selective breeding that illustrated mechanistically how natural selection could operate (Darwin, 1859). In many cases a conscious-based process leads to a problem of teleology in that the architects of domestication would be required to be insightful about the consequences of their selective pressures without any precedent. It is also questionable whether in the case of processes involving large stands of plants the proto-farmers would be able to observe the phenotypes of the rare mutations associated with domestication. Of course there are contrasting examples in which domestication was likely both conscious and rapid. Where there were not large stands of organisms, it is more reasonable to suppose that proto-farmers could have observed useful characteristics and breed those on (Sauer, 1965). An example of such a crop is squash in which bitterness appears to have been rapidly selected out in the domestication process (Dillehay *et al.*, 2007).

Mechanisms to explain the domestication process need to provide unconscious pathways in many cases, conscious pathways in others, and the often-slow pace of change. Integral to these considerations are the consequences and drivers of human agency. The shift from a hunter-gatherer to an agrarian-based diet is associated with a diminution of body size (Frayer, 1980), adaptation to a starch diet (Perry *et al.*, 2007), and rising disease load (Wolfe *et al.*, 2007). The reasons why hunter-gatherer societies would take on an agrarian lifestyle were complex and not likely to have been due to an obvious and immediate gain in nutritional benefit. Indeed, evidence shows that European hunter-gatherer and farming societies coexisted with exchange and interbred for thousands of years while maintaining their cultural differences (Haak *et al.*, 2015; Lazaridis *et al.*, 2014; Smith *et al.*, 2015). Models to explain the possible mechanisms of the evolution of domestication have correspondingly become pluralistic with an increased emphasis on natural adaptive processes in the earlier stages, followed by more conventional artificial selection in later stages.

Animal domestication has been categorized into three principal pathways (Zeder, 2012). The three pathways are termed commensal, prey, and directed, respectively, which occurred progressively overtime and provide a framework in which mechanisms of unconscious selection can be invoked (Larson and Fuller, 2014). In the first pathway, the principal agents are the animals themselves, which are attracted to aspects of the human environment such as food waste and adapt to it without any human intervention. To adapt to such an environment such animals would be required to reduce aggression and develop a level of tameness. Such animals are termed synanthropes, and the evolutionary pathway would lead to habituation, commensalism and then to a reciprocal interaction between humans and the animals, which would eventually lead to selective breeding controlled by humans. Companion animals such as cats and dogs are believed to be examples, and represent the earliest known interactions between humans and animals that led to domestication. Pigs and chickens are also considered to have become domesticated

this way. Many animals only proceeded a small way along this pathway and remain commensal today, examples including pigeons, house sparrows, and various rodents such as rats and mice. Notably, hunter-gatherer communities were responsible for the domestication of some commensals such as the dog. Unlike the commensal pathway, the prey pathway is instigated by human action. In this case the initial intention is not to domesticate either but to increase hunting efficiency, which may have had unintended consequences (Larson and Fuller, 2014). Human activities include overkilling males of a species and leaving females to produce more offspring so increasing selection for fecundity, an adaptation currently seen in animals in zoo captivity (Gilligan *et al.*, 1997). Generally, overhunting progressed to herd management, with consequences of increased docility possibly as the most aggressive males were killed off preferentially, and increased fecundity. The archeological record indicates that overhunting occurred prior to the domestication of cows (Marom and Bar-Oz, 2013). The last pathway differs from the previous two in that it is driven by human intentionality and consciousness, occurring sometime after the initial episodes of domestication. In this pathway humans are aware that animals can become domesticated and target them. Examples include horses, donkeys, camels, and rabbits, many instances occurred in the last few hundred years.

Similar natural adaptive explanations occur for the early stages of plant domestication. Like animals, plants can be considered as commensal or domesticated in the human environment. The human agency mechanism transitioned from gathering wild stands of plants to actively cultivating plants closer to settlements. It is likely that seeds dropped by gatherers would have resulted in plants growing in and around settlements, and a selective advantage would be conferred to plants that prospered with the qualities of that environment. Plants would be favored that thrived in a disturbed environment and that could take advantage of the richer nutrient supply from waste. Early cultivation of these plants manifest in the archeological record as a preponderance of wild species within human settlements (Weiss *et al.*, 2006; Willcox, 2005). These early gathering and cultivating practices acted on a community of plants rather than single species in isolation that included weeds as well as plants used for food. Both weeds and proto-crops adapted to the human environment through the manifestation of domestication syndrome traits. The different syndrome traits arose at different times, possibly in response to changing cultural practices (Fuller, 2007; Fuller *et al.*, 2010). The earliest indications of the transition to domesticated forms in the case of cereals was increased seed size, followed later by a rise in the loss of shattering (Fuller, 2007). It is thought that these adaptations were responses to cultivation and harvesting pressure respectively, wherein larger seeds survive burial more easily and convey an advantage in seedling competition, and failure to disperse increases the probability of successfully becoming part of the harvested stock which then goes on to seed the next generation (Fuller, 2007). Even gathering of seeds in the wild is likely to lead to increased frequency of loss of seed dispersal in wild stands through the reduced efficiency of gatherers to retrieve non-shattering seeds as they shake or strip the seeds off plants. Such selection pressures could theoretically have stretched far into the Pleistocene many millennia before the onset of domestication. Loss of shattering is also observed in weeds such as rye brome, darnel, and corncockle (Howard *et al.*, 2011; Senda *et al.*, 2006; Spahillari *et al.*, 1999) which have also adapted to the human environment but remain as commensals. Some commensals from this plant community subsequently transitioned to food crops, classic examples being oats and rye which were weeds for some considerable time before later becoming food crops (Küster, 2000). The coevolution between the humans and the environment they construct and plant communities is further apparent in the observation that different early Neolithic societies were specifically adapted to different ecologies (Banks *et al.*, 2013).

In both the cases of plants and animals there are numerous examples of evolutionary dead-ends, domestication trajectories that ceased. For instance, despite being on the prey trajectory of animal domestication, neither gazelles nor zebras became domesticated (Diamond, 2002; Zeder, 2006). Species of pea, lentil, rye and bean are among a catalog of plants that appear to have reached early stages of the domestication trajectory but were abandoned (Fuller *et al.*, 2011). These may represent instances where the organisms failed to adapt to the human environment sufficiently, or simply that human settlements moved into new environments leaving these natural communities behind. The suitability of an organism for domestication to certain extent is determined by its adaptability, which in turn is determined by the underlying genetic diversity and genomic structure of the species. It has been noted that genes contributing to domestication syndrome traits are often colocated in the genome despite their physiological level of irrelevance to each other in the normal functioning of the organism (Gepts, 2004). This may be a contributing factor to the ease in which an organism can become domesticated because desirable traits are less likely to be broken up by gene flow between wild and cultivated populations. Debate also concerns the nature of the genetic variation upon which the selection process acted. New mutations may have occurred within the population of organisms within the human environment that gave rise to domestication-associated adaptations, or alternatively, selection in the human environment may have acted on standing variation in the wild (Weber *et al.*, 2007). Often the traits that distinguish wild and domesticated forms are diametrically opposed in their selective advantages in each environment giving a tension between the two types of selection pressure. Under these circumstances one might expect it to be more likely that a mutation associated with such a domestication syndrome trait would be very rare in the wild or derived *de novo* in the cultivated environment. However, if the trait in question is essentially neutral in the wild environment but advantageous in the human environment, then such standing variation in the wild may be expected to occur at much higher frequencies. This latter type of adaptation is unlikely to leave a signature of selection in the genome, caused by a large genomic region of low genetic diversity, even if the selection associated with it is very strong indeed (Innan and Kim, 2004; Teshima *et al.*, 2007). Many of the known genetic mechanisms that underlay syndrome traits involve loss of function mutations, such as in the case of loss of seed dispersal or coat color in animals (Fuller and Allaby, 2009; Larson and Fuller, 2014), and as such would be expected to be recessive. The length of time required to select for

the same selection strength recessive traits varies greatly with mating system. Species that have propensity to self-cross (selfing), will rapidly produce individuals homozygous for a recessive trait and therefore manifest the trait phenotype. Selfing, mostly associated with plants rather than animals, is often an adaptation to colonizing new and disturbed environments (Baker, 1955; Massol and Cheptou, 2011), and so reflects well the plant communities that adapted to the human environment that included cereals. In the case of these communities it is possible that the genetic variation required for the adaptation spontaneously formed within those communities in the human environment. Conversely, outcrossing species would take a timescale longer than that of the rise domestication to select recessive traits because of the relatively high frequency of heterozygote individuals. It is probable then that outcrossing species by necessity must adapt on the basis of standing genetic variation if the traits involved are recessive. This applies to all domesticated animals, and it is notable that examples of high-standing genetic variation for domestication syndrome associated loci have come from outcrossing plants such as maize (Weber *et al.*, 2007; Nesbitt and Tanksley, 2002), but is not restricted to outcrossers (Jones *et al.*, 2008).

Post Domestication Evolution

An emerging consensus among scholars is that domestication should be considered an evolutionary trajectory rather than a discrete event in time (Larson *et al.*, 2014). A corollary is that the sequence of adaptations to the human environment is ongoing, and at which point one could consider a plant or animal domesticated is a point of legitimate debate and varies between species. A convenient milestone for plants is the loss of seed dispersal trait, or the loss of dormancy has also been suggested as a similar threshold (Abbo *et al.*, 2014). Less discrete parameters occur for animals, but general traits such as size reduction can be applied (Larson *et al.*, 2014).

Further adaptation in domesticated plants and animals include 'improvement' traits, such as the increased row number in barley associated with increased protein content (Komatsuda *et al.*, 2007), the development of fragrant forms of rice (Kovach *et al.*, 2009), or the development of a rich array of breeds in dogs (vonHoldt *et al.*, 2009). Such improvements are still ongoing today, arguably into the age of genetically modified (GM) crops. A second category of adaptation in plants and animals is in response to the dynamic nature of the human environment. Agriculture spread from the 20 or so regions of the world into new environments and latitudes. For plants this required the adaptation to changing environmental cues and stresses. The longer day lengths of higher latitudes can cause earlier flowering, which can result in stunted plants with under-resourced propagules. Plants that require a cold winter period before initiating flowering, the vernalization requirement, found winters increasingly difficult to survive at higher latitudes (Fuller and Allaby, 2009). Typically, plants have responded to these pressures by losing sensitivity to environmental cues, such as photoperiod insensitivity in rice, barley, and wheat and loss of the vernalization requirement allowing crops to be planted after the harsh winter period (Fuller and Allaby, 2009). Although there are many losses of function mutations for these traits, often one is sufficient to achieve the phenotype.

The pace of the spread of agriculture from domestication centers was likely limited by the capability of the broader human ecology to adapt to new environments. A hiatus in agricultural spread for several hundred years in the southern Balkans is thought to have been due not only to the lag associated with latitudinal adaptation, but also adaptation to the changing community assemblage emphasizing the interdependence of domesticated and commensal plants on each other (Colledge *et al.*, 2005; Coward *et al.*, 2008). Despite this adaptation, it is likely that spread was still too rapid for the package to adapt, and the general trend in the archeological record is one of repeated collapse throughout Europe as agriculture arrived and then disappeared, often for long periods of time up to the millennium scale (Stephens and Fuller, 2012; Shennan *et al.*, 2013). This time required for adaptation may be a contributing factor to explain why Mesolithic hunter-gatherers retained their lifestyle for a long period of time after becoming aware of the existence of the agrarian way of life (Smith *et al.*, 2015).

See also: Commensalism, Amensalism, and Synnecrosis. Evolution and Agriculture II. Evolutionary Applications to Breeding

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Evolution and Agriculture II. Evolutionary Applications to Breeding

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Glossary

Additive genetic variance The genetic variance that is due to the average additive effects of alleles.
Association mapping It is also called linkage disequilibrium mapping. It is the mapping of traits to loci using historical linkage disequilibrium in the population.
Breeders' equation $R=h^2S$ relates the response to selection (R) in a single generation to the narrow sense heritability of a trait (h^2) and the selection differential (S).
GWAS Genome-wide association mapping. See association mapping.
Heritability The ratio of genetic variance in the population to its phenotypic variance. The 'narrow sense' heritability is the additive genetic variance divided by the phenotypic variance.
Indirect genetic effect Effect from the genetic composition of socially interacting conspecifics on the focal individual.
Linkage disequilibrium The correlation of allele frequencies at different sites.
Quantitative trait locus (QTL) mapping The use of markers to map continuous traits to regions of chromosomes.

Introduction

Although humans have been domesticating animals and plants for many thousands of years, widespread use of selective breeding to consciously alter traits is a much more recent phenomenon, dating back to the last few centuries (Smith, 1998). This selective breeding has led to tremendous variation in many animals, including notably dogs, but the largest contribution of selective breeding has been in agriculture.

Professional breeding programs for crops and livestock improvement remains a cornerstone of agricultural research. Despite worldwide population increases and a substantially decreased percentage of people in developed countries involved in farming, food supply in developed countries substantial improved in quality and quantity over the last half century and breeding programs are largely responsible for these improvements (e.g., Havenstein, 2006). For instance, growth rates and sizes of chicken markedly increased between 1957 and 2001, and at least 85% of that improvement was owing to genetic factors, with the remainder being dietary (e.g., Havenstein *et al.*, 2003).

The agricultural industries cannot rest on these accomplishments. In its '2015 Revision of World Population Prospects' (1), the United Nations estimated the 2015 world population at 7.3 billion and predicted that number will increase to 9.7 billion by 2050 and 11.2 billion by 2100. Feeding these additional billions will require further agricultural improvement.

Historical Overview

Darwin and Wallace in the Nineteenth Century

The relationship between breeders and evolutionary biologists is characterized by a long history of rich reciprocal exchanges of information, ideas, and approaches. Moreover, many luminaries have made substantial contributions to both fields (Hill, 2014; Hill and Kirkpatrick, 2010).

This mutualistic relationship between evolutionary biologists and breeders starts with Charles Darwin. A breeder of pigeons and other animals and plants himself, Darwin had extensive correspondence with various breeder communities, and used insights from them in his development of evolutionary theory (Browne, 2002; Secord, 1981). Notably, Darwin made the analogy between what breeders did in selecting some variants while discarding others – a process that would be called 'artificial selection' – to what occurs in nature, what Darwin called 'natural selection' (Darwin, 1859). Darwin (1868) also produced a two-volume series on variation under domestication. Darwin used this book to continue to present evidence for evolution, but also went in new directions. Among other things, Darwin (1868) discussed differences between wild and domesticated animals, attempted to trace back the lineage of dogs to the progenitor, and suggested a 'provisional hypothesis' of 'pangenesis' to discuss how offspring inherited traits from their parents.

Although the late Victorian scientific community quickly embraced Darwin's ideas about common ancestry, they were more reluctant about his main explanation for evolutionary change – natural selection. Indeed, natural selection remained controversial for several decades after his death (Provine, 1971). Evolution via natural selection requires heredity – that offspring resemble their parents. While Darwin had evidence for heredity, his mechanism of pangenesis was sharply criticized (Mayr, 1982). Moreover, he lacked a quantitative theory of heredity (Provine, 1971).

Development of such a quantitative theory of heredity begun with Darwin's cousin, Francis Galton, who was obsessed with measurement and had passion for understanding how heredity works (Crow, 1993). Among Galton's major contributions was the development and application of the related concepts, correlation and regression (Stigler, 1989). Galton noticed a phenomenon called 'regression to the mean' (also called 'regression to mediocrity') wherein offspring of parents with extreme values of a trait tended to be less extreme than their parents (Lynch and Walsh, 1998, p. 8; Provine, 1971). For example, the offspring of extremely short parents

tend to be shorter than average, but not as short as their parents. This concept was an important early step in the foundation of what is now known as quantitative genetics, and would lead to advances in breeding.

Why does the regression to the mean occur? Traits of individuals have both heritable and non-heritable (mainly environmental) components, and by definition, only the heritable component is transmitted to the offspring. Extremely short individuals are likely to be short because they have 'short' heritable factors and 'short' non-heritable factors. Their offspring, however, have 'short' heritable factors but not the 'short' non-heritable factors; and thus, the offspring should be taller than their parents, but shorter than the average of the original population. The early quantitative geneticists at the turn of the twentieth century realized that the extent of the regression to the mean could explain how much a population could respond to selection (Lynch and Walsh, 1998). This understanding would lead to the development of formal treatments of heritability, the 'breeders' equation' (see below), of great importance for both evolutionary quantitative genetics and breeding.

Fisher and Wright in the Twentieth Century

The interexchange between evolutionary biologists and professional breeders continued through the twentieth century. Between World War I and World War II, a new science called population genetics developed and matured, unifying Mendelian genetics and Darwinian evolution. Two early leaders in population genetics, Sir Ronald Fisher and Sewall Wright, strongly influenced breeding programs (Hill, 2014).

Fisher (1918) showed that patterns of correlation between relatives for continuous characters could be explained by Mendelian genetics. This was an important milestone for both the synthetic theory of evolution and breeding programs. Fisher (1918, 1930) developed a model of evolution that assumed traits were determined by huge numbers of genes, each with small effect. This so-called infinitesimal model became widely used in both evolutionary quantitative genetics and in breeding, in part because of its mathematical tractability. In particular, the use of the infinitesimal model allows one to easily apply normal (Gaussian) distributions and standard methods of multiple regression and linear models in addressing expected evolutionary change (Lynch and Walsh, 1998; Hill, 2014). Not all traits conform to the infinitesimal model, but many do; for example, human height, which is determined by at least several hundreds of genes with tiny effect, comes very close (Lango Allen *et al.*, 2010).

Fisher was particularly interested in responses to short-term selection, which has obvious breeding implications. Notably, he formulated what he called 'the fundamental theorem of natural selection' in which he stated, "the rate of increase in fitness of any species is equal to the genetic variance in fitness" (Fisher, 1930, p. 46; see also Price, 1972, for a derivation). The genetic variance that Fisher referred to is the additive genetic variance (the part due to average additive effects of alleles, see Lynch and Walsh, 1998, p. 69) and not the total genetic variance (Price, 1972). Robertson (1966), in what is known as the secondary theorem of natural selection, extended Fisher's theorem to show that changes in traits equaled their genetic covariance with fitness. Later, George Price (1970, 1972) would extend and generalize this theorem in what is known as the Price equation. Fisher's fundamental theorem and its extensions have been influential in both evolutionary genetics (Price, 1972; Frank, 1995) and breeding (see Heritability and the Breeders' Equation below) because they illustrate and quantify the relationship between additive genetic variation and the response to selection.

Wright, who spent the first decade of his career (1915–25) at the United States Department of Agriculture (USDA), was extraordinarily influential in both developing evolutionary theory and applying it to agricultural programs (Provine, 1986). One of Wright's major contributions from his time at the USDA was in ascertaining patterns and effects of inbreeding in livestock, particularly cattle (e.g., Wright, 1922, 1969). Wright devised coefficients of inbreeding, which could be calculated from pedigrees, and developed patterns of mating that would minimize inbreeding (Wright, 1969). Minimizing inbreeding and its deleterious effects (inbreeding depression) continues to be of great importance both in agriculture (Leroy, 2014) as well as in conservation biology. Moreover, inbreeding depression has been a persistent topic of interest in general evolutionary biology (Charlesworth and Charlesworth, 1987; Hartl and Clark, 2007).

Wright (1931, 1969) also made fundamental contributions to the study of random genetic drift, the sampling that occurs each generation due to the finite size of populations. Genetic drift can pose challenges for breeding for two reasons: (1) it reduces genetic variation and thus reduces how well populations can respond to selection and (2) it can lead to the fixation of slightly deleterious alleles (Wright, 1931, 1969; Hartl and Clark, 2007). Drift is also strongest in small populations, which are typical of breeding populations. In his investigation of drift, Wright realized that its strength depends not just on the current size of the population, but also the population's sex ratio, fluctuations in population size, and the extent of variance in the numbers of offspring. Thus, he devised the metric of the effective population size (N_e), which standardizes the expected effect of genetic drift experienced by actual populations under different circumstances (Wright, 1931, 1969; Hartl and Clark, 2007; Crow, 2010).

Fisher and Wright both developed seminal statistical methodologies that laid the foundation for biometry, influencing both studies of evolution and breeding. Fisher's analysis of variance (ANOVA), a staple of biometry, uses properties of variances to determine statistical significance of differences between the means of various samples (Sokal and Rohlf, 1995). Wright (1921, 1968, Chapters 13 and 14) developed path analysis, an extension of multiple regression, that attempts to tease apart causation from correlation by measuring the influence of different factors on others in a system.

Heritability and the Breeders' Equation

Galton attempted to parcel out the influences of heredity (nature) and environment (nurture). Building on Galton's foundation, Fisher (1918) and Wright (1920) independently

(and through different methodology) formalized the concept of heritability as a quantitative measure of the proportion of phenotypic variation that is due to genetic factors (Lynch and Walsh, 1998; Visscher *et al.*, 2008).

There are several different types of heritability, but the one of greatest importance to breeders is 'narrow sense' heritability. This quantity is defined as the additive genetic variance divided by the total (phenotypic) variance and is usually represented as h^2 (Lynch and Walsh, 1998, pp. 170–175). Narrow sense heritability can be estimated from parent–offspring regressions, and is often used in making predictions about short-term responses to selection (Lynch and Walsh, 1998). Although nonadditive effects of genes are important in evolutionary genetics (Wright, 1968; Wade and Goodnight, 1998; Lynch and Walsh, 1998), only additive effects are directly transmitted from parent to offspring (Visscher *et al*, 2008). Because it is based on the additive genetic variance, narrow sense heredity is also directly related to Fisher's fundamental theorem.

Heredity is a concept fraught with misconceptions (Visscher *et al.*, 2008). A high heredity does not equate to genetic determinism because environmental changes could alter the phenotype. Moreover, heredities can and do change over time. In fact, a heredity value is specific to a given population with its distribution of genotypes, phenotypes, and environments (Lynch and Walsh, 1998; Visscher *et al.*, 2008).

That caveat aside, narrow sense heredities are informative in making short-term predictions about responses to selection. Jay Lush (1937), who was inspired by Wright and especially Fisher, expressed a quantitative expectation for the response to selection in each generation based on the narrow sense heritability of the trait and the strength of selection (Hill, 2014). Lush's expression, which is now known as the breeders' equation, remains an important concept in both breeding programs and evolutionary quantitative genetics (Lynch and Walsh, 1998).

A simple formulation of the breeders' equation is $R=h^2S$, where R is the response to selection in a single generation and S is the difference between the mean of the selected individuals and the mean of the population. For instance, suppose that a population of tomatoes has a mean weight of 150 g and the selected individuals have a mean weight of 180 g. If the heritability is 0.3, then the response should be $0.3*(180-150)$ or 9 g, and the next generation should have a mean weight of 159 g.

Another formulation of the breeders' equation is $R=\sigma_A^2 i$, where σ_A^2 is the additive genetic variance and i is the selection intensity (the selection differential divided by the phenotypic variance) (Roff, 2006). This formulation emphasizes the importance of additive genetic variance to the selection response.

Traits do not evolve in a vacuum, but instead are often associated and evolve with other traits. Using the phrase 'correlation of parts,' Darwin (1859) had noted that selection on one trait could influence the evolution of other traits. This correlated response is now known to be due to pleiotropy (genes having multiple effects) and/or genetic loci for different traits being genetically linked (Roff, 2006).

Both breeders (Hazel, 1943) and evolutionary quantitative geneticists (Lande and Arnold, 1983) have developed methods to deal with correlated responses to selection. In the multivariate breeders' equation, $\Delta z=\mathbf{G}B$, the expected responses to selection (Δz) is a vector and B represents the vector of selection intensities. **G**, which is sometimes called the G matrix, is the additive genetic variance–covariance matrix (Lande and Arnold, 1983; Roff, 2006). See **Box 1** for more detail about the multivariate breeders' equation.

Some traits change with age and condition. Both breeders (e.g., Kirkpatrick and Heckman, 1989) and evolutionary quantitative geneticists (e.g., Schaeffer and Dekkers, 1994) have developed methods to incorporate such traits in the breeders' equation.

Box 1 The multivariate breeders' equation

Consider the case where average weight of pigs (trait 1) and their protein content (trait 2) are negatively correlated, with a covariance of -0.5 units. The additive genetic variance of traits 1 and 2 are 0.8 and 0.2 respectively and the selection intensity is 0.2 for trait 1 and 0.1 for trait 2.

If there were no covariance between the traits, the response to selection for weight would be 0.16 and the response for protein content would be 0.02.

Expanding the breeders' equation $\Delta z=\mathbf{G}B$

$$\begin{bmatrix}\Delta Z_1\end{bmatrix} = \begin{bmatrix}\mathrm{Var}_1 & \mathrm{Cov}_{1,2}\end{bmatrix}\begin{bmatrix}\mathrm{B}_1\end{bmatrix}$$
$$\begin{bmatrix}\Delta Z_2\end{bmatrix} \quad \begin{bmatrix}\mathrm{Cov}_{1,2} & \mathrm{Var}_2\end{bmatrix}\begin{bmatrix}\mathrm{B}_2\end{bmatrix}$$

Substituting the values gives

$$\begin{bmatrix}\Delta Z_1\end{bmatrix} = \begin{bmatrix}0.8 & -0.5\end{bmatrix}\begin{bmatrix}0.2\end{bmatrix}$$
$$\begin{bmatrix}\Delta Z_2\end{bmatrix} \quad \begin{bmatrix}-0.5 & 0.8\end{bmatrix}\begin{bmatrix}0.1\end{bmatrix}$$

With matrix algebra, we find that $\Delta Z_1=0.11$ and $\Delta Z_2=-0.08$. With the negative covariance, the response to selection for weight is reduced and the response to selection for protein content is actually in the opposite direction of the selection. The extent to which these covariance structures constrain evolution and how G matrices themselves evolve in natural populations is still debated among evolutionary geneticists (Roff, 2006).

Breeding Value

Breeders aim to alter the genetic composition of populations over the course of generations to obtain desired phenotypes. In making decisions about which individuals to choose, breeders attempt to estimate the effects of the individual's genes on the trait of interest. Recall from Fisher's fundamental theorem and the breeders' equation above, that short-term selection responses are due to primarily to the additive effects of genes. The sum of the additive effects the individual's genetic loci define its 'breeding value' (Lynch and Walsh, 1998, p. 72). It is this quantity that breeders attempt to estimate.

Breeding values are seldom readily apparent, but instead need to be estimated, generally based on phenotypic information from the individual as well as that of its relatives. Charles Henderson, who as a graduate student at Iowa State was influenced by Lush and Hazel, pioneered estimation of breeding values. Henderson's (1950, 1975) best linear

unbiased prediction (BLUP) remains an important tool in breeding value estimation (Lynch and Walsh, Chapter 26).

Marker-Assisted Selection

The standard approach to estimating breeding value has been to use phenotypic and pedigree information (Henderson, 1950; Lynch and Walsh, 1998). Marker-assisted selection incorporates predictive phenotypic information from particular genotypes when making decisions about which individuals to select (Hayes and Goddard, 2010).

QTL Mapping

The goal of quantitative trait locus (QTL) mapping is to map loci that contribute to the value of a trait to small regions of chromosomes. Despite the name 'locus' in the name, QTL analysis generally maps traits to regions of chromosomes that often contain many (possibly hundreds of) genes. Although the idea of mapping quantitative traits to regions of chromosomes had been around since the early days of Mendelian genetics, widespread implementation of what is now called QTL mapping did not begin until the mid-1980s when molecular marker and computer algorithm technology had sufficiently matured (Lander and Botstein, 1989; Lynch and Walsh, 1998).

QTL analysis usually begins with crosses between parental lines that differ substantially in value for the trait. Although QTL analysis can be done with F_2 and/or backcross offspring, contemporary QTL analysis usually involves the creation of lines of numerous individuals each with similar genotypes at a particular region of the chromosome. Commonly used types of lines are recombinant inbred lines (RILs) and nearly isogenic lines (NILs). Molecular markers are generally used in the creation of these lines to identify genotypes (Lynch and Walsh, 1998, Chapter 14; Rothschild *et al.*, 2007). Next, individuals from these lines are evaluated for the trait(s) of interest. Given data on the traits from various lines, computational tools evaluate the value of the contributions of different chromosomal regions to each of the traits and their statistical significance (Lynch and Walsh, 1998). See **Figure 1** for an example of QTL results.

Given knowledge of which QTLs affect traits of interest, breeders can genotype individuals and use this genotypic information in assessing breeding value. The extent to which the added QTL information improves breeding value estimation depends on the quality of the analysis and the proportion of phenotypic variation that is explained by the QTL (Hayes and Goddard, 2010).

QTL analyses are limited in several respects. First, traditional QTL analyses require crossing between extreme lines and work best with the generation of inbred lines such as RILs and NILs. Such preparation is especially challenging for livestock. QTL analysis is also prone to bias from several sources (Lynch and Walsh, 1998). One such bias is that QTL analyses frequently substantially overestimate the magnitudes of effect sizes, especially when sample sizes are modest (Beavis, 1994). This so-called 'Beavis effect' is a statistical artifact that is a source of concern for both breeders and evolutionary biologists attempting to understand the genetic basis of their traits of interests (e.g., Slate, 2013). Another concern is that for most traits, QTL analyses capture only a fraction of the phenotypic variance (Hayes and Goddard, 2010).

Association Mapping and Genome-Wide Association Mapping

Evolutionary geneticists, medical geneticists, and breeders also use a related strategy to genetically map traits. This mapping analysis, which is known as association mapping, does not require crosses between extremes and the generation of inbred lines, but instead relies on past recombination events that have occurred in the population.

Association mapping looks for associations between alleles at molecular markers and the traits of interest. If the markers are from across most or all of the genome, the study is known as genome-wide association mapping or GWAS. These markers may not have any causative bearing on the trait, but they are associated with trait. This association arises because the markers are correlated with the actual causative genetic variants. This genetic correlation, which is known as linkage disequilbrium (LD), makes markers useful to evolutionary geneticists and breeders. (For this reason, association mapping is sometimes called LD mapping.) Given knowledge about the allele an individual has at a marker locus in LD with the causative genetic variant, one can infer which allele at the causative locus the individual has. Thus, these markers can inform estimation of breeding values (Hayes and Goddard, 2010).

Development and application of GWAS requires understanding LD patterns, which in turn requires population genetic theory (Hartl and Clark, 2007). For instance, the effective population size (Ne) plays a major role in shaping LD patterns. Because allele frequency changes via genetic drift generate LD, the extent of LD is inversely related to Ne (Sved, 1971; Hartl and Clark, 2007). The application to association mapping has brought LD from obscure theory to one of the fundamental concepts of population genetics (Slatkin, 2008).

Genome Selection

Like traditional QTL analyses, GWAS poses statistical challenges. Often in GWAS, the total variance explained by statistically significant markers is modest. This limits the application of GWAS for estimating breeding values (Hayes and Goddard, 2010). One way around this limitation is to use all of the markers from GWAS, and not just the statistically significant ones, in calculation of breeding values. This approach is known as genome selection (Meuwissen *et al.*, 2001; Hayes and Goddard, 2010).

Genome selection will be particularly useful for certain traits: (1) those that are expressed only after breeding decisions have been made, including traits whose measurement require destruction of the individual, (2) those that are expressed only in a single sex (e.g., milk yield), and (3) those that are difficult to measure (Hayes and Goddard, 2010). Because it uses information directly from genotypes instead of patterns of relatedness, genome selection should minimize the effects of

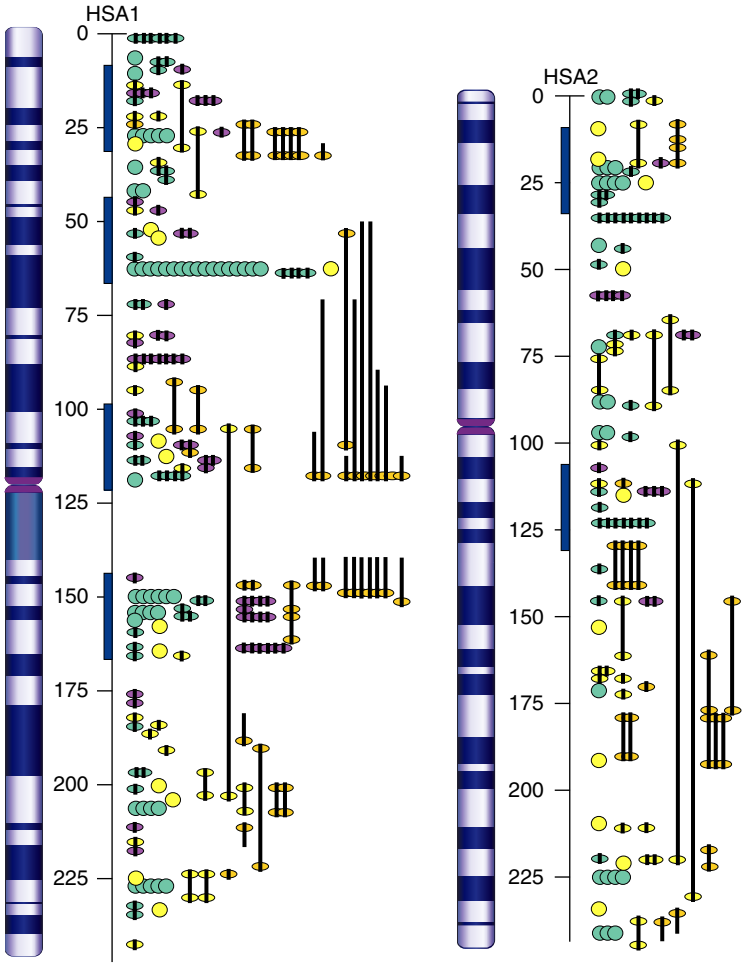

Figure 1 The results of QTL analysis. For each column, on the left are positions mapped on the various chromosomes with affected traits on the right. Note only two chromosomes are shown here. Reproduced from Rothschild M.F., Hu, Z.I., Jiang, Z., 2007. Advances in QTL mapping in pigs. International Journal of Biological Sciences 3, 192–197.

inbreeding and associated inbreeding depression in comparison to methods that employ pedigrees to estimate breeding values (Daetwyler *et al.*, 2007).

Hayes *et al.* (2009) present a promising use of genome selection to ameliorate the negative effects of human-induced climate on agricultural crops and livestock. One particular concern is how milk yield of cows in hot, arid regions, like much of Australia, will be affected by further increases in temperature. Hayes *et al.* (2009) noted that genetic variation exists in cattle with respect to sensitivity to heat. They then used GWAS to demonstrate how genome selection can be used to select for more heat-tolerant cattle. This analysis revealed several SNPs that are associated with lower slopes between milk yield and the temperature–humidity index, and thus indicative of greater tolerance. Breeding programs now can use this additional information.

Long-Term Selection

The breeders' equation and its extensions were formulated to make short-term predictions about responses to selection, both artificial and natural. These tools were not designed for predicting or explaining long-term selection responses (Barton and Turelli, 1989; Lynch and Walsh, 1998; Roff, 2006).

Although evolutionary biologists are generally more interested in long-term selection than are breeders, there are exceptions. Long-term artificial selection is important to on-going breeding programs, and thinking about how to implement them. For instance, how intense should selection be? Robertson (1960) noted a potential trade-off between short-term and long-term selection. More intense selection will generally lead to a faster short-term response, but this will come at a cost: intense selection reduces the effective population size of the breeding population and that limits the potential long-term response. Evolutionary theory shows that extent to which selection will reduce Ne will be inverse proportion to the recombination rate (Hill and Robertson, 1966, 1968; Hartl and Clark, 2007).

One well-known long-term experiment is the Illinois long-term selection experiment (Dudley, 2007). Started by C.G. Hopkins in 1896, it has been selecting on maize traits for well over a century, with a single generation per year. Selection has been imposed for both increases and decreases in both oil and

protein content. The lines selected for decreased oil (low oil) and decreased protein (low protein) stopped responding to selection after about 75 generations, but the lines selected for increased oil (high oil) and increased protein (high protein) continue to respond. The selection response has been dramatic. At the start, the lines contained about 5% oil. The high-oil lines now have around 20%, and the low lines have around 1%.

Why can responses in the Illinois long-term selection experiment continue? Genetic variation should have been exhausted a long time ago. Yet, additive genetic variation in the high-oil and high-protein lines is still abundant (Dudley, 2007). Likely multiple factors explained the continued response. These include the founding population being conducive to long-term selection because it had ample variation with many loci at low allele frequency contributing to the traits. Additional factors likely include the input new mutations and the conversion of genetic variance caused by gene interactions (epistasis) into additive genetic variance (Dudley, 2007; see also Goodnight, 2004). This conversion of genetic variation due to epistasis into additive genetic variance is an on-going subject of evolutionary genetics (Wade and Goodnight, 1998; Visscher *et al.*, 2008).

Social Interactions and Indirect Genetic Effects

Organisms interact with conspecifics and these interactions can substantially traits. These social interactions are part of the individual's environment, and both affect the evolution of individuals and can be shaped by evolution (Bleakley *et al.*, 2010). In social evolution, an individual is not only influenced by its own genes, but also by genes of conspecifics. Such influences are known as indirect genetic effects. Recent evolutionary theory has incorporated indirect genetic effects into the breeders' equation (reviewed in Bleakley *et al.*, 2010). Many agricultural researchers have found social interactions giving considerable contribution to traits of interest, including growth rate in pigs and survival time of laying hens (reviewed in Wade *et al.*, 2010). Interestingly, body weight in cannibalistic quail has a large variance due to indirect genetic effects and there is a negative correlation between direct and indirect genetic effects, complicating selection experiments (Wade *et al.*, 2010).

Selecting on the performance of groups of individuals (instead of individual performance) is a way to incorporate social evolution into breeding designs without direct estimation of indirect genetic effects. In a seminal evolutionary genetic study, Wade (1977) showed that selection on groups of flour beetles for productivity led to responses that could not have been predicted by responses from individual-level selection.

Muir (1996) used an approach similar to Wade's studies of flour beetles to select on groups of chickens. Here, hens from each sire family were caged together and the cage performance (egg production) was used to select or reject the group as a unit. Over the course of this experiment, group egg production increased dramatically, while mortality declined. The responses in multi-hen cages were faster than those in single-hen cages, illustrating the power of such group-level selection. Other examples of agricultural applications of group selection can be found in Wade *et al.* (2010).

Genomics and Effects of Domestication

Evolutionary genomic studies can reveal the genes that have been under selection during domestication as well as the overall genomic effects that occurred due to domestication. Flint-Garcia (2013) found that domestication of maize from teosinte led to a modest decrease in overall genetic variation, but a severe loss of genetic variation in the loci that were presumably selected during domestication.

Similarly, a recent study of sunflowers found that cultivated varieties have accumulated more missense mutations and putatively deleterious mutations compared with wild varieties (Renaut and Rieseberg, 2015). Determination of which mutations are likely to be deleterious is based on bioinformatic criteria commonly used in molecular evolution. Interestingly, the presumptively deleterious mutations were clustered in regions of low recombination (Renaut and Rieseberg, 2015), consistent with evolutionary theory (Hill and Robertson, 1968; Hartl and Clark, 2007). In maize (Flint-Garcia, 2013), sunflowers, and likely other crop species, the progenitor species could be useful as a source of genetic variation to be used for further breeding studies.

Concluding Remarks

The mutualistic relationship between breeders and evolutionary geneticists that began in the mid-nineteenth century is likely to persist over the coming decades. Both are engaged in comparative genomic and population genomic studies, and the development of the associated tools and resources. Improving association mapping studies continues to be a pressing need across many scientific communities. Long-term predictions of responses to selection and how the **G** matrix evolves will continue to interest both breeders and evolutionary geneticists.

Despite the mutualistic relationship, the aims of breeders and evolutionary geneticists are not perfectly aligned. As Hill and Kirkpatrick (2010, p. 2) note: "The breeder is looking forward and wants to know how to make rapid and continuing changes in desired directions, whereas the evolutionary biologist is primarily looking back and wants to understand what forces caused the changes that have occurred and shape the current population".

See also: Artificial Selection. Conservation Biology, Evolution and. Evolution and Agriculture I. The Evolution of Domestication. Evolutionary Medicine I. An Overview and Applications to Cancer. Multivariate Quantitative Genetics. Synthetic Theory of Evolution, History of

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Evolutionary Biology, History of

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Introduction

Evolutionary biology is widely considered to be the scientific discipline devoted to understanding the origin and maintenance of biological diversity; it is the area of inquiry directly concerned with evolutionary processes leading to the origin of new species, and with descent from shared ancestors. Because of its central location in the disciplines of knowledge, the belief is commonly held that evolutionary biology serves a unifying function, integrating the diverse disciplines of knowledge, although the extent to which it unites, or reduces the social sciences and even humanities to the biological and physical sciences has drawn more than its share of controversy (see Wilson, 1998, for one instance of how these unifying properties are thought to inform the humanities; see Mayr, 1982, 2001, for more on evolution and its unifying properties).

Although the study of evolution is most commonly associated with names such as Charles Darwin (1809–1882) and Alfred Russel Wallace (1823–1913) in the nineteenth century, it reached the status of scientific, unifying discipline embodying rigorous methods including experimentation and quantification, only in the first few decades of the twentieth century, and accompanied the establishment of the modern or 'synthetic' theory of evolution during the period of 'evolutionary synthesis' (Smocovitis, 1996; and see Mayr and Provine, 1980, for more on the 'evolutionary synthesis').

Etymological research into the origins of the term 'evolutionary biology' confirms its recent origin. The first recorded use of the term, more precisely the identifying label, 'evolutionary biologist' appears to have been in 1881, in a passage from English naturalist Grant Allen's *Vignettes from Nature*, in a passage reading: "and it is these self-same odd, overgrown outer flowers which make the guilder rose so interesting a plant in the eyes of the evolutionary biologist" (Allen, 1881, p. 93). The term was first used in 1912 in the title of the book *Outlines of Evolutionary Biology* by English zoologist Arthur Dendy and then again in 1938 by English embryologist Gavin de Beer in his *Essays on Aspects of Evolutionary Biology Presented to E. S. Goodrich* (de Beer, 1938). Thus, though the term was used in obscure contexts as early as the late nineteenth century, and had some limited use in the first couple of decades of the twentieth century it began to gain currency and used in its present disciplinary formulation during the interval of time between 1930–1950. It was used especially heavily by English zoologist, Julian Huxley (1887–1975), whose 1942 book titled *Evolution: The Modern Synthesis*, announcing the synthesis between Mendelian genetics and Darwinian selection gave name to this critical period in the history of evolutionary thought (Huxley, 1942).

The Origins of Evolutionary Thought

Evolutionary thought itself had a much earlier origin and grew out of a concern with the problem of change, especially organic change, beginning in antiquity and associated with thinkers like Heraclitus (*c*. 540–*c*. 480 B.C.E.), who believed that change was a fundamental property of the universe, and Empedocles (*c*. 490–430 B.C.E.), who formulated a crude but dynamic theory for the origin of life that suggested organic change. Concern with organic change fell into disfavor with the philosophical worldview of Aristotle (384–322 B.C.E.), his many translators, commentators, and supporters, who believed in a static universe and that the world had been created by a designer (the so-called Demiurge of Plato's *Timaeus* or the biblical Creator) that then remained essentially unchanged. The diversity of life on earth was frequently seen in terms of idealized types, or species that were arranged hierarchically in the 'ladder' of creation, or what came to be called the *scala naturae*. During the early modern period, this metaphor began to give way to the 'great chain of being,' which referred to a progression of living forms, or species, linked chain-like in an orderly fashion. Extinction, or the sudden disappearance of a species was therefore unthinkable as it meant a break in the chain of life. Belief in the fixity of species and in idealized types seen in strict relation to each other dominated thinking about living organisms and was most clearly demonstrated in the modern classification scheme associated with Carolus Linnaeus (1707–1778) (Mayr, 1982; Bowler, 2009).

The belief in species change or 'transmutationism,' as it came to be called, began to emerge in the period of the Enlightenment after the growing recognition that the earth was much older than previously believed and that fossils, long thought to be curiosities or 'sports of the creator,' were in fact of organic origin. The shift toward a progressive view of the world in both social and scientific terms also helped fuel the belief in transmutationism as did the many geological theories that began to entertain uniformitarian instead of catastrophist views (Bowler, 2009).

Uniformitarianism and transmutationism were associated most closely with the French naturalist George-Louis Leclerc, Comte de Buffon (1707–88) whose forty-four volume treatise in natural history known as *L'histoire Naturelle* (or *Natural History*) suggested a theory of species change (Roger, 1997). Although Buffon was a respected naturalist, he did little to convince the community of his insights, in part because of his own writing style which often appeared confused, but also because he provided no real convincing evidence in support of his theory of species change. He was also undercut by the philosophical teachings of his successor Georges Cuvier (1769–1832), who was a staunch catastrophist and anti-uniformitarian and who thought successive revolutions had given rise to the pattern and diversity of life on earth. Ironically, he was the first to recognize the phenomenon of extinction, or the view that biological species had disappeared from the biological record, while all the while upholding the fixity of species.

The first viable theory of transmutation was associated with another French zoologist, Jean-Baptiste de Lamarck

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(1744–1829). He was a contemporary of Cuvier's and faced notable opposition from him. Trained in botany, Lamarck was given the task of organizing the invertebrate collection at the Musée National d'Histoire Naturelle in Paris. It was while organizing the material, that his attention was drawn to particular trends in the group that appeared to be progressive in nature. He was especially intrigued by the phenomenon of adaptation, or the manner and process by which organisms adapted to their environment in both physiological and morphological terms. He became especially interested in how well-adapted organs originated and for this reason is most closely associated with the example of the adaptive modification in the neck of the giraffe, which became elongated in response to stretching during feeding on the leaves of trees on the African savannah. According to Lamarck, the use or disuse of such organs could lead to the origin of novel, but well-adapted traits, the cumulative effect of which could eventually lead to new species. Lamarck never really provided a cogent mechanism by which this transformation happened, though he drew on contemporary theories of physiology to explain the physical transformation of the organ (Burkhardt, 1977). Lamarckian transmutationism, which became known as 'Lamarckism' or the inheritance of acquired traits, was subsequently shown to be wrong because such changes lacked heritability, though it resurfaced periodically despite the celebrated experiments of the German biologist August Weismann (1834–1914) who famously cut off the tails of hundreds of mice hoping to generate tailless mice.

Transmutationism became increasingly acceptable in the early decades of the nineteenth century and could be seen in the thinking of the French anatomist Étienne Geoffroy Sainte Hilaire (1772–1844) whose interest was in the science of birth defects and their sudden origin, as well as Erasmus Darwin (1731–1802), Charles Darwin's grandfather, who suggested that life had originated from 'one living filament' in the *Zoonomia* (1794–96). Remarkably, a number of individuals began to search for mechanisms of species change that closely resembled natural selection well in advance of Charles Darwin. As early as 1813, for example, William Wells suggested that new human races originated when groups moved into new environments requiring adaptation to conditions of life and in 1831 Patrick Matthew came even closer to formulating a view of natural selection in an obscure treatise *On Naval Timber and Arboriculture*; Matthew suggested that catastrophic extinctions of species took place leaving behind hardy survivors that would in turn diversify, giving rise to better adapted species. But by far the outstanding example of the importance of transmutationism in the first half of the nineteenth century, was a book published in 1844, at first anonymously, by Robert Chambers and titled *Vestiges of the Natural History of Creation.* Marketed widely, the book became an instant sensation to very wide Victorian audiences who entertained a provocative, and at times scandalous theory for the origins of the solar system and the origins of humanity, which postulated evolution from the apes (Secord, 2000). Though it was widely discussed, it also drew heavy criticism from the scientific community. The criticism was so devastating that this was one reason that Charles Darwin, who by then had formulated his own theory of species change, was dissuaded in delaying publication of his own theory for some 15 years (Browne, 1995).

Charles Darwin and Descent with Modification by Means of Natural Selection

Charles Darwin was, of course, the leading transmutationist of the nineteenth century. He sought a general explanation for his observations of the natural world following his celebrated 5 year voyage aboard the H.M.S. Beagle. Between 1837 and1838, regarded as the crucial years in the formulation of his theory that followed his return to England, Darwin read Thomas Malthus's (1766–1834) *Essay on the Principle of Population*. This essay provided Darwin with the backdrop for his theory by suggesting that competition for natural resources was a fact of life and that populations remained stable as a result of a complex set of processes involving checks and balances. In such a competitive world, those organisms with favorable heritable variations would survive and reproduce themselves. With enough time, such a process would lead to divergence, with new species arising from ancestral forms. The novelty of Darwin's theory thus lay in his appropriation of the competitive struggle for existence from Malthus, but also in his emphasis on heritable variation that conferred an advantage, and that would ultimately be transmitted to the progeny (Browne, 1995).

Darwin's own theory, which he called 'descent with modification' by means of the primary, though not sole means of natural selection therefore drew heavily from his many predecessors, and did not emerge in a sudden 'aha' moment while making observations in the Galapagos during his celebrated voyage on the H.M.S. Beagle. Recent scholarship on Darwin and the history of evolutionary thought has given us a more nuanced and complex portrait of how his theorizing emerged in the context of engaging the work of not only his predecessors but also his contemporaries like the geologist and uniformitarian Charles Lyell (1797–1875) and naturalists like Robert Edmond Grant (1793–1874), along with scores of other naturalists like German-Brazilianist Fritz Müller (1821–97) who worked on a staggering diversity of living forms and geographic contexts (West, 2003). On a more domestic front, Darwin also followed popular breeding practices, especially tracking the pigeon fancy craze in Victorian England; this was to provide him with immediate familiar examples of artificial selection in demonstration of his own natural selection (Secord, 1981). He also embarked on a careful study of the systematics of barnacles at this time. This was likely meant to buttress his own theory but also earn him his credentials as someone who had committed to a close taxonomic study of a group of organisms.

The 20 years roughly between the formulation of his theory and its final publication was thus well spent on sharpening his thinking, earning his credentials, and garnering support as well as evidence to buttress what he knew would be a controversial theory. He also spent a great deal of time anticipating criticism and forming arguments in defense of his theory, well in advance of receiving it. Indeed, had he not been approached in benign and casual manner by Alfred Russel Wallace (1823–1913) a younger naturalist, who had independently formulated his own theory of species change resembling Darwin's, he probably would not have published *On the Origin of Species* in 1859, which was merely intended to be an 'abstract' of what he was ambitiously planning to be a larger work.

After consultation with a group of distinguished Victorian naturalists that included Darwin supporters, such as Charles Lyell, and Kew Garden botanist, Joseph Hooker (1817–1911), the two jointly published an early formulation of their theory in 1858. With good reason, some scholars now refer to the 'Darwin–Wallace' theory of evolution, which is inclusive of Wallace's many contributions especially on the topic of island biogeography, which is now informing contemporary understanding of conservation biology (Quammen, 1996; Fichman, 2004). Evolution itself, as a term for the general theory of descent with modification was not used by either individual initially, but gained currency only shortly after the publication of Darwin's book in 1859. Its currency is owed to the use of the term by Darwin's contemporaries like social evolutionist Herbert Spencer (1820–1903) as well as Darwin's most famous advocate and celebrated 'bulldog,' Thomas Henry Huxley (1825–95). A little known fact is that only at the very end of his long treatise did the word 'evolve' appear; until then the term evolution, which meant 'unrolling or unfolding' was mostly associated with embryology (Bowler, 1975, 2009).

Because of his keen insights, abundant examples, and his literary and rhetorical flourish, Darwin is now regarded as the giant of evolutionary theory and his name is nearly synonymous with it. But clearly he was not alone in this and drew heavily from the community of scientists engaged with similar concerns. Nor was his theory entirely complete; he spent the remainder of his life collecting more evidence in support of it or in highlighting special concerns revising and rewriting his *On the Origin of Species* into some six editions. Most of Darwin's original books after 1859 were actually botanical in nature, some heavily experimental, and some meant to bolster his theory (Browne, 2002). His studies on orchids, for example, not only capitalized on the availability of the plants in an era obsessed with them, but was also meant to study the adaptive functions of the many 'contrivances' in a group known for its stunning morphological diversity. His attempt to apply his theory to humans and to human social evolution led to the publication of his *Descent of Man* in 1871, which was also a prolonged discussion of sexual selection, a kind of amendment to natural selection. Here it should be noted, that he held a fundamental difference of opinion with Wallace (Gayon, 1998).

Most importantly Darwin embarked on an examination of the study of variation and its origins, publishing *The Variation of Animals and Plants under Domestication in 1868*, which included his celebrated 'provisional hypothesis of pangenesis,' a theory of heredity, that while appropriate to his thinking, turned out to be wrong. Darwin did not actually have a viable theory of heredity, one that required a particulate instead of a blending theory, and that remained one of the fundamental weaknesses of his theory. Remarkably, he also did not have adequate direct support for natural selection. His examples in *On the Origin of Species*, for example, were either 'imaginary' illustrations or were indirect in nature such as the evidence from biogeography. Thus, though Darwin, along with others – convinced the world that species change and organic evolution had taken place in a mechanistic and orderly fashion, he lacked deep knowledge of heredity as well as good examples of the actual means by which that took place. Darwin in fact included several other means by which species change could take place, including the inheritance of acquired characters, or Lamarckism. By the end of the century, even advocates of Darwin's theory offered a number of amendments, interpretations, and alternative theories. Indeed so many people extended, appropriated, or reinterpreted Darwin's own views, especially as they applied to humans and to human social evolution, sometimes inventing entire areas of science, or more correctly pseudosciences such as eugenics (a theory of human 'improvement') or social Darwinism that many scholars have drawn the distinction between Darwin, Darwinism, and Darwinisticism (Peckham, 1959).

The Eclipse of Darwin and the Modern Synthesis of Evolution

The interval of time after Darwin's death, and around the turn of the century is thus regarded as a historical moment characterized by widespread discussion of Darwinism, that was often confused, and at times even anti-Darwinian in outlook (Bowler, 1983). A number of theories were proposed including a return to a belief in the inheritance of acquired characters, or neo-Lamarckism, or directed evolution, which upheld the view that evolution was guided by an internal driving force; and even creative evolution, a kind of mystical evolutionary theory put forth by French philosopher Henri Bergson (1859–1941). Indeed, some have even claimed that some of these theories were non-Darwinian or at times even anti-Darwinian (Bowler, 1988). This was so much the case that Julian Huxley (1887–1975), the grandson of Thomas Henry Huxley, designated this interval of time as the 'eclipse of Darwin' in 1942 (Huxley, 1942; Bowler, 1983). This was certainly true of the mutation theory (also called mutationstheorie) a popular theory associated with the Dutch botanist Hugo De Vries (1848–1935) who argued for rapid or saltationist species change due to the phenomenon of mutation pressure. This formulation thus gave Darwinian natural selection merely an eliminative instead of a creative role, and contrasted with Darwinian views of evolution taking place slowly and gradually on small, individual differences. It is one of the ironies of the history of science that de Vries was one of the three codiscoverers of Mendel's theory of heredity in 1900, along with Eric Tschermak (1871–1962) and Carl Correns (1864–1933), so that Mendelism, which actually supplied the particulate theory of inheritance needed by Darwin, was instead seen to bolster a rival mutation theory. Mutation theory also had the additional strength of being experimental in methodology and therefore more rigorous; Darwinism, was increasingly seen as descriptive and came to be associated with statistical data collection and analysis. It was associated with the school of Biometricians, who, for methodological, intellectual, and even personal reasons began to clash with the proponents of Mendelism. Thus ensued one of the most celebrated and counter-productive episodes in the history of evolution, which witnessed a number of feuds and misunderstandings, over the means and mode of evolution (Provine, 1971). The truth was that variation, and its origin, was incompletely understood, hence the hypothesizing over 'mutation pressure' as a way of generating biological novelty.

Only after a series of developments, that included the death of many of these argumentative individuals, and the

development of new mathematical techniques and methods, such as the 'Hardy–Weinberg Equilibrium Principle,' which determined the conditions under which there would effectively be no evolutionary change, did a proper synthesis between Mendelian genetics and Darwinian selection theory began to take place. The application of mathematical and indeed statistical methods, which viewed natural selection, genetic drift, and even mutation as interacting variables within varied population structures, eventually set the stage for what would become the new or modern synthesis of evolution and lead to a kind of restoration of a modified or synthetic view of Darwin's original theory of 1859. The work of three mathematical population geneticists in particular, R. A. Fisher (1890–1962), Sewall Wright (1899–1988) and J. B. S. Haldane (1892–1964) working in tandem with field practitioners and experimentalists such as E. B. Ford (1901–88) and Theodosius Dobzhansky (1900–74), thus laid the foundations for the synthetic theory of evolution (Provine, 1971). Though there were notable differences of opinion about the relative importance of these variables within varied population structures, all agreed that the alternatives proposed at the turn of the century were diminished if not eliminated outright; indeed they became alternative, once natural selection was established as the primary mechanism of evolution.

Although historians of science have tended to favor the contributions of one or more of these individuals, most agree that Theodosius Dobzhansky, a Russian émigré, played an especially critical role in establishing the new field of evolutionary genetics (Adams, 1968, 1980). Trained in systematics in Russia in what was a kind of ecumenical and population-oriented approach to understanding variation in natural populations, Dobzhansky moved to study classical genetics in the laboratory of Thomas Hunt Morgan (1866–1945) in the United States (Provine, 1981). He then took this latest laboratory understanding of transmission or classical genetics to the field through his studies of the genetical structure of field populations of varied related species of *Drosophila*. Engaging in a close collaboration with the theoretical understanding of evolution by the mathematical population geneticist Sewall Wright, Dobzhansky brought evolutionary theory to the field and to natural populations, gaining new insights into population structure and in the genetic basis of evolutionary processes such as speciation (Provine, 1986). Dobzhansky's (1937) book, the culmination of his efforts to understand speciation in natural populations and titled *Genetics and the Origin of Species*, was thus intended to redress the absence of the genetic basis for evolutionary change in Darwin's (1859) book. It was widely read, and highly influential, shaping an entire generation of younger workers entering what was an emerging field that drew on both genetics and systematics and emphasized the populational aspects of species change. This was so much the case, that it also functioned as the first textbook of evolutionary biology bringing new practitioners to the field.

The Convergence of Biological Disciplines and the Emergence of Evolutionary Biology

Dobzhansky's book also functioned as a kind of catalyst for the publication of other synthetic books that built on, amended, or extended the views set forth in *Genetics and the Origin of Species* and that served to bring a number of related disciplines to consensus (Mayr and Provine, 1980). These books included Ernst Mayr's (1904–2004) 1942 book *Systematics and the Origin of Species*, that brought systematics into the synthesis and set forth the modern biological species concept; George Gaylord Simpson's (1902–84) 1944 *Tempo and Mode in Evolution* that brought the paleontological record, a serious problem for Darwin, in line with the modern synthesis of evolution; and G. Ledyard Stebbins's (1906–2000) 1950 *Variation and Evolution in Plants*, that offered a synthesis of knowledge that had accumulated in plant genetics, systematics, and evolution and that resolved fundamental problems of variation in the plant world. Finally, Julian Huxley's (1942) book, *Evolution: The Modern Synthesis* appeared, signaling the emergence of a new and modern view of Darwinian evolution. Indeed, more than any other, this book heralded the new synthesis of evolution between Mendelian genetics and Darwinian selection theory and it did so in a way that supported a progressive but also mechanistic view of evolution that was palatable in terms of its sociopolitical outlook. The book reached wide audiences and gave the name 'modern synthesis' to the new understanding of evolution that incorporated Mendelian genetics and Darwinism in a populational way of thinking.

The growing consensus on evolution by means of natural selection along with the integration of a number of disciplines grounded in evolutionary genetics and looking to each other for new insights and methods, was also accompanied by new organizational activities that provided opportunities for intellectual exchange. Starting as early as the mid-1930s, groups of workers came together informally at first, to discuss some of the new trends in evolution (Smocovitis, 1994). In the United States (increasingly the locus for evolutionary activities), in the San Francisco Bay area, a group of primarily systematists looking to new understanding from genetics and ecology organized themselves into an informal scientific society they called the 'Biosystematists.' Echoing the call from Julian Huxley for a 'new systematics' that integrated these new approaches, the group included people like Theodosius Dobzhansky who was a frequent visitor to the Bay area. In 1939, many of these systematists were active participants at a meeting of the American Association for the Advancement of Science, where they organized a formal society for the new systematics called Society for the Study of Speciation. In 1943, a group on the east coast comprised mostly of paleontologists formed the Committee on Common Problems of Genetics and Paleontology, later changing their name to the Committee on Common Problems of Genetics, Paleontology, and Systematics. During the war years, when meetings were difficult, members shared their conversations with each other and interesting new insights in the form of mimeographed bulletins that they circulated with each other. The very last bulletin included a special notice from G. G. Simpson, newly returned from service in the war, that 'a field common to the disciplines of genetics, paleontology, and systematics' had recently come into existence that was 'beginning to be defined.' (Simpson, 1944). In 1946, shortly after the war, members from these varied groups came together in St. Louis, Missouri in agreement to found a formal scientific organization devoted

to the study of evolution known as evolutionary biology. After much deliberation they chose the name of Society for the Study of Evolution, which sponsored the new journal titled *Evolution*. It was the first international journal devoted to the subject (Smocovitis, 1994, 1996).

Shortly thereafter in 1947, on the occasion of Princeton University's Bicentennial, an international assemblage of evolutionists from a staggering diversity of backgrounds convened at a meeting devoted to the new synthetic science, and began to explicitly reidentify themselves as evolutionary biologists. The proceedings of the meeting, edited by Glenn L. Jepsen, G. G. Simpson, and Ernst Mayr titled *Genetics, Paleontology and Evolution* published in 1949 made reference to this new 'synthetic field' which represented a fusion of a number of independent disciplines. Writing one of the better known essays in the collection, geneticist Hermann J. Muller (1890–1967) stated that there had been a "convergence of evolutionary disciplines." He noted that there had been convergence of evolutionary types, such as between paleontologists and geneticists to form a 'synthetic' type of evolutionist (Muller, 1949:421). Most importantly, he noted what had made this possible was a 'common ground of theory' that served to unite formerly disparate areas. The fundamental points of agreement included that: (1) natural selection was the primary mechanism of evolutionary change; (2) it operated at the level of small individual differences, making evolution a slow, gradual process; (3) the same processes that operated at lower levels also accounted for higher order phenomena; in other words there was a continuum between microevolution and macroevolution. Muller then echoed Julian Huxley's insights in his 1942 book noting that a more unified science of evolution had emerged and that it served as the unifying principle of the whole of the biology – hence the choice of name evolutionary biology, for the new discipline that came to be commonly used around this time (Smocovitis, 1996).

The Watershed: The Darwin Centennial and After

By 1959, most of the critical developments leading to a scientific discipline of research called evolutionary biology had therefore taken place. This was just in time for the 100th anniversary of Darwin's *On the Origin of Species* that also corresponded with the 150th birthyear of Charles Darwin. At this time, members of the new synthetic science of evolutionary biology came together all over the world in contemplation, reflection, and celebration over Darwin's theory. Until then, little actually existed in the way of historical scholarship on Darwin and his theory, but after the Darwin Centennial, a number of biographies of Darwin began to appear and a rich body of literature began to accumulate (Smocovitis, 1999).

So too, a number of other developments happened to bring in additional disciplines to resolve long standing concerns. Human evolution, for example, had been barely mentioned by Darwin in his 1859 book, and was not properly included even in the evolutionary synthesis of the preceding decades. This, despite the fact that individuals like R. A. Fisher were attempting extensions of the synthetic theory to areas like eugenics. In the 1940s, it fell to Dobzhansky to help integrate biological anthropology into the synthetic theory (Dobzhansky, 1941). Again, it was his emphasis on populations and his background drawn from Russian population genetics that facilitated this. He drew on the notion of 'gene pool,' (a concept derived from his Russian mentors) as a replacement for 'race' or 'racial type.' In doing this, Dobzhansky deftly eliminated the criticism made by cultural anthropologists like Franz Boaz, and his influential school of anthropology, that evolutionary theory attached to humans necessarily essentialized and racialized humanity (Smocovitis, 2011).

By 1959, an additional set of new of disciplines – and individual practitioners – were drawn to evolutionary study. After the articulation of the structure of DNA in 1953 and after the celebrated experiments of Stanley Miller (1930–2007) and Harold Urey (1893–1981) established the biochemical basis for the origins and evolution of early life in the same year, even more scientists entered the study of evolution. This was evident at the many events organized in honor of the Darwin centennial, at places like Cold Spring Harbor, but especially at the University of Chicago that hosted the largest celebration of all. Organized by anthropologist Sol Tax, the five-day event was designed to bring all the scientific disciplines together in a spirit of unity made possible by the synthesis of evolution, but to also allow the process of unification between disciplines to continue especially between areas like anthropology as well as to open discussion of topics like the evolution of mind, culture, and even the evolution of the physical world (Smocovitis, 1999).

From the large number of attendees, to the international attention garnered by the Darwin Centennial at the University of Chicago, indications were that it was a great success, and for the most part consensus appeared to dominate discussions. Some participants, however, viewed this as an unhealthy sign of a growing orthodoxy. Years later a similar point was made by paleobiologist Stephen J. Gould (1941–2002) who saw the 1959 consensus around natural selection as the 'hardening' of the synthesis around a selectionist core (Gould, 1980, 1983). But while consensus and unity appeared to exist, some disciplines such as embryology, or developmental biology appeared to be left out of the synthetic theory (Waddington, 1953; Amundson, 2005); and even paleontology appeared to be incompletely integrated (Olson, 1960). Criticisms aside, the Darwin centennial celebration does reveal the extent to which evolutionary biologists, indeed evolutionary scientists, had endorsed evolution by means of natural selection, who began to also recognize that evolution served as a unifying or organizing principle of the whole of the biological sciences, and indeed within a unified theory of knowledge. Taken as a whole, the year 1959 and the opportunity to reflect on Darwin and his science represented a kind of watershed demarcating the struggle to establish Darwin's theory of evolution by means of natural selection with the emergence of the new science of evolutionary biology. That new science was growing rapidly, especially in the wake of the Darwin centennial year. By the end of the 1950s, membership in the Society for the Study of Evolution began to spike, despite the increasing resistance, especially in the United States of America from creationists who were galvanized into action as a result of the lavish attention given to Darwin and evolution (Smocovitis, 1994, 1999).

Evolutionary Biology in the Post-Sputnik Area and Challenges to the Synthetic Theory

The biological sciences as a whole began to boom in the Cold War era. Funding for research began to increase significantly especially in the United States of America, which dominated the world stage at this time. New directions in biological research especially from the discovery of the structure of DNA in 1953, as well as new techniques made available in sciences like biochemistry and genetics were at first only slowly integrated by evolutionary biology. Indeed, in some circles, the emphasis on molecular biology and its philosophy of reductionism caused initial tumult for many evolutionary biologists who felt that the autonomy of biology was challenged as it stood to be engulfed by the physical sciences (Mayr, 1982; Wilson, 1996). For some, a series of 'molecular wars,' broke out between the younger molecular biologists and biochemists and the older, established evolutionary biologists, as the two disciplines appeared to collide. The friction between the two areas – and the protection of the lesser funded evolutionary biology – was resolved by a process of fission separating or cleaving the two halves of biology, one molecular and the other organismic (Smocovitis, 1996). As a result, beginning in the late 1960s, entire departments of organismic biology began to spring up, many focusing on 'ecology and evolutionary biology,' or 'ecology, evolution, and behavior,' offering doctoral degrees in the area, and enrolling even more practitioners to evolutionary biology.

Whatever the initial tension between the two disciplines, newer methods and critical insights made their way into evolutionary biology leading to a new area of research traveling under the new banner of molecular evolution. It began to dominate the study of evolution, posing a series of challenges to the synthetic theory of evolution, and culminating with the views of Japanese geneticist Motoo Kimura (1924–94) and others who argued that the vast majority of variation seen at the molecular level was due to random genetic drift and mutation and not to natural selection, which acted merely in a negative or eliminative role. They began to cast doubt on the primacy of natural selection as the driver of evolutionary change. In 1983, for example, in a book titled *The Neutral Theory of Molecular Evolution*, Kimura went so far as to claim that the synthetic theory was but an 'orthodox view' that had held back understanding of evolution at the molecular level. Debates between Kimura and his supporters called 'neutralists,' and those who favored a more conventional positive or creative role for natural selection ensued, and continue to the present with little hope in the way of resolution.

Organismic biologists came through the period of the 'molecular wars' intact, rallying around Dobzhansky's oft-heard assertion that 'nothing in biology makes sense, except in the light of evolution' (Smocovitis, 1996). The centrality of evolution and its organizing, unifying properties made its way into textbooks of evolution that reached both secondary school and university level students, bolstering the discipline of evolutionary biology and its inclusion into the biological sciences curriculum; this was increasingly the case all over the world. It was also a powerful argument against creationist attacks on science, which began to dominate discussions over the teaching of evolution especially in the 1970s and 1980s (Dobzhansky, 1973). As a result of the inclusion of evolution, often as an undergirding theme, in widely used textbooks like the Biological Sciences and Curriculum Study (the 'BSCS') a new generation of students received their primary biological training with a view that evolutionary biology was a foundational science in the biological sciences (Smocovitis, 1996).

Challenges to the synthetic theory continued, however, especially in the 1970s and began to peak in the 1980s. Drawing a great deal of attention was a reform of Darwinian evolution promoted by paleobiologists endorsing 'punctuated equilibrium,' a theory of species change that read the fossil record in terms of punctuated events and that involved a revision of the rates of evolution as being slow and gradual. Examining the fossil record, proponents of this theory saw instead long periods of stasis, punctuated by periods of rapid phenotypic evolution. Publishing two papers on the topic first in 1972 and then in 1977, Stephen Jay Gould and Niles Eldredge called for a reform of the synthetic theory and precipitated a round of heated debates, many of which unfolded in a public settings (Eldredge and Gould, 1972; Gould and Eldredge, 1977). In 1980, Gould added more heat to the fire of controversy by suggesting the possibility of evolutionary change taking place in terms of radical reorganization at the genic level which implied that different kinds of evolutionary mechanisms operated below and above the species level (Gould, 1980). As a result, Gould and a number of proponents began to call for a decoupling of micro- from macroevolution (Smocovitis, 1996; Sepkoski and Ruse, 2009). Along with a critique of 'the adaptationist program' launched with population geneticist Richard C. Lewontin (1929), which challenged the 'hyper-selectionist' rhetoric and its overly uncritical applications to biology, Gould was instrumental in challenging some of the fundamental tenets of the synthetic theory (Gould and Lewontin, 1979). Along with a group of other 'young Turks' with paleontological backgrounds, he was also part of an effort to create another discipline or subdiscipline related to evolutionary biology, namely paleobiology (Smocovitis, 1996; Sepkoski and Ruse, 2009).

Adding even more fuel to fires that raged around evolutionary biology in the 1970s and 1980s, were debates over group selection, sociobiology and 'selfish genes' (Wilson, 1975; Dawkins, 1976), a return to Lamarckism under the guise of 'somatic selection' (Steele, 1981), and a reform of the principles of systematics launched by Willi Hennig's systematic manifesto and its application (see Hull, 1988, for many of the controversies over 'schools of classification').

By the next decade, furthermore, long standing concerns pertaining to development, which had not been part of the formulation of the synthetic theory began to take center stage as discussions over 'deep homology,' the recognition that distantly related organisms may share a kind of toolkit of developmental genes, gained acceptance. It was with good reason that beginning in the 1990s one commonly began to speak of an exciting area of research that integrated perspectives on evolution with genetics and with development, known as 'evo-devo,' and as the century drew to a close the possibility of integrating ecology began to be entertained as the term 'eco-evo-devo' began to gain traction (Futuyma, 2013; Gilbert, 2013).

Despite the heated discussions that remained about the importance of 'evo-devo,' or what molecular evolution meant for the synthetic theory, and despite a number of challenges from outside of science such as the 'intelligent design,' or ID advocates, the century drew to a close with a much broader view of evolutionary biology. Indeed by the middle decades of the 1990s, evolutionary biology was already being wedded to medicine, and especially epidemiology as interest in emerging pathogens grew and as the principles of evolutionary biology were increasingly applied to more practical ends not only in medicine, but also in agriculture, computer sciences, and even robotics. For good reason, evolutionary biologists now sometimes speak in terms of theoretical and applied evolutionary biology, indicating that the scientific discipline continues to prosper as it reaches out to new areas of research.

See also: Adaptation, History of. Biogeography, History of. Darwin's Finches, the Galapagos, and Natural Laboratories of Evolution. Darwin–Wallace Theory of Evolution. Directed Evolution, History of. Evolutionary Genetics, History of. Hardy–Weinberg Equilibrium and Random Mating. Molecular Evolution, History of. Natural Selection, Introduction to. Paleobiological Revolution, History of. Schools of Classification. Sociobiology, History of. Synthetic Theory of Evolution, History of

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Evolutionary Computation

L Altenberg, The Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg, Austria

Glossary

A-Life 'artificial life' Computational systems in which replication, natural selection, and ecological interactions are not set by the designer but emerge from the dynamics of the system.

EA 'evolutionary algorithm' An algorithm (sequence of mathematical operations on a data set, a computer program) that incorporate analogs of (1) natural or artificial selection and (2) variation generation.

EC 'evolutionary computation' The name for the entire research and engineering fields centered on evolutionary algorithms.

EP 'evolutionary programming' Evolutionary algorithms where the search space is a set of finite-state automata, originated by Fogel in 1964.

ES 'evolution strategies' A class of evol00utionary algorithms originated by Rechenberg and Schwefel in 1964, where the search space is Euclidean space and the variation operators are Gaussian random perturbations.

GA 'genetic algorithm' An evolutionary algorithm that incorporates recombination as a variation operator, originated by Holland in 1975, emphasizing homologous crossover on bit string encodings of the search space.

GP 'genetic programming' An evolutionary algorithm in which the objects being evolved are executable data structures, originated by Koza in 1990, where programs are represented as parse trees of operator and variable symbols, and the principal variation operator is exchange of subtrees between programs evolved in a population.

Encoding A representation of points in the search space that may change from a native representation to an alternate representation, such as encoding real numbers as a binary sequence.

Fitness In EC, 'fitness' is used as a shorthand for 'value of the objective function,' rather than the population genetics usage where it means the expected number of offspring.

Fitness proportional selection Selection in which the expected number of offspring of a candidate solution is proportional to the value of its (possibly rescaled) objective function.

NFL 'no free lunch' theorems The result from Wolpert and Macready in 1995, that all search algorithms have equal average performance when averaged over all problem sets.

Object function A measure of how good a candidate solution is to producing a desired outcome.

Representation The way that candidate solutions in a search space are mapped to the data structures that represent them in the algorithm.

Search space The collection of all candidate solutions to be searched through by the evolutionary algorithm.

Simulated annealing An evolutionary algorithm with a population of one parent plus one mutant offspring ($1+1$ EA), where the offspring replaces a less fit parent with probability one, and it replaces a more fit parent with probability $e^{-(w_P - w_O)/T}$, where w_P is the parent fitness, w_O is the offspring fitness, and T is a 'temperature' which is successively lowered during a run of the search (the 'annealing').

Introduction

Evolutionary computation is an approach to engineering and optimization in which solutions, instead of being constructed from first principles, are instead *evolved* through processes modeled after the elements of Darwinian evolution. Evolutionary computation is one of the principal methods in what is called 'nature-inspired computing.' Nature-inspired computing, which also includes artificial neural networks, swarm intelligence, and fuzzy logic, has also been called 'soft computing,' or 'computational intelligence' to distinguish it from symbolic artificial intelligence. Technically, evolutionary computation is as an example of heuristic search, i.e., search by trial and error, where in EC the 'trials' are candidate solutions, and the 'error' is the measurement of how far a trial is from a desired outcome. The error is used to select which trials are to be used to generate further trials. The fundamental rule-of-thumb is that the best chance to further reduce the error is to generate new trials by making modifications to the previous trials that had the lowest errors.

The *evolutionary algorithm* is the main object of interest in evolutionary computation. There is a problem to be solved, and the solution is conceived to lie somewhere in a space of possible candidate solutions – the *search space*. The evolutionary algorithm searches for good solutions in the search space using this typical structure:

1. Initialization: Randomly generate a population of samples from the search space.
2. Iteration:
   (a) Evaluation. Compute the value of the objective function for each sample.
   (b) Selection operator. Use the values of objective function computed for the evaluated samples to select the samples to be used in the next step 2(c).
   (c) Variation operators. Apply variation operators to the selected samples to transform them into additional samples from the search space.
3. Termination: If the termination criteria are met, halt the computation; if not, return to step 2(a).

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The problem to be solved usually determines in an obvious way what the search space is, and what the objective function is. For example, if one is trying to find the maximum value of $f(x,y)=\sin(x^2-2x-4)\ \cos(-3y+y^2+1)$ on the intervals $-1\le x\le 1$ and $0\le y\le 1$, the search space is simply the intervals, and the objective function is $f(x,y)$ itself. To give a more elaborate example, when trying to evolve artificial neural networks that implement a desired map between inputs and outputs, the search space is the set of weights and topology of the network connections. The objective function is a measure of how closely a candidate map matches a desired map, using a measure of closeness such as mean squared error over a test set of inputs.

Exploration of the search space is performed by using previously sampled and evaluated points to generate new points to be evaluated. The points in the search space are concretely represented by some data structure in the algorithm, and variation operators that randomly perturb these data structures generate the new points.

The classical variation operators are mutation and crossover (i.e., recombination), based on Mendelian genetics and thus called 'genetic operators.' Mutation is implemented by altering the state of symbols in a string, such as flipping a bit in a binary string from 0 to 1 or vice versa. Crossover is implemented by recombining parts of one parent with parts of another. Crossover may be analogous to homologous chromosomal recombination when the positions in the data structure are fixed, as in a fixed-length bit string, or in a fixed set of real-valued parameters. But there are other search spaces where it is impossible to make the analogy to linear chromosomes. For example, in the Traveling Salesman problem, the search space consists of all tours of a set of cities. Taking parts of one tour and recombining them with parts of another tour is likely to produce a path that repeats some cities and misses others. Special variation operators are used which ensure that a path is a tour.

Choosing which samples to apply the variation operators to is the task of the selection operator. Here, a population structure needs to be introduced. In what is called the '$1+1$ EA,' the population consists of a single sample of the search space. A new sample is generated by applying a variation operator to that sample, to generate a population of size 2. The values of the objective function for the two samples are evaluated and compared, and the sample with the better objective function is selected to generate the next sample. Simulated annealing is a simple modification of the selection operator where inferior offspring may be selected with a certain probability (see Glossary). Larger populations may be used, in which case they may maintain enough variation so that recombination may be used as a variation operator. Many different selection operators are possible in large populations. Both the Wright–Fisher multinomial sampling model and the Moran sampling model have been used.

The selection operators that carry out the computational analog of selective breeding may in fact be the exact same operators used in agriculture, such as culling, or ranking.

History of Evolutionary Computation

Evolutionary computation developed in three phases: the first phase consisted of numerous independent experiments in evolutionary algorithms in the 1950s and 1960s. Principal researchers during this period were Nils Aall Barricelli in the early 1950s, George E. P. Box and Alex S. Fraser in the late 1950s, and Hans J. Bremermann in the early 1960s.

The second phase began in the late 1960s and early 1970s where, out of these multiple lines of investigation emerged three lineages that persisted and produced academic offspring: Fogel (1964) established *evolutionary programming*, Rechenberg (1964) and Schwefel (1965) established *evolution strategies*, and Holland (1975) established *genetic algorithms*. Deriving from the genetic algorithm lineage, Koza (1990) established the field of *genetic programming*.

The third phase came when these four lineages, which had established their own conferences, merged in the mid-1990s into a unified evolutionary computation community. Out of this convergence the field gained its first journals, *Evolutionary Computation* at MIT Press in 1993, *IEEE Transactions on Evolutionary Computation* in 1997, and *Genetic Programming and Evolvable Machines* at Springer in 2000. Before the advent of these journals, research in evolutionary computation appeared mainly in the proceedings of the International Conference on Genetic Algorithms, the Evolutionary Programming conferences, the Parallel Problem Solving from Nature conferences in Europe, and scattered articles throughout academic journals. Because the journal citation index Web of Science does not currently index many of these sources, a large body of the founding literature in evolutionary computation remains invisible to it.

The founding papers in the field from 1953 to 1997 have been collected by Fogel (1998) into a single volume, *Evolutionary Computation: The Fossil Record*.

The history of evolutionary computation differs markedly from that of the field of *artificial intelligence*. Artificial intelligence had a specific birth as a new field in a proposal for a 1956 Dartmouth conference organized by Dartmouth mathematician John McCarthy, Marvin Minsky at Harvard, Nathaniel Rochester at IBM, and Claude Shannon at Bell Labs McCarthy *et al.* (1955 (2006)). John Holland, it should be noted, was one of the invitees. In the founding of evolutionary computation, the Ivy League played almost no role, and the research took root in diverse state universities and industrial research labs in the United States, and universities in Europe and Australia.

Evolutionary computation has developed largely independently from the established field of theoretical population genetics. Yet interchanges between the evolutionary computation and evolutionary biology communities occurred as early as 1980 in Europe during a workshop on the 'Evolution of Evolutionary Mechanisms' held in Göttingen, West Germany (Wagner, 1981), while in the United States, interactions between the two communities were fostered by the advent of the Santa Fe Institute, in which John Holland from the University of Michigan and evolutionary theoretician Marcus W. Feldman from Stanford University were key participants. Paixão *et al.* (2015) have made a recent effort enhance the flow of theoretical results between fields by creating a modeling framework that encompasses both evolutionary algorithms and computational models of evolution.

Variety in Evolutionary Computation Techniques

Designers of evolutionary algorithms have explored a very large space of techniques in the hope of improving the performance of evolutionary algorithms over the very wide variety of engineering problems to which they have been applied. Explorations include the values of algorithm parameters such as mutation and crossover rates, selection strength, and population size, encodings, spatial population structure, generation overlap, niching, fitness sharing, multi-objective optimization methods, feasibility constraints, etc. The theory of evolutionary dynamics has yet to reach the point where one can convert the information one has about an optimization problem into the choices of which techniques and parameters to use. For specific models however, progress is being made in obtaining rigorous theoretical results (portal papers include Oliveto *et al.*, 2007; Valiant, 2009).

Evolutionary algorithms employ a great palette of special operators which may have no analogy in biology. One area under extensive development is multi-objective evolutionary optimization. Another example that has burgeoned into a research program is the method called *estimation of distribution* algorithms (EDAs) introduced by Mühlenbein and Paass (1996). An early method was to merge all of the genomes in the population into a single gene pool and sample the alleles at each locus to generate a new genotype, so that the entire population serves as the parent (Syswerda, 1993). EDAs derive from an abstract view about what the operators of selection, mutation, and recombination are actually doing: they produce (implicitly) a probability distribution from which the genotypes of offspring are sampled. EDAs produce these probabilities explicitly, as a dynamical system on probability models whose parameters are adjusted by the objective function values of sampled candidate solutions, in the hope of concentrating the probability on the best candidate solutions. EDAs readily incorporate techniques from machine learning to generate the probability models (Lozano *et al.*, 2006; Pelikan, *et al.*, 2015).

A significant development in evolutionary computation came with the advent of genetic programming, because the data structures representing candidate solutions are no longer of fixed length, but can grow in complexity. Genetic programming evolves code structures that are themselves executable. Programs can be represented as parse trees, and pairs of parse trees can be recombined by exchanging their subtrees. Figure 1 shows an illustration of how a mathematical function can be represented as a parse-tree in genetic programming, and how subtree exchange between two copies of a program (in this case, for a Gaussian distribution) produces an offspring that encodes a different function. Many other techniques have been developed for variation operators in genetic programming, for example, by placing the executable structures back one step from the variation operators through 'cellular encoding' (Gruau, 1994) or 'developmental encoding' (Mouret and Tonelli, 2014), in which the executable structures are constructed from the encoding through an ontogenic development process.

Contrasts Between Evolutionary Computation and Organic Evolution

The motivation behind the development of evolutionary algorithms is optimization – finding approximate or exact

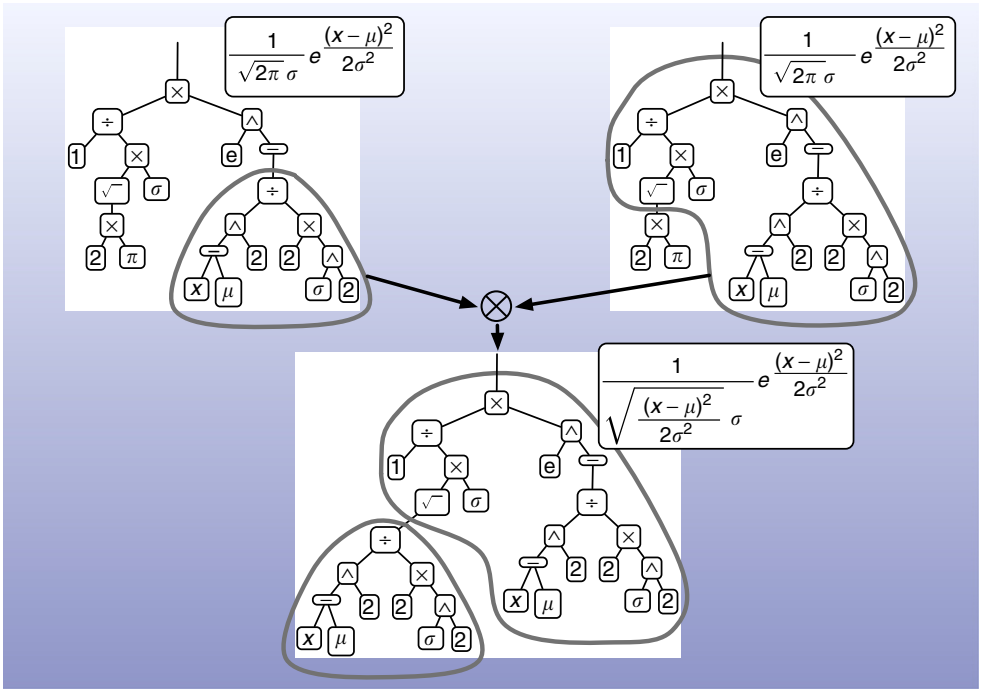

Figure 1 Genetic programming subtree exchange between two parse trees. Two identical parent trees encode the algorithm for the Gaussian normal density. Their recombinant offspring encodes a new mathematical expression.

solutions to diverse problems within the constraints of the computational resources available. The amount of computation required to find an optimum or to achieve a desired level of approximation is therefore a central concern. Such rate of improvement per computation can be considered a measure of *evolvability*. In organismal evolution, everything is conditioned on the survival of the lineage, as pointed out by Palmer and Feldman (2012), and evolvability may conflict with survival if it is tied to increased production of deleterious variation, or leads to the evolution of short-sighted traits that sabotage long-term survival (Nunney, 1989; Altenberg, 2005) or reduces total population size (Frank, 2013). Lineage survival is precluded as an issue in evolutionary optimization because the algorithms are fundamentally search algorithms, and a 'population' is simply the set of previously sampled points from which new samples are to be generated. In other words, population persistence is guaranteed by construction. Evolvability is thus the primary performance goal of evolutionary algorithms.

In terms of sheer computational power, computer-based evolutionary algorithms cannot compare with the number of evolutionary operations taking place in the biosphere (reproduction, mutation, recombination, natural selection). There are an estimated 3×10^{27} cells of the ocean cyanobacterium *Prochlorococcus marinus* alive at any one time (Flombaum *et al.*, 2013) (on the order of the number of atoms in a ton of gold), and with a once-daily cell division, a genome size of 2×10^6 base pairs, and an estimated mutation rate of 5.4×10^{-7} mutations per base pair (Osburne *et al.*, 2011), there are some 3×10^{27} mutations generated daily. That is enough to sample all of the four-mutation neighbors of a parental sequence. The raw information content of organismal genomes, which ranges on the order of 10^5 to 10^{10} bits, is matched by very few instances of the data structures searched with evolutionary algorithms. The current record is set by some special examples in which genetic algorithms have been performed on one-billion-bit strings (Iturriaga and Nesmachnow, 2012).

A run of an evolutionary algorithm typically begins with a population constructed from uniformly sampled random points in the search space. In organic evolution, by contrast, the initial condition was the origin of life itself. At some point life evolved into organisms with DNA-based genomes, but none of the scenarios for the origin of nucleotide-based genomes propose that the initial sequences were uniformly sampled random sequences. So the initial stage in a run of an evolutionary algorithm, where recombination, mutation, and selection are being performed on a population of random sequences, has no parallel in organic evolution, where the processes are always observed in populations that have been evolving for a very long time.

In evolutionary algorithms, there is a clean separation between logical parts of the algorithm: the code for the objective function and the variation and selection operators is entirely separate from the code for the data structures representing the search space, and the entire algorithm is contained in application code separate from the operating system and hardware of the computer. In organic evolution we see the exact opposite: these logical elements are all physically enmeshed – the molecular machinery that produces mutation and recombination and organismal survival and reproduction is all a product of the information in the genome, cytoplasm, and whole organism, interacting with its environment. Some efforts have gone in to blurring the logical separations in evolutionary algorithms, notably the encoding of mutation and recombination rates into the genomes of the individual (see Section Genetic Modifiers, below). The field of *artificial life* distinguishes itself from evolutionary algorithms in that it places a priority on getting reproduction, replication, mutation, recombination, fitness, spatial movement, and ecological interactions to all emerge from the computational system instead of being written by hand in separate parts of the program, emulating the enmeshment of logical levels found in biology (see Section Artificial Life, below).

Contrasts Between Evolutionary Computation and Computational Models of Evolution

When evolutionary algorithms are compared with computational models of evolution, the most obvious difference is that computational models of evolution place a priority on biological realism, while evolutionary algorithms place a priority on computationally efficient optimization. As 'nature inspired' computing, EC has drawn upon many biological and population processes as a source of ideas to try out in algorithmic form, but little priority is given to keeping them biologically realistic.

In the drive toward more biological realism, computational models of evolution have gone beyond the rather limited set of constructs used traditionally in population genetics models, such as additive, multiplicative, low-order epistatic, random, and Gaussian fitness functions. Kauffman and Levin (1987) introduced the more structured, tunably-rugged NK fitness landscapes. Knibbe *et al.* (2007) included insertions, deletions, and duplications in a model with a complex genotype–phenotype map. Computational models of RNA folding (Schuster *et al.*, 1994), protein folding (Lobkovsky *et al.*, 2011), and gene regulatory networks (Wagner, 1994) have provided more biologically grounded fitness landscapes for exploring evolutionary dynamics. More recently, laboratories have been able to obtain fitness estimates for small regions of fitness landscapes, and computational models of evolution on these empirical fitness landscapes have been utilized to explore and even predict evolution (de Visser and Krug, 2014).

The fitness landscapes encountered in evolutionary algorithms are derived from a vast variety of real engineering problems, which may be seen as 'wild' rather than constructed. Evolution on these fitness landscapes ranges beyond the standard population genetics models and the special cases explored thus far in computational models of evolution. They may therefore have value as models to expand the palette of observed evolutionary phenomena.

Parallel Discoveries of Evolutionary Phenomena in Evolutionary Computation

There are several phenomena that were discovered in the field of evolution in parallel with their discovery in the evolutionary biology community. Here, several principal examples will be

given, under the names given to the phenomena in the evolutionary biology literature.

The Problem of Evolvability

The earliest experiences with evolutionary algorithms showed that the combination of Darwinian selection with random variation does not necessarily work to produce adaptation. Friedberg (1958) and Friedberg *et al.* (1959) attempted to evolve computer programs through selection and variation operators, but found that the process frequently stagnated. Conrad (1974a) pointed out that in computer programs, 'slight changes in such a rule (e.g., in the pattern of internal inputs) result in radical changes in the behavior of the system.' In organisms, the physical nature of molecular dynamics makes gradual changes in function possible, and Conrad (1974b) even proposed that gradualism could itself be increased in evolution.

But even where gradualism is abundant, epistasis, multimodality, and deception can stymy evolutionary algorithms from finding global optima or even good approximations. Good performance of evolutionary algorithms was found early on to depend critically on the encoding or representation of the search space, and on parameters of the algorithm such as mutation rate, population size, and selection operators.

A key theoretical breakthrough was the paper, 'No Free Lunch Theorems for Search' by Wolpert and Macready (1995, 1997). They showed that over the space of all problems, all search algorithms have the same average performance. The only way that a search algorithm such as an evolutionary algorithm can have superior performance is if the set of problems is matched to the algorithm, or said in another way, that the algorithm has implicit knowledge about the search space. Concretely, the variation operators have to be able to generate candidate solutions with ever better objective function values as the population evolves. This depends on how the variation operators acting on the representations relate to the objective function (Altenberg, 1995).

Genetic Modifiers

In 1967, Nei (1967) introduced a model for the evolution of recombination in which a modifier locus controls the rate of recombination between two other loci under viability selection. Feldman (1972) gave the first evolutionary stability analysis of the model. Simultaneously, on the evolutionary computation side, Reed *et al.* (1967) included genetic modification for mutation and crossover rates and mutation size in their genetic algorithms. Rechenberg (1973) began to incorporate genetic control of the 'strategy parameters' for his evolutionary algorithms, which included mutational step sizes, and this was termed 'self-adaptation' by Schwefel (1987).

Constructive Neutral Evolution

An early discovery in the field of genetic programming was that the size of evolved programs would keep growing in time, even though there was no explicit selection for larger programs. This was dubbed 'bloat.' One of the explanations proposed by Langdon and Poli (1997) to account for code bloat in genetic programming was that the number of long programs that could implement a given function was much greater than the number of short programs. Offspring produced by exchanging subtrees of parent programs would often have identical fitness to the best parent, and so evolution along neutral networks was possible. Longer programs would evolve as an entropic phenomenon of evolution along neutral networks. As described by Langdon and Poli (1997):

> In general variable length allows many more long representations of a given solution than short ones of the same solution. Thus (in the absence of a parsimony bias) we expect longer representations to occur more often and so representation length to tend to increase. That is fitness based selection leads to bloat.

On the evolutionary biology side, Covello and Gray (1993) proposed that RNA editing may have evolved through a mechanism that Stoltzfus (1999) named 'constructive neutral evolution,' in which needlessly complex mechanisms evolve through a process of neutral evolution simply because there may be a much greater number of complex ways to produce a phenotype than simple ways. Stoltzfus (1999) includes in this list the phenomenon of 'scrambled genes' in ciliates, and the process independently called 'subfunctionalization' by Force *et al.* (1999) in which independently functioning modules within a gene become separated into multiple genes, another essentially entropic process. Echoing the explanation by Langdon and Poli (1997) of bloat, we find the explanation by Stoltzfus (1999) for gene scrambling:

> Given such a buffer against the otherwise adverse effects of micronuclear gene rearrangements, a long-term net increase in scrambling would be expected, simply because there are many more scrambled than unscrambled configurations.

The Evolution of Genetic Robustness

As described above, program bloat is one of the emergent properties discovered in evolving computer programs through genetic programming. Before Langdon and Poli (1997) proposed their entropic mechanism for bloat, an earlier hypotheses was that the code bloat gave programs a 'defense against crossover' (Singleton and Keenan, 1993). They discovered that much of the code in bloated programs could be deleted or exchanged without consequence for the program's behavior. The bloated programs were thus phenotypically robust to the variation operator, and the extra code diverted the crossover operator from hitting sensitive parts of the program. Altenberg (1994) proposed that inherently neutral code would proliferate in the long-term evolution of populations of programs. Nordin and Banzhaf (1995) examined the distribution of fitness effects of subtree exchange (**Figure 2**) and found that, indeed, the proportion subtree exchanges with no effect on program performance (neutral crossovers) increased several-fold during the population evolution.

On the evolutionary biology side, interest in computational models of RNA folding revealed the existence of large mutationally connected networks of RNA sequences that

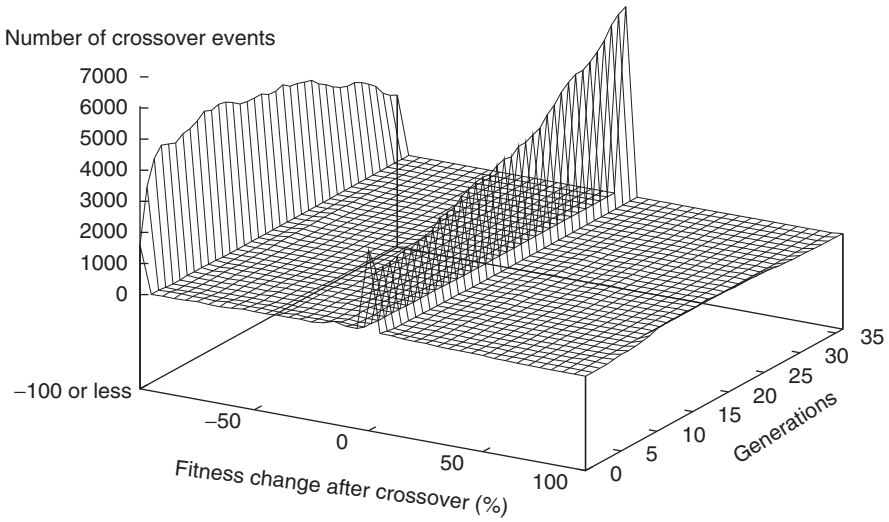

Figure 2 Distribution of fitness effects of recombination during an evolutionary run with genetic programming, Reproduced from Nordin, P., Banzhaf, W., 1995. Complexity compression and evolution. In: Eshelman, L. (Ed.), Genetic Algorithms: Proceedings of the Sixth International Conference. San Francisco, CA: Morgan Kaufmann, pp. 310–317. The proportion of neutral crossovers increases during the run.

folded into the same secondary structure, leading to questions about how evolution would proceed on such neutral networks. In 1999, two papers (Bornberg-Bauer and Chan, 1999; Nimwegen *et al.*, 1999) showed that evolution on neutral networks could move a population to genotypes with greater probability of producing neutral mutants, the same outcome of increased genetic robustness observed in genetic programming by Nordin and Banzhaf (1995).

Artificial Life

The field that has come to be known as 'artificial life' (Langton, 1984, 1986) also focuses on computational systems that exhibit Darwinian evolution. But the motivations behind artificial life and the design of its evolving systems depart significantly from the field of evolutionary computation. In its most ambitious form, the goal of artificial life is to create new 'instances of life' in addition to the actual biological world – i.e., systems that exhibit all the essential features of life – in order to advance our understanding of life by having more than the single 'data point' of terrestrial living systems. The means to this goal is to create artificial systems that exhibit the principal features of life: structures whose activities result in the maintenance and reproduction of the structures (autopoeisis, Varela *et al.*, 1974), ecological interactions, and reproduction with variation that allows Darwinian evolution to occur. Another widespread goal is to design artificial systems that exhibit an unbounded increase in complexity in time.

As stated by a founder of the field, Christopher G. Langton (1986):

> The ultimate goal of the study of artificial life would be to create 'life' in some other medium, ideally a virtual medium where the essence of life has been abstracted from the details of its implementation in any particular hardware. We would like to build models that are so life-like that they cease to be models of life and become examples of life themselves.

The root document of the field is von Neumann (1966) 'Theory of Self-Reproducing Automata.' It spawned numerous efforts to construct cellular automata in which configurations of cell states would self-reproduce through the automaton dynamics. Langton (1984) discovered such a cellular automaton, called the 'Langton loop.' A different approach was the successful construction by Ray (1993) of a virtual machine, Tierra, where a specific configuration of instructions would compute its own replication. By including error prone operators, the self-reproducing configuration could evolve. The evolutionary dynamics of Tierra produced emergent phenomena such as parasitism, where mutants parasitized other programs for their own replication, and a rich structure of ecological interactions. Several other virtual machine systems have been subsequently developed, including Avida (Adami and Brown, 1994) and Amoeba (Pargellis, 1996).

In these systems, sets of instructions in a virtual machine language perform the computation that replicates the set of instructions. Replication is not performed by separate variation or selection operators. Thus, replication emerges from the computational dynamics. Selection – the differential survival and replication of structures in the virtual machine – is also emergent from the computation, when some sets of instruction (the 'digital organisms') outcopy others. Mutation, however, is typically hand-coded into the construction of the virtual machine by making some instructions probabilistic. Also, until recently, the programmer had to design by hand the initial self-replicating configuration of instructions that founds the population. But LaBar *et al.* (2015) searched through 3 billion randomly generated programs in the Avida system and discovered 170 programs that produce self-replication.

Another distinction between evolutionary computation and artificial life is that the latter do not employ objective functions for use by selection operators. There is no 'goal' or outcome to optimize; the digital organisms are not 'candidate solutions' to some exogenously defined problem as is usually the case with evolutionary algorithms. Selection in A-Life systems could be called 'natural' in that differential survival and reproduction of the digital organisms is an emergent outcome of the computations in the virtual machine. The emergent properties of artificial life systems such as Avida have made them useful for exploring the dynamics of evolution, for example the phenomenon of 'survival of the flattest' (Wilke *et al.*, 2001), the evolution of the germline/soma division (Goldsby *et al.*, 2014), and the evolution of complexity (Lenski *et al.*, 2003).

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See also: Evolvability, Quantitative Genetics of. Robustness and Evolvability in Molecular Evolution

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Evolutionary Genetics, History of

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Glossary

Epistasis Epistasis occurs when the alleles at two or more loci combine to produce genotypes whose trait values differ from the values expected if the loci combined in a simple additive manner. The synergism across loci can be such that the multi-locus genotype has a trait value either more or less than expected if the loci had combined additively.

Genetic load The genetic load is the difference between the relative fitness of the most-fit genotype and the average relative fitness in a population, scaled to the average fitness (i.e., divided by the average fitness). It can be described heuristically as the number of offspring that the most-fit genotype must produce to compensate for the deaths distributed among the less-fit genotypes in order to avert a population crash.

Identity by descent Two copies of a gene are identical by descent when they are descended from the same ancestral copy, which occurs with probability 1/2N in a randomly mating diploid population.

Linkage disequilibrium Linkage disequilibrium occurs in a system of two (or more) loci when the frequencies of two-locus (or multi-locus) gametes are not the products of their constituent allele frequencies. Linkage disequilibrium implies that some two-locus (or multi-locus) genotypes are more (less) common than the combinations of their constituent allele frequencies would suggest. It can arise through random drift or selection on two-locus (or multi-locus) systems with epistasis across loci in fitness.

Quasi-linkage equilibrium The frequencies of the four gametes in a model of two-locus evolution with epistatic fitness relationships are said to be in quasi-linkage equilibrium when the variance in fitness contributed by linkage disequilibrium cancels the pairwise epistatic contributions to the variance in fitness. When this happens, the rate of change in mean fitness is proportional to the additive genetic variance in fitness.

Introduction

Evolution is a genetic phenomenon; if it is to be understood, its genetic underpinnings must also be understood. This was obvious to Darwin and his critics (see Reznick, 2010) and for decades after publication of *On the Origin of Species*, the slow accumulation of genetic understanding hindered the acceptance of Darwinian evolution (Provine, 1971).

In this article we trace the path from Darwin and his proposed mechanism of natural selection to the sophisticated evolutionary genetics that validated Darwin's ideas. Our article covers about a century, from the years after publication of *On the Origin of Species* to the publication, in 1970, of Crow and Kimura's *An Introduction to Population Genetic Theory*, which represents the synthetic climax to the first century of evolutionary genetics.

The Origin of Evolutionary Genetics

The Argument over Variation

Darwin argued that the driving force of evolution would be a natural process of selection, acting on variations among individuals within a population and causing some individuals to leave more offspring than others. The notion of a 'natural' selection had been suggested prior to Darwin to explain the persistence of certain populations and the extinction of others through episodes of environmental change (Eiseley, 1958). In these cases, selection was posited to act on traits that were shared by all of the members of a population. Darwin's genius lay in perceiving that selection could act on the *differences* among individuals.

For Darwinian selection to generate evolutionary change, some of the trait variation among individuals must be inherited. Moreover, the mechanism of inheritance must preserve variation in the absence of selection. Blending inheritance, which was the mechanism postulated in Darwin's time, would quickly homogenize heritable variation among individuals. Under blending inheritance, any observable variation among individuals would be environmentally induced and Darwin's postulated mechanism would be unable to produce any lasting change.

The discovery of particulate inheritance did not immediately vindicate Darwin's ideas (Provine, 1971). In fact, it bolstered his critics because the limited genetic variation in one-locus, two-allele systems constrains how much change selection can catalyze. The rapid attainment of limits to selectively driven change in such cases suggested that mutation, and not natural selection, would be the major driving force of evolution. Further, if there were a one-to-one relationship between a gene and a single, discrete phenotypic trait, selection would require enormous periods to alter many of an organism's features because the process would be limited by the rate at which mutations for each feature appeared. In this view, Darwinian selection might explain small, occasional changes in organismal features but could not possibly be a driving force for significant evolution.

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The Emergence of Evolutionary Genetics

Particulate inheritance of discrete traits has mathematical precision: crosses between individuals with different features produce progeny with a predictable statistical distribution of those features. The earliest studies of inheritance uncovered these predicted distributions in various circumstances: one locus with two alleles, two independently segregating loci each with two alleles, and, eventually, two linked loci (Edwards, 2012).

Once the statistical nature of inheritance was understood, scientists turned their attention to describing the distributions of allele frequencies in large natural populations. It quickly became apparent that with particulate inheritance, variation would not be blended away (the Hardy–Weinberg–Yule equilibrium). Moreover, it became clear that Darwinian selection would act by altering the distribution of allele frequencies over time. Among the earliest of these demonstrations was Castle's (1903) description of how deleterious recessives would be eliminated from a population and Norton's calculations of the number of generations required for selection to change allele frequencies from one set of values to another (presented in Punnett's 1915 book (Provine, 1971)).

There was still controversy. While the mathematics of inheritance was clear for discrete phenotypic traits, attempts to characterize the inheritance of continuous traits, which characterized a species and defined its distinction from other species, had produced no similarly simple mathematical rules. Without such a theory, it was unclear how natural selection could drive changes in such traits.

In a monumental paper in 1918, R. A. Fisher produced that theory (Fisher, 1918). This paper derived precise mathematical predictions for correlations among relatives of specified degrees and demonstrated that those predictions were entirely general, regardless of how many loci were involved, how many alleles segregated at each locus, and what levels of allelic dominance prevailed. Fisher's paper also defined a more sophisticated approach to genetics, a general mathematical representation of a class of problems instead of an arithmetical description of one type of problem. Evolutionary genetics had been born.

The Maturation of Evolutionary Genetics

By the mid-1930s, evolutionary genetics had evolved its current *bauplan*. A student of evolutionary biology today could return to a class in population genetics in the mid-1930s and feel at home; the subject matter, terminology, mathematical machinery, and basic themes had already taken on their modern shapes.

Common Ground: A Theory of Evolutionary Genetics

Science and human nature being what they are, much has been written about the disagreements among the founders of evolutionary genetics. However, it is important to appreciate the broad swaths of theory on which there was general agreement.

Whereas Fisher's (1918) paper can be considered the foundation of quantitative genetics, his 1922 paper, entitled 'On the Dominance Ratio,' became the foundation for evolutionary genetics (Fisher, 1922). In this paper, Fisher took the same approach to describing evolution that he had taken to describing variation in continuous traits: he developed general mathematical representations of entire classes of problems. For example, he developed the general mathematical model for viability selection from which most of population genetic theory would descend.

The genius of this paper was in the sections in which Fisher introduced and developed the idea of evolution as an inherently stochastic process. In Section 2, "Survival of individual genes," of the paper, Fisher calculated the probability of extinction of a new mutant gene, which he showed to be independent of population size and approximately independent of selection. He noted:

> …Only when the number of individuals affected becomes large will the effect of selection predominate over that of random survival, though even then only a very small minority of the population may be affected.

This was the first quantification of the role of chance in evolution and the first application of the theory of stochastic processes in biology (de Vladar and Barton, 2011). In Section 3, "Factors not acted on by selection," Fisher described how the distribution of allele frequencies would change over time due to random factors alone. In Section 4 he extended his calculations to include mutation, providing the first formal statement of mutation-drift dynamics. In Section 5 he mathematically addressed the random loss of alleles due to chance extinction in finite populations, which had been first suggested by Hagedoorn and Hagedoorn (1921). Fisher calculated the rate of loss of genetic variation due to drift as 1/4N, which Wright subsequently showed should have been 1/2N.

In the final, monumental portion of the paper, Fisher studied the combined effects of selection, mutation, drift, and assortative mating. Two novel conclusions emerged. First, Fisher articulated the concept now called 'effective neutrality,' which describes how the relative influence of selection and drift depends on the population size. More specifically, he showed that the smaller the population, the larger must be the selective advantage of an allele for its dynamics to be driven by selection. Second, Fisher showed that the level of genetic variation expected when mutation and selection are in balance is proportional to the ratio of mutation rate to the strength of selection.

Fisher continued to develop novel theory for natural selection and evolution, culminating in 1930 with the publication of *The Genetical Theory of Natural Selection*. This book remains the most important work in evolutionary biology since *On the Origin of Species*. While it summarized much of Fisher's prior work, it also offered three original, important results. First, Fisher demonstrated the relationship between fitness and reproductive value, which is the weighing of an individual's potential future reproduction by its expected future survival. Not only did this idea link evolutionary genetics with demography, it provided the foundation for an eventual evolutionary theory of aging. Second, he identified negative

frequency-dependent selection, in which the fitness of a genotype decreases as its relative fitness increases, as the mechanism that stabilizes the sex ratio at nearly 1:1. Finally, he articulated what has become one of the most durable explanations for the evolution of recombination and sex (the Fisher–Muller model): recombination permits beneficial mutations to come together in the same genome, thereby greatly speeding the rate of adaptive evolution.

Theory for the stochastic components of the evolutionary process was developed in more detail by Sewall Wright. Whereas Fisher came to evolutionary genetics from a background in mathematics, Wright was trained as a geneticist and always considered himself primarily a physiological geneticist (Provine, 1986). He initially developed statistical methods to understand complicated patterns of inheritance and then applied those methods to more general problems. For example, agriculturalists had long been aware of the benefits and drawbacks associated with inbreeding (as was Darwin), and by 1920 there was a body of literature on how the genetic composition of populations evolved under various systems of nonrandom mating. Wright's initial mathematical work was directed toward a general method for studying nonrandom mating, which resulted in his development of the inbreeding coefficient, *f* or F, and the method of path analysis.

Wright's initial foray into evolutionary genetics was his interpretation of the selection experiment on coat color in hooded rates conducted by his mentor, William Castle (Provine, 1986). Wright's analysis revealed the effectiveness of mass selection on genes of small effect, thereby offering an empirical complement to Fisher's development of selection theory.

Wright developed his own approach to evolutionary genetic theory and published his synthetic work in 1931 (Wright, 1931). Beyond the formal mathematical exposition on evolution in finite populations, two fundamental contributions in this paper stood out. First, he showed that the rate of decay of genetic variation in a finite, random mating population will be 1/2N per generation, drawing on a probabilistic argument foreshadowing Malécot's development of identity by descent (see below). Second, he defined the genetic effective population size, *Ne*, a measure of how many individuals actually leave offspring behind, and illustrated its role in the stochastic distribution of allele frequencies.

J. B. S. Haldane rounds out the great triumvirate of the founders of mathematical population genetics. Haldane published a series of papers in the 1920s, collectively titled 'A mathematical theory of natural and artificial selection,' in which he characterized a wide variety of selective scenarios, culminating in 1932 in his own synthetic book, *The Causes of Evolution*. Haldane is most appreciated for two results. In 1927, he showed that in a sufficiently large population, the probability of fixation of a new beneficial mutation is approximately twice its selection coefficient (Haldane, 1927). Later (Haldane, 1937), he showed that the reduction in fitness when mutation and purifying selection are at equilibrium is approximately equal to twice the genome-wide mutation rate and is approximately independent of the strength of selection.

The Russian geneticist Sergei Chetverikov is not as well-known as Fisher, Wright, and Haldane, but he independently reached similar conclusions with respect to many of the important basic results mentioned above, which he published in 1926. This paper was available only in Russian until Theodosius Dobzhansky published some excerpts in English translation in 1959 and the full translation by Malina Barker appeared in 1961 (Chetverikov, 1961). His influence on evolutionary genetics in this period was exerted primarily through his students and others who were inspired by his original papers. Chetverikov was among the first evolutionary biologists to design his empirical work as an explicit attempt to test mathematical theory. His argument that natural populations harbored extensive genetic variation would inspire a tradition of field-based research that, ultimately, infused what Mayr (1988) called "population thinking" into genetics. By "population thinking," Mayr referred to considering a natural population to be a collection of genetically variable individuals. The triumph of "population thinking" would ultimately change the focus of evolutionary genetics from describing how selection altered genetic variation to how so much genetic variation could be maintained.

Contention and Argument: Which Theory Does Nature Follow?

While a general theory of evolutionary genetics was in place by the late 1930s, the major architects of that theory, Fisher and Wright, did not agree on how it applied to nature. Fisher envisioned nature as the laboratory for weak selection (i.e., very small differences in fitness among genotypes) acting in extremely large, panmictic populations (Provine, 1986; Ewens, 2004; Crow, 2010). Under these conditions, selection could sift all combinations of alleles at different loci; those alleles contributing most to the combinations that produced the highest fitness would increase in frequency. Fisher saw mass selection on the average effect of an allele across all possible genetic backgrounds as an efficient means of ensuring that the best possible genotype would predominate.

Wright thought this view unrealistic on two grounds (Wright, 1980; Provine, 1986). First, he expected natural populations to be sufficiently limited in size that all possible allelic combinations would never occur. Second, Wright thought that synergistic effects of gene combinations – dominance between alleles, epistasis between alleles at different loci – were prevalent and would prove more important than the average effects of an allele. For Wright, these two issues demanded a wholly different mechanism for increase in the frequency of alleles that combine to produce the best available genotypes.

Wright's view, which came to be called his shifting balance theory of evolution, was that a species consisted of numerous populations of limited size that were connected by modest levels of migration and genetic interchange (Wright, 1980; Provine, 1986; Ewens, 2004). The random drift within each population would generate a variety of allelic combinations at different loci on which selection could act. The partial isolation among populations would allow different statistical distributions of these combinations to emerge and be sampled by selection in different populations. Once favorable combinations rose to appreciable frequencies in each population, migration between populations, enhanced by higher

population sizes resulting from increased fitness, would spread the best combinations and thereby ensure that the best possible genotype will predominate in the species. For this mechanism to perform as postulated, there must be a precise combination of population sizes and migration rates (Ewens, 2004). How often nature finds that combination is an empirical matter that remains unresolved.

These dramatically different views of nature led to each man's taking different positions on issues like the evolution of dominance and whether inbreeding and genetic drift should be regarded as separate processes (Crow, 2010). Wright and Fisher fought bitterly over the evolution of dominance precisely because they perceived that a defeat on this topic would falsify one or the other's entire conception of evolution (Provine, 1986). While they agreed that dominance was an evolved property and not a fixed attribute of genes (Wright, 1977, p. 571), they proposed very different theories for that evolution. Fisher posited that dominance at one locus evolved via changes in allele frequency at a second locus, whose alleles modified the expression of a mutant allele at the primary locus when the mutant allele at the primary locus was in heterozygous condition. Wright was critical of this hypothesis. He argued that because heterozygotes for mutant alleles will be rare, selection on the modifier locus will be extremely weak. This will render the process ineffective except in very large populations. Moreover, this mechanism would require that the modifier gene have no pleiotropic effects on other characters or epistatic interactions with other genes. It is obvious that Fisher's postulate follows directly from his view of nature and, of course, that Wright would find this theory wholly inadequate. The plausibility of Fisher's theory for altering dominance within balanced polymorphisms, in which heterozygotes are common, was never in question (Charlesworth, 1979) and, indeed, new discoveries about balanced polymorphisms may vindicate Fisher's view in this context (Billiard and Castric, 2011).

Wright argued that dominance evolved via more complicated interactions among genes in a common metabolic pathway (Wright, 1977). His theory assumes strong interactive effects among genes at different loci as enzymatic reactions regulate the concentrations of substrates in a pathway. In particular, his theory, which has been expanded in theories of metabolic control (Kacser and Burns, 1981; Bagheri, 2006), predicted the negative association between the degree of dominance of a mutant allele and the magnitude of selection against the allele seen in studies of mutants in *Drosophila* (Charlesworth, 1979) and yeast (Phadnis and Fry, 2005).

Evolutionary Genetics Permeates the Study of the Evolutionary Process

The Dominance of Evolutionary Genetics

By the mid-1930s, the study of evolution was becoming a genetic discipline. At one level, many field naturalists devoted themselves to studying the distribution and dynamics of visually conspicuous genetic variation. The school of 'ecological genetics' associated with E. B. Ford (1975) is one example. Ford's field studies had been shaped by his interactions with Fisher. Together, they applied evolutionary genetic theory to data on the spatial distribution and temporal dynamics of Mendelian pattern variations in moths with an eye toward demonstrating that natural selection was the predominant influence on the distribution and dynamics of gene frequencies. Ford's and Fisher's students extended this approach to studying polymorphisms in snails, butterflies, and even plumage patterns in birds.

On the other side of the Atlantic, Dobzhansky and his collaborators, including, for a period, Wright, were engaged in the same kind of work. Dobzhansky had been trained in Russia in the tradition championed by Chetverikov and brought that sensibility to the United States, where he began studying the genetics of *Drosophila*. He was struck by what he saw as Wright's more realistic view of nature and focused his work on understanding how natural selection and genetic drift combined to drive the distribution and dynamics of genetic variation. Dobzhansky and his colleagues studied a variety of genetically controlled traits, primarily in *Drosophila pseudoobscura* but also in other organisms (including the desert plant *Linanthus parryae*), combining observational data from natural populations and laboratory experiments with theory and models.

Beyond the explicit study of genetics, practitioners studying other aspects of biological evolution drew inspiration from ideas in evolutionary genetics or interpreted their data in the language and concepts of evolutionary genetics. An example of the former is Simpson's (1944) depiction of the adaptive landscape in paleontology, which he borrowed from Wright's graphical depictions of genetic fitness surfaces within his shifting balance theory (described in Provine, 1986). An example of the latter is Huxley's (1942, Chapter 5) extensive discussion of clinal variation in continuous characters.

Every phase of the evolutionary process was being described and studied in genetic terms, from microevolution to systematics. However, the emergence of evolutionary genetics as a discipline in itself was pivotal for advances in two subjects that were crucial, but poorly understood, components of Darwin's theory of evolution: the origin and maintenance of genetic variation and the genetic changes that drive the formation of new species.

The Randomness of Mutation

The fundamental principle of Darwinism is that selection acts on preexisting random variation among individuals, i.e., selection itself does not influence the creation of new variation. By the time the unification of Darwinian selection with Mendelian genetics was complete – the 'Modern Synthesis' (Smocovitis, 1996) – almost all biologists working with higher organisms accepted that mutation was random. That was not true of biologists working with microbes, many of whom continued to entertain the possibility that the external environment acted to create its own favored variants, i.e., 'directed' mutations. That idea was put to the test first by Luria and Delbruck (1943) and later by Lederberg and Lederberg (1952) and others in studies of mutations in *Escherichia coli* that promoted resistance to bacteriophage or antibiotics. Those experiments showed that mutations promoting

resistance were already present in the bacterial populations prior to the exposure to those agents of selection. These were crucial vindications of Darwin's basic precept.

The more provocative challenge to the Darwinian paradigm has been the recent discovery that there are conventional mechanisms through which apparently directed mutations can occur, at least in principle. These include transcriptional mutations (Whitehead *et al.*, 2008) in which a transcribed variant RNA sequence may be integrated into the genome by a process of reverse transcription. This process offers the possibility of bringing epigenetic processes into the mainstream of evolutionary thought.

Genetics and the Formation of New Species

Evolutionary genetics transformed the study of speciation. In the decades since Darwin, a major issue in the study of speciation was whether differences in traits between pairs of closely related species had been molded by natural selection's favoring different features in different species (Provine, 1986). Because many of the characters used to reliably distinguish species seemed of no ecological value, many biologists argued that speciation occurred solely as a consequence of two populations being isolated from each other long enough for them to lose the ability to interbreed, with little to no role for natural selection.

This view of speciation was a direct challenge to Darwin's view that reproductive isolation was itself a product of divergent natural selection in different populations. Darwin had argued that there would be a continuum between incipient and complete isolation; where a pair of populations would be found on that continuum would depend on the magnitude of the divergent selection pressures driving them apart and the time over which those pressures will have been operating. Darwin's view began to get its empirical support when Lancefield (1929, described in Provine, 1986) showed that reproductive isolation between stocks of *D. pseudoobscura* was indeed a continuum of effects based on the distribution of genes affecting hybrid sterility. This work inspired Theodosius Dobzhansky to initiate his far-reaching studies of the genetics of hybrid sterility (Dobzhansky, 1951). Dobzhansky's work, in turn, set an entire research agenda for others who followed him in examining the genetics of hybrid fitness and the genetic nature of species differences.

Four patterns emerged from these efforts. First, as Lancefield's work had suggested, there were gradations of reproductive isolation between pairs of stocks, populations, or closely related species. Second, the reduction in hybrid fitness was, quite often, more pronounced in one gender than the other, usually the gender with heterogametic gender determination, a result predicted by Haldane (1922). Third, in many cases, there were asymmetries in hybrid fitness in which crosses between populations in one direction (i.e., from which population the female was drawn) produced normal progeny whereas crosses in the other direction produced dramatically reduced hybrid fitness. Subsequent research has shown that this is caused by incompatibilities between genes inherited from only one parent, for example, mitochondrial genes, and genes inherited from both parents (Turelli and Moyle, 2007). Fourth, reduced hybrid fitness could often be shown to be caused by the effects of mixing two distinct sets of epistatically acting allelic combinations (which are now called Bateson–Dobzhansky–Müller incompatibilities; Coyne and Orr, 2004).

Studies on the genetics of speciation led to other discoveries. The role of chromosomal changes in the speciation process, including chromosomal mutations and the formation of polyploid species, became a major focus in the study of plants and some animals (Stebbins, 1950; White, 1954). The formation of new species via hybridization and introgression was beginning to be recognized (Anderson, 1949). The variety of genetic patterns of species differences inspired new ideas about how speciation could occur and how isolating mechanisms could be classified (Mayr, 1963).

By the early 1960s, the study of speciation had been transformed almost beyond recognition from what it had been 40 years previously. The transformation was so complete that, in 1970, Mayr would write:

> The essence of speciation, we now realize, is the production of two well-integrated gene complexes from a single parental one. All early attempts to explain the genetics of speciation missed this essential point, since they were concerned entirely with the problem of the origin of the difference. To be sure, the differences between species are due to mutation and selection, but demonstrating that does not explain how species split. Mayr (1970, p. 296)

The Next Generation of Evolutionary Genetics

The Expansion of Theory

The second wave of evolutionary genetic theory expanded into three broad topics. The first of these was a reconsideration of the stochastic population genetic theory initiated by Fisher and Wright. This phase of theoretical development began, somewhat retroactively, in 1948 with the publication of Gustave Malécot's book *Les Mathématiques de l'Hérédité*. Malécot's contributions to theoretical population genetics were slow to be appreciated by the wider community because he published in French and his book was not translated into English until 1969 (Epperson, 1999). Malécot put much of the stochastic theory initially developed by Fisher and Wright onto rigorous probabilistic footing. He developed the concept of identity by descent of gene copies (as did Cotterman, independently, in 1940). This concept clarified the relationship between inbreeding and random genetic drift, over which Fisher and Wright were bitterly divided. Much of the theory for the effects of migration, gene flow, and population subdivision is due to Malécot. His work anticipated coalescent theory (Slatkin and Veuille, 2002) and he was the first to develop the so-called infinite alleles model of mutation (Epperson, 1999).

The expansion of stochastic population genetic theory accelerated through the work of Motoo Kimura, whose broad contributions to evolutionary genetics are second only to Fisher's (see Takahata, 1994). In 1955, he published the first complete mathematical solution to the process of random genetic drift, and in subsequent papers he extended that treatment to drift with mutation, various forms of migration, and selection (Kimura, 1955, 1962). In essence, Kimura finished what Fisher had begun 40 years earlier: the Wright–Fisher model was now a general model of evolution.

Kimura also led the exploration of the second broad topic in this second wave, the evolutionary dynamics of multilocus systems. In 1956, he produced what might be considered the first investigation of the evolution of the genome by finding the conditions under which linkage between selected loci would evolve (Kimura, 1956). Kimura (Kimura and Crow, 1964) also showed that if epistasis and linkage are weak, that a population under selection attains a state of "quasi-linkage equilibrium," in which case the additive genetic variance is the best predictor of evolutionary change under selection. This result was far-reaching because it validated the standard quantitative genetics practice of using only the additive genetic variance to predict the response to selection (Crow, 1995). And in 1960, Lewontin and Kojima introduced a formal analysis of polymorphism in two-locus systems and initiated an investigation of the role of epistasis in maintaining genetic variation (Lewontin and Kojima, 1960).

An even more fundamental discovery about multilocus systems emerged from the work of Alan Robertson and William Hill (Robertson, 1960, 1967). They described how selection at one locus would reduce the efficiency of selection at all other linked loci (the so-called Hill–Robertson effect). That finding ranks among the most profound in evolutionary genetics, with ramifications for the evolution of sex and recombination (Felsenstein, 1974; Keightley and Otto, 2006), the maintenance of genetic variation, and the relationship of polymorphism within species to the divergence among species (Comeron *et al.*, 2008).

The third great stream of postwar evolutionary genetics involved what has come to be called 'levels of selection.' This set of ideas begins with the premise that what is favored by selection at one hierarchical level (e.g., the gene) may be opposed by selection at some other level (e.g., the individual organism). This question came into sharp focus initially in considering the evolution of sociality, explaining the evolution of altruistic behavior in which individuals sacrifice opportunities for reproduction in order to increase the fitness of other individuals or the overall fitness of the group, family, or colony within which they live.

While the arguments on this topic can be traced to Darwin, Fisher, and Wright (see Williams, 1966), it was the great English biologist W. D. Hamilton who translated them into mathematical theory. Hamilton demonstrated that the marginal fitness of an allele is a function not only of its effects in the focal individual carrying that allele but of its effects in all other individuals carrying that same allele with which the focal individual interacts – i.e., the individual's relatives. Hamilton referred to this phenomenon as inclusive fitness and showed that an altruistic trait can evolve if the indirect fitness benefit to the altruistic individual's relatives outweighs the direct fitness cost borne by the altruistic individual (Hamilton, 1963, 1964). The more general conclusion was that selection operating at different hierarchical levels can lead to counterintuitive evolutionary outcomes. This line of reasoning has proven hugely influential in evolutionary biology, not only with respect to understanding the evolution of sociality but also to more overtly genetic problems concerning the evolution of 'selfish' genetic elements.

Empirical Discoveries Challenge Theory: New Problems or Deeper Questions?

In the early 1960s, evolutionary genetics felt the impact of the revolution in molecular biology. Previously, the genetic characters chosen for empirical study were a highly nonrandom subset, usually chosen because they produced visually conspicuous phenotypes. This made the distribution and dynamics of these characters easily studied. But convenience carried an epistemological cost because it was impossible to know whether the levels of genetic variation for such characters, or the magnitude of selection on their associated genotypes, were representative of the genome as a whole. As biologists became adept at determining the sequence of amino acids in proteins and identifying allozymic forms of proteins, it became possible to study a largely unbiased sample of genetic variation.

The results ignited an intellectual revolution. In 1962, Zuckerkandl and Pauling observed that the rate of amino acid substitutions in hemoglobin, inferred from pairwise species differences and the fossil record of their separation times, appeared to be relatively constant across orders of mammals (Zuckerkandl, Pauling, 1962). Subsequent studies with other proteins and taxa reinforced that finding, leading to the suggestion that protein evolution proceeded according to a "molecular clock" (Margoliash, 1963; Zuckerkandl and Pauling, 1965). At about the same time, the first large-scale surveys of allozyme variation in proteins within species revealed unexpectedly high levels of standing variation in *Drosophila melanogaster* (Lewontin and Hubby, 1966) and *Homo sapiens* (Hopkinson and Harris, 1966).

Taken together, these observations suggested something was terribly wrong with evolutionary genetic theory. Specifically, in the context of existing theory, they implied that natural populations would be carrying impossible levels of genetic load. The rates of allelic substitution through time, inferred from the amino acid sequence data, appeared far higher than could be supported by the most generous estimates of sustainable genetic loads. Similarly, the observed levels of polymorphism at many loci simultaneously would impose a genetic load in every generation that was so large that no population could bear it.

Kimura (Kimura, 1968) was the first to explicitly connect the dots between the seemingly intolerably high rate of protein evolution and the seemingly intolerably high heterozygosity. Both observations could be explained by a single postulate, that the majority of molecular variants were selectively neutral so that their evolutionary dynamics would be governed only by mutation and drift. This was the so-called neutral theory of molecular evolution. This theory led to two important predictions. First, the substitution rate at any locus should be equal to the neutral mutation rate at that locus. Second, the level of standing genetic variation should be proportional to the product of the effective population size, a measure of how many individuals contribute genes to the next generation, and mutation rate.

Almost 50 years on, these predictions remain at the epicenter of evolutionary biology because they describe the role of random chance (mutation and drift) in creating evolutionary patterns. Distinguishing the patterns created by

natural selection from those expected from random change remains the core challenge of evolutionary biology (Felsenstein, 1985; Gillespie, 2000; Lynch, 2007).

The Syntheses of Evolutionary Genetics

Several synthetic treatments of evolutionary genetics appeared in the 30 years after Fisher's (1930) monograph. However, nearly all of them were empirically oriented. Some of these books were focused on evolution in particular groups of organisms (e.g., Anderson, 1949; Stebbins, 1950) while others were focused on the evolutionary process per se (e.g., Dobzhansky, 1951 and earlier editions; White, 1954).

There were few synthetic treatments of theory in this period. Haldane's book (Haldane, 1932) appeared in 1932, shortly after Fisher's, and Wright did not begin to publish his own synthesis until 1968 (Wright, 1968). Malecot's monograph appeared in 1948 but was not translated into English until 1969. The only comprehensive treatment of evolutionary genetic theory in this period was C. C. Li's (1955) textbook, *Population Genetics*, which was published originally in 1948 in Chinese. Li's book covered topics from the effects of inbreeding and assortative mating to the stochastic distribution of allele frequencies under the joint action of selection and drift, as it was known through about 1950.

Three books that appeared between 1960 and 1970 summarized the achievements of evolutionary genetics and laid the foundation for the next generation of theory. The mathematically oriented monographs by Moran (1962) and Ewens (1969) followed in the tradition of Malécot (1948), establishing a rigorous mathematical framework for traditional formulations and pointing toward new approaches to longstanding problems. Crow and Kimura's (1970) textbook, *An Introduction to Population Genetics Theory*, offered a synthetic presentation of the full spectrum of evolutionary genetic theory, from traditional topics like the effects of selection on variation at a single locus to the newly developed neutral theory of molecular evolution. A full third of the pages are devoted to random processes and the stochastic distribution of genetic variation, testimony to Fisher's, Wright's, and Malecot's views on evolution as a stochastic process and evidence of the mathematical sophistication that now characterized the discipline. A little over a century after Darwin articulated his hypotheses about the evolutionary process in words, evolutionary genetics, in theory and data, had validated them.

See also: Adaptive Landscapes. Shifting Balance Theory, Sewall Wright and. Synthetic Theory of Evolution, History of

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Evolutionary Medicine I. An Overview and Applications to Cancer

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Glossary

Biotic interactions Environmental interactions with other organisms.
Drivers Mutations that are selectively advantageous for the tumor. These generally promote tumor growth (see also Passengers).
Genetic hitchhiking The process by which neutral or deleterious variants that are linked to an advantageous variant increase in frequency in a population.
Genomic conflict Conflict that arises among different parts of the genome of an individual because their evolutionary interests do not coincide.
Genomic imprinting Differences in DNA methylation patterns distinguishing maternally and paternally inherited alleles.
Linkage disequilibrium The correlation (association) of allele frequencies at different sites on a chromosome.
Metastasis The process by which cells from the primary tumor detach and form new tumors (metastases).
Microbiome The collection of microorganisms that live inside or on the surface of the body of an organism.
Neoplasm An abnormal growth of tissue.
Oncogene A gene, for which over-expression can lead to tumor formation or continued progression.
Passengers Mutations that are either neutral or deleterious to the tumor, but accumulate via hitchhiking with selectively advantageous mutations (drivers).
Peto's paradox The observation that rates of cancer and body size are not well correlated, despite cancer being connected to cell division.

Overview of Evolutionary Medicine

Four general principles underlie evolution.

1. Evolution is historical. Evolution has shaped features of organisms over historical time. All organisms (including humans) share an evolutionary history due to shared ancestry. Moreover, this shared history is nested hierarchically: a given organism shares more recent ancestry with some organisms than it does with others.
2. Evolution requires heredity, the transmission of information from ancestors to descendants.
3. Evolution takes place in populations, and is necessitated on the presence of variation within populations. Evolution involves changes in frequencies of variants. Natural selection, genetic drift, and other processes of evolution act on variation within populations.
4. Evolution occurs in an ecological context, involving interactions of the organism and the environment. This environment includes both abiotic (physical characteristics) and biotic (other organisms) components.

These basic evolutionary principles have implications for human health and how we treat disease. It can also inform treatment in a number of cases.

The evolutionary medicine articles in this encyclopedia each tackle at least one of these evolutionary principles. Kelly's article (see Section See also) focuses on the first principle. It explores how we employ the concept of shared (and not shared) evolutionary history between humans and nonhuman animals in biomedical research. The interaction of history, genetics, and environment is at the center of Low and Gluckman's article (see Section See also). Scarpino's article (see Section See also) involves the ecological context as well as the processes underlying evolution of populations of disease-causing microbes. Cancer, which is treated in this article, encompasses all four principles.

Why We Get Sick

Evolutionary approaches have long been applied to medically related topics. Hermann Joseph Muller, who made substantial contributions to evolutionary genetics (Carlson, 1981; Johnson, 2002), won the 1946 Nobel Prize in physiology and medicine for his pioneering studies of mutagenesis in flies. These investigations led him to consider the implications of mutations to human health and human evolution (Muller, 1950). Mutation rates and their ramifications for human health continue to interest evolutionary biologists (e.g., Lynch, 2010). The basic principles for an evolutionary theory of aging were also laid out in the 1950s and 1960s (Williams, 1957; Hamilton, 1966). In the 1970s, Cairns (1975) and Nowell (1976) put forth seminal evolutionary theories of cancer progression. Yet, prior to the 1990s, these evolutionary treatments of human health and disease were done piecemeal. A general framework was lacking.

In the 1990s, a collaboration between a psychiatrist (Randolph Neese) and an evolutionary biologist (George Williams) laid out a general framework for evolutionary treatment of human health and disease in a review article (Nesse and Williams, 1991) and then a popular book, *Why We Get Sick* (Nesse and Williams, 1996). This framework has grown into the interdisciplinary field of evolutionary (or Darwinian) medicine (e.g., Stearns *et al.*, 2010).

In their popular book *Why We Get Sick*, Nesse and Williams (1996, pp. 8–11) present their ideas about the major reasons for illness. Infection is one of the reasons, but so are side effects of our defense systems against infection. Another cause is the

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Table 1 Possible causes of human disease

Category	*Example(s)*
Infection	Malaria (Hedrick, 2011) HIV-AIDS (Rambaut *et al.*, 2004)
Defense side effects (physiological)	Fever (Nesse and Williams, 1996, pp. 26–29) Low levels of iron (Nesse and Williams, 1996, pp. 29–31)
Defense side effects (genetic)	Sickle cell anemia as a genetic defense against malaria (Hedrick, 2011)
Mismatch	Type II diabetes prevalent when humans have a high-sugar diet (Glickman and Hanson, 2006) Cancers of certain reproductive tissues are higher in modern western societies in part because women in these societies undergo more menstrual cycles than they did throughout much of evolutionary history (Aktipis and Nesse, 2013)
Historical legacies	Inverted retinas (Nesse and Williams, 1996, pp. 127–129)
Conflicting selection pressures	Selection will generally favor alleles that increase survival when young, even if the alleles decrease survival when the individual is older (Nesse and Williams, 1996, pp. 112–118)
Genomic conflict	Prader–Willi syndrome (see main text) (Haig and Wharton, 2003)

Note: Some of these examples (notably fever and low levels of iron) are hypotheses to be tested.

mismatch between the novel environments we now inhabit and the environments in which our species evolved (see Section See also). Related to mismatch are historical legacies. Conflicting selection pressures, conflicts between different genes in our genome (genomic conflict), and design compromises also contribute to human illness. Deleterious mutations, as Muller (1950) had noted, are still a factor (see **Table 1** for a list of examples of these categories). Note that the items presented in **Table 1** are not mutually exclusive, and that some diseases may be attributed to more than one cause. For instance, sickle cell anemia is prevalent in people of African descent because heterozygotes for the sickle form of hemoglobin B have protection against malaria (Hedrick, 2011). This is an example of both genetic defense for infection and conflicting selection pressures. Note also that each of these reasons for illness involves at least one and usually more of the principles of evolution above.

Biotic Interactions and Human Health

Research in each of these categories of causes for disease that Nesse and Williams (1996) identified has continued over the last two decades. Many of these advances have focused on how our interactions with the biotic environment. Unlike features of the physical environment, other species are also subject to evolutionary pressures; thus, coevolution of humans and other species becomes a possibility. Coevolution can follow many paths, including the evolution of mutualism, wherein both the human and nonhuman populations evolve traits that benefit the other. Coevolution can also result in host–parasite arms races wherein parasites evolve traits that help them better exploit humans and humans evolve counter-measures to defend against the parasites. Understanding infectious disease and humans' responses to it in this case requires an understanding of the evolutionary history of humans and the disease-causing organism, as well as the populational processes and heredity of both species.

Coevolutionary arms races between humans and disease-causing organisms can involve tradeoffs wherein the adaptations of one species are detrimental in some way. In a fascinating study, Kaiser *et al.* (2007) showed that adaptations in humans to defend against certain viruses could have increased human vulnerability to HIV. This study used phylogenetic methods to reconstruct ancestral viruses that humans were likely exposed to and found that human immune protein TRIM5α can destroy those viruses, but at the expense of humans' being less able to defend against HIV. It is not just humans that are subject to tradeoffs; so are our natural enemies. For instance, evidence is accumulating supporting the hypothesis that the reason avian influenza is extremely difficult to be transmitted from human to human because the virus has evolved adaptations for living in domestic chickens (Long *et al.*, 2014).

Perhaps the most unexpected development in evolutionary medicine from the last decade is the increased appreciation for the role the microbiome plays in human health. The microorganisms of the microbiome (microbiota) are not just passive by-standers residing in our bodies. Instead, they play myriad roles in our physiology and, amazingly, even our psychology (see reviews in Finlay, 2013; Heintz and Mair, 2014). These studies are addressed in great detail in Knight (see Section See also). Comparative studies of the microbiota in nonhuman primates could reveal insights into the human microbiome. An interesting recent study demonstrated that social interactions, in particular grooming networks, strongly influence microbiota composition in baboons (Tung *et al.*, 2015). Note also that this work on baboon microbiota could only be done given the rich evolutionary demography studies by Jeanne and Stuart Altmann and their colleagues.

Once thought to be a vestigial organ of no current evolutionary value, the role of the appendix is being revised (Johnson *et al.*, 2012). Bollinger *et al.* (2007) contend that the appendix may be a storehouse for beneficial microbiota. Taking a phylogenetic approach to the evolution of the appendix in a variety of mammals, they find evidence supporting this role for the human appendix. Inflammation of the appendix, however, may be due to possible mismatch between current human diets and microbiota and previous environments in which we evolved (reviewed in Johnson *et al.*, 2012).

Genomic Conflict

Another recent major development in evolutionary medicine is the accumulating evidence supporting genomic conflict as a

major contributor to human disease (Haig, 2004; Crespi, 2010, 2011). Genomic conflict occurs when the evolutionary 'interests' of different parts of the genome do not perfectly coincide (Burt and Trivers, 2006; Rice, 2013). For instance, because mitochondria are strictly maternally inherited, a mitochondrial allele that benefitted daughters at the expense of sons would be evolutionarily favored. Accumulation of such male-detrimental alleles would be contrary to the evolutionary interests of autosomal genes, and thus selection would favor autosomal genes that counteract the mitochondrial alleles. Like biotic interactions, genomic conflict can lead to persistent and intense coevolutionary arms races (Crespi, 2010; Rice, 2013).

One area of genomic conflict that seems to be particularly intense is in maternal–fetal interactions and these conflicts often involve different interests regarding the allocation of resources (Haig, 2004; Burt and Trivers, 2006; Crespi, 2010, 2011; Boddy *et al.*, 2015). Evolutionary biologists have long been aware of the divergent evolutionary interests of parents and offspring (Trivers, 1972). Consider the optimal level of resources a mother should provide for her fetus (or infant). From the perspective of the fetus' interests the fetus should receive as much from the mother as possible without seriously endangering her health. Whereas, the mother's evolutionary interest will depend on her health, but also her prospects of having and supplying resources to other children. Because of this, optimal resource allocation is different for the mother than for the child (Trivers, 1972; Burt and Trivers, 2006).

Given at least some departure from strict monogamy, the evolutionary interests of mothers and fathers would diverge (Haig, 2004; Burt and Trivers, 2006). In resource allocation disputes, the father's evolutionary interests would favor more resources be given to the particular fetus than would be optimal according to the mother's evolutionary interests because he may not be the father of the mother's other current and future children. Given this conflict, the evolutionary interests of the paternal alleles of the fetus (the ones inherited from the father) would favor a higher resource allocation level than the interests of the maternal alleles of the fetus. This complex conflict has likely led to patterns of genomic imprinting wherein paternal and maternal alleles for particular genes (many of which are involved in resource allocation) are differentially methylated and differentially expressed (Haig, 2004; Burt and Trivers, 2006).

In general, the arms race associated with conflict between paternal and maternal alleles reaches resolution in an uneasy truce, but mutations can reveal the evidence of the battlefield (Haig, 2004; Burt and Trivers, 2006). Loss or reduced expression of maternal alleles results in paternal alleles exerting control, and this should lead to the fetus taking far more than the optimal level of resources from the mother. In contrast, reduced expression of paternal alleles should lead to the fetus taking far less than the optimal level of resources too little. Prader–Willi syndrome, caused by deletion of part of paternal chromosome 15, is an example of the latter (Haig and Wharton, 2003). Although the excess food-seeking behavior of postweaning Prader–Willi individuals is better publicized than other symptoms, fetuses and infants with Prader–Willi are generally lethargic and actually have reduced food intake (Haig and Wharton, 2003).

Preeclampsia, or increased blood pressure in pregnant or postpartum women, may also be due to conflicts among the mother, the father, and the fetus and may be a function of selection within the human lineage (Crespi, 2010). Although the evidence supporting an evolutionary explanation for preeclampsia is not fully established, some evidence does support this hypothesis. This evidence includes the rarity of the disorder in nonhuman primates, the increased prevalence of the disorder in first pregnancies, the association between preeclampsia and a change in sexual partners, and the positive association between increased gestational blood pressure and maternal blood flow into placental tissues (Crespi, 2010).

Related to maternal–fetal conflicts is the phenomenon of fetal microchimerism, wherein women often retain some cells from their offspring many years or even decades after their pregnancy. The overall effect of such fetal microchimerism is not quite clear, as it seems to have both apparent advantages and detriments to the woman (Boddy *et al.*, 2015). Evolutionary explanations based on conflict and those based on cooperation are being applied to address this unexpected phenomenon (Boddy *et al.*, 2015).

Genomics and Evolutionary Medicine

At the time of the publication of *Why We Get Sick*, not a single eukaryotic genome had been sequenced! As of 2014, genomes from tens of thousands of human individuals have been sequenced (Field and Davies, 2015). The accumulation of full genome sequences from many humans from multiple populations, Neanderthals and other archaic humans, and nonhuman primates is a dramatical change. Insights from this genomics revolution to evolutionary medicine are still being processed, but some interesting data and ideas have already come of it, with the promise of much more.

Due to advances in genomics, researchers can more efficiently identify associations between genes and diseases. In some cases, these advances can also tie the genetic associations with disease to our evolutionary history. Population genomics of human populations combined with comparative genomics of nonhuman primates, as well as Neanderthals and other archaic humans, enables the detection of selective forces that have operated (Johnson, 2007; Crespi, 2011).

Bernard Crespi (2010, 2011) raised the fascinating proposition that the genetic changes that 'made us human' (contributed to human-specific brain and cognitive traits) also made us sick. As suggestive evidence, he notes that genes showing signs of positive selection since humans diverged from Neanderthals are significantly more likely to be associated with a host of neurological diseases such as schizophrenia, mood disorders, and autism.

Crespi (2010) also noted that cognitive and emotional traits, reproductive traits, and traits related to the increased life span in humans are overly represented in human disease. Related to these three types of phenotypes, Crespi contends that certain tissues appear particularly vulnerable to human disease. These include brain as well as reproductive tissues (breast, ovary, placenta, testis, and prostrate). These tissues are likely to be affected by genomic conflict (see above) as well as be influenced by genes that have been under strong selective

pressures. The evolutionary changes at such genes may have strong side effects (Crespi, 2010). These ideas, while intriguing, have generally not been confirmed, but are useful as hypotheses to be tested.

Related to Crespi's hypotheses, Moalic *et al.* (2010) argue that more knowledge of the genes that show signatures of positive selection in the human lineage could be applied to drug discovery, especially for neurological diseases. The study of these genes can reveal new processes about human cognition and how it can go awry, and this knowledge can then be used to develop novel drugs.

Evolutionary Medicine and How Humans Are Evolving

The answer to the question 'Are humans still evolving?' is yes, definitively. Humans vary in traits, those traits are heritable, and are linked to differential survival and reproduction. A more interesting question is 'how are humans evolving?'

Evolutionary medicine can address this question by examining how traits affect viability and fecundity within human populations. For instance, the Framingham Heart Study has been collecting reproductive, physical, and physiological data on people (mainly European-Americans from eastern Massachusetts) since 1948. Byars *et al.* (2010) applied standard evolutionary quantitative genetics theory to the Framingham data to project changes in the population over the next 10 generations (approximately 250–300*x* years). Fertility is the major selective factor, and the fertility period is expected to continue increasing due to later menopause. Assuming that the patterns of selection and the environments remain the same, women in that population are expected to be somewhat shorter, heavier, with lower cholesterol, and blood pressures levels.

Cancer as an Evolutionary Disease

In a prescient, and far-reaching article, Peter Nowell (1976) articulated a view of cancer as an evolutionary process with each tumor usually having a unicellular origin. Nowell also envisioned bouts of selection being associated with tumor progression. In the decades since, the connections between cancer and evolutionary biology have grown substantially. Several recent reviews highlight the increasing awareness that evolutionary biology can inform the study of cancer biology and can even be of therapeutic relevance (Merlo *et al.*, 2006; Podlaha *et al.*, 2012; Pepper *et al.*, 2009; Attolini and Michor, 2009; Gerlinger and Swanton, 2010; Sprouffske *et al.*, 2012; Aktipis *et al.*, 2015). Numerous approaches from evolutionary biology are used, including phylogenetic analysis (Campbell *et al.*, 2008; Aktipis *et al.*, 2015), experimental evolution with microbes (Sprouffske *et al.*, 2012), evolutionary epidemiology (Attolini and Michor, 2009), and mathematical modeling (Attolini and Michor, 2009). Moreover, cancer inherently involves consideration of different levels of selection (e.g., cells and tissues vs. the individual organism) (Pepper *et al.*, 2007; Aktipis *et al.*, 2015), a perennial topic in evolutionary biology.

Although numerous forms of cancer exist, each with their own particular characteristics, different cancers share several commonalities (Podlaha *et al.*, 2012; Hanahan and Weinberg, 2011). Cancer tissues sustain signals to proliferate, while evading growth suppressors and resisting cell death. They bypass limits normally imposed on cell division, and thus achieving replicative immortality. In addition, they obtain a blood supply. Finally, primary tumors often invade and metastasize new regions of the body. See **Figure 1** for an overview of cancer progression.

These 'hallmarks of cancer' (Hanahan and Weinberg, 2011) involve evolutionary change during cancer's progression as well as defenses that the body has evolved for countermeasures. One possible example is the finding of certain cases wherein too much expression of some oncogene signals can lead not to progression of cancer, but to cell senescence (reviewed in Hanahan and Weinberg, 2011). Could this be the result of a mechanism that evolved to guard against cancer?

Although cancer progression is typically a multistep process involving the evolution of several genetic changes, knocking out the expression of a single oncogene often halts or retards the tumor. Because it appears tumor is dependent on the oncogene much like an addict, this phenomenon has been called 'oncogene addiction' (Weinstein and Joe, 2008). Oncogene addiction and redundancies in the pathways underlying the different hallmarks of cancer (Hanahan and Weinberg, 2011) suggest the relationship between genotype and phenotype (and fitness) in cancer progression is complicated, and that sophisticated evolutionary genetics that is becoming more adept to deal with such complexities (e.g., Weinreich *et al.*, 2013) can be useful. There are also implications for therapy as suggested by Weinstein and Joe (2008).

Why Cancer?

Biologists and medical researchers have learned much about the progression of cancer within an individual organism by viewing it an evolutionary process. Why we have cancer in the first place is also an evolutionary question.

Cancer and its suppression are outcomes of selection at different levels (Aktipis *et al.*, 2015). Evolution via natural selection will occur provided (1) variation exists among entities that affect their survival and/or reproduction and (2) offspring inherit the traits of their parents. The entities need not even be biological as computer programs can evolve via these principles (Lenski *et al.*, 2003). Thus, selection can occur on cell lineages: those replicate more and ones that do not respond to cues to stop replicating would have a selective advantage, and would increase in frequency. This selection within the organism is at odds with the evolutionary interests of the multicellular organism, which would be under selective pressure to suppress potential cancers. Examining the outcomes when different forms of selection are acting at different levels is a mainstay of evolutionary biology (Wade, 1978; Michod, 1999; Novak, 2006) that can be applied to understanding both why cancer occurs and why it is rare (Aktipis *et al.*, 2015).

Cancer is a consequence of breakdowns in the cooperation needed to sustain multicellularity (Aktipis *et al.*, 2015). Multicellular organisms have evolved multiple and independent times in the evolution of life, and cancer and/or cancer-like phenomena are found in each of the independent lineages that evolved multicellularity (Aktipis *et al.*, 2015). Multicellular organisms require cooperation among the

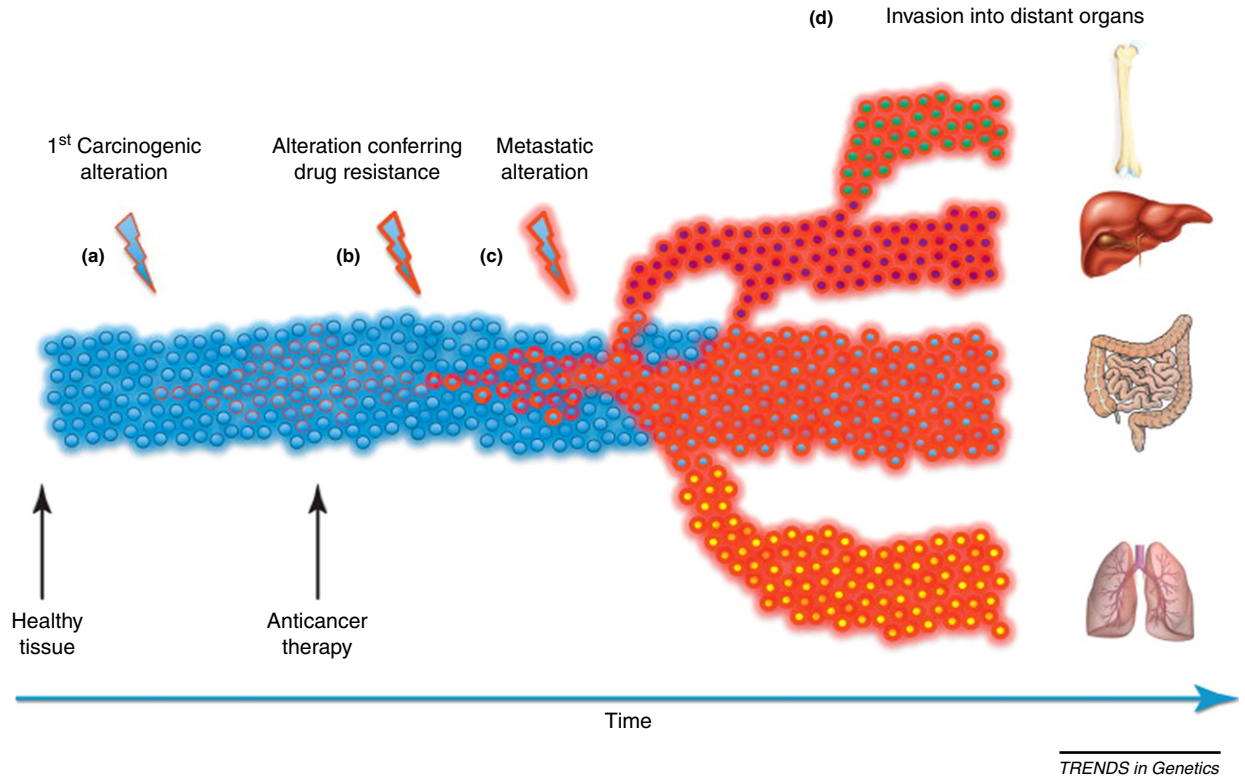

Figure 1 Key steps in the progression of cancer. Adapted from Podlaha, D., Riester, M., De, S., Michor, F., 2012. Evolution of the cancer genome. Trends in Genetics 26, 155–163.

different cell lineages in each individual organism (Michod, 1999). In a multicellular organism, cell division patterns of each particular cell lineage need to be tightly controlled. Multicellular organisms also require particular cells to undergo programmed cell death on schedule. Proper functioning also requires appropriate allocation of resources within the organism (Aktipis *et al.*, 2015).

The hallmarks of cancer (Hanahan and Weinberg, 2011) reflect different types of breakdowns – 'cheating' – of the cooperation (Aktipis *et al.*, 2015). Cancer progression is marked by continued proliferation and failure to respond to signals to undergo programmed cell death. This is an example of demographic cheating, in the terminology of Aktipis *et al.* (2015). Another hallmark of cancer is the diversion of blood supply by the growing tumor for its own use – a form of economic cheating (Aktipis *et al.*, 2015). Examining these forms of cheating as well as how they are countered by the organism should lead to better understanding of cancer.

Cancer Susceptibility

Lifetime risk of cancer in developed countries is about 40% in men and 30% in women (Varki and Varki, 2015). There are two main questions about cancer susceptibility and evolutionary genetics: (1) Why are humans more or less susceptible than other animals? and (2) Why are some genotypes within humans more susceptible than others?

As noted above, cancer is a disease of cell lineages failing to cooperate with the rest of the organism. With each cell replication, the possibility of progression to cancer exists. Yet, the relationship between body size and cancer incidence is weak at best; for example, humans and mice have similar lifetime cancer risks, despite the gigantic differences in size and number of cell divisions (Caulin and Maley, 2011; Aktipis *et al.*, 2015) This puzzling pattern is known as Peto's paradox. A likely explanation is that larger organisms have evolved various mechanisms to suppress cancer (Caulin and Maley, 2011; Aktipis *et al.*, 2015). A prediction of this explanation is that animals much larger than humans (e.g., whales and elephants) should have evolved even better cancer suppression systems.

Despite the general lack of correlation between size and cancer risk among species, variation in size within species is associated with cancer risk. In humans, a 10-cm increase in height results in a 10–15% increased cancer risk, and similar patterns exist in other mammals (Aktipis and Nesse, 2013). The association of cancer risk with size within species suggests that the cancer suppression mechanisms are not genetically linked to size within species.

Mammals appear to have higher overall cancer rates compared with birds and reptiles (Aktipis *et al.*, 2015). Within mammals, cancer rates also vary: at one extreme, mole rats appear to have extraordinarily low cancer rates (Seluanov *et al.*, 2009; Aktipis *et al.*, 2015); at the other, most Tasmanian devils (which are carnivorous marsupials) have an infectious cancer that is usually transmitted via biting (Grueber *et al.*, 2015).

Unfortunately, we know little about the cancer in nonhuman primates. Although the incidence of carcinomas (malignancies arising from epithelial cells) appears to be very low in captive apes, insufficient data precludes a conclusive finding (Varki and Varki, 2015). There are hints of genetic differences that may underlie cancer susceptibility differences between humans and other primates, but nothing definitive has been established. One such putative reason for the likely increased cancer susceptibility in humans is in the differences between humans and other primates in the siglecs, the sialic-acid-binding immunoglobulin-like lectins that have immunological and other functions (Varki and Varki, 2015). Clearly, much more study in nonhuman primates is needed, especially on the quantification of cancer and its genetic basis.

Numerous susceptibility genes have been located. Some of these show signatures of selection in the human lineage. For instance, Ding *et al.* (2008) show an association for both the signature of selection and increased cancer susceptibility in fragile histidine triad gene (FHIT). This is a tumor suppressor gene that is involved in apoptosis after environmental damage (e.g., radiation exposure). In many human cancers, its protein expression is diminished or lost. Moreover, single nucleotide polymorphisms (SNPs) have been found that affect prostrate cancer risk. Ding *et al.* (2008) found a complex pattern of selection operating on the gene, with some SNPs showing signatures of positive selection in one or more populations, and other SNPs showing signals of balancing selection. They did find an association between the variant in the intron of FHIT associated with both enhanced prostrate cancer risk and also positive selection in European-American and Japanese populations.

Crespi (2010) notes a negative correlation between incidence of three human-specific diseases (Parkinson's disease, Alzheimer's disease, and schizophrenia) and cancer risk. As these diseases are likely involved in the evolution of the social brain of modern humans, the epidemiological link to cancer is most intriguing.

Tumor Heterogeneity

Although tumors usually originate as a single cell, different cells in most human tumors generally vary considerably in morphology, gene expression patterns, cell surface receptor expression, and numerous other phenotypic ways. This tumor heterogeneity presents challenges to cancer biology, but also illustrates the value of viewing cancer from an evolutionary perspective (Marusyk and Polyak, 2010). Heterogeneity is just another word for variation, and evolutionary genetics has intensely studied the causes and consequences of variation since its conception (e.g., Dobzhansky, 1937; Lewontin, 1974; Hartl and Clark, 2007; Corbett-Detig *et al.*, 2015).

Although much of tumor heterogeneity is due to genetic changes, some of it arises from environmental differences (Park *et al.*, 2000; Marusyk and Polyak, 2010). For instance, in addition to causing genetic mutations, ionizing radiation may be carcinogenic due to its remodeling of the extracellular matrix (Park *et al.*, 2000). The phenomenon of the same genotype leading to different phenotypes is known as phenotypic plasticity, an area of intense research in evolutionary genetics. This research shows that the extent of phenotypic plasticity is both of a product of past evolution and can influence future evolutionary trajectories (e.g., Pigliucci, 2001; West-Eberhard, 2003).

Coevolution between the tumor cells and cells in surrounding tissues often occurs, with each influencing the other's evolutionary trajectory (Polyak *et al.*, 2009). The 'normal' cells in the microenvironment of a potential tumor often participate in the evolution of cancer due to the signals they produce. For instance, circulating levels of signaling proteins such as integrins and transforming growth factor-*B* appears to influence the early stages of tumor progression (Marusyk and Polyak, 2010). The tumor cells likewise influence the surrounding tissue (Polyak *et al.*, 2009). Coevolution is a vital area of mainstream evolutionary biology, and studies of such coevolution could be useful for studying cancer.

Examining and quantifying tumor heterogeneity poses challenges in sampling the variation (Marusyk and Polyak, 2010). These challenges are similar in some respects to those of evolutionary biologists sampling variation, especially those engaged in metagenomics (e.g., Kembel *et al.*, 2011).

In some cases, the extent of heterogeneity strongly correlates with tumor progression, and thus can be used as a predictive clinical biomarker (Pepper *et al.*, 2009; Marusyk and Polyak, 2010). One such case is Barrett's esophagus, a premalignant neoplasm that arises from chronic inflammation. Some people who have Barrett's esophagus develop malignancies, but most do not. Maley *et al.* (2006) used measures of clonal diversity commonly used by ecologists and evolutionary geneticists and found that indeed, higher levels of clonal diversity were predictive of progression to cancer, even after controlling for other risk factors. They also hypothesized that genomic instability from telomere shortening led to increased genetic diversity. Consistent with their hypothesis, telomere length was negatively correlated with one of the metrics of clonal diversity, the Shannon index (Maley *et al.*, 2006). In general, this and similar studies highlight how an evolutionary perspective can inform when malignancies will develop (Pepper *et al.*, 2009). Tumors are generally spatially heterogeneous. This heterogeneity sets up the prospect of selection pressures varying in different locations, and the heterogeneous selection pressure would likely lead to yet more heterogeneity (Marusyk and Polyak, 2010). This feedback loop is similar to what was seen in an experimental ecosystem of microbes (Rainey and Travisano, 1998).

Drivers and Passengers

Not all of the genetic changes that occur during the evolution of a tumor are advantageous to the tumor. Indeed, most are neutral or somewhat deleterious. Cancer biologists call the mutations that are driven to high frequency by selection 'drivers,' and the neutral and deleterious mutations that also accumulate 'passengers' (see references in Attolini and Michor, 2009; Sprouffske *et al.*, 2012; McFarland *et al.*, 2014).

The idea that neutral or even deleterious variants can accumulate in populations undergoing adaptation along with advantageous ones is an old idea in evolutionary genetics, and was coined 'hitchhiking' by Maynard Smith and Haigh (1974).

The reason that hitchhiking occurs is that some neutral/deleterious variants are associated with the advantageous variants. This association is known as linkage disequilibrium. Even though recombination erodes linkage disequilibrium each generation, selection increases the frequency of both the advantageous variant and variants that are in linkage disequilibrium with it. Linkage disequilibrium is very important in evolutionary genetics as well as in applied field (Hartl and Clark, 2007; Slatkin, 2008).

Cancer is a largely clonal process, and recombination is generally absent. With the absence of recombination, hitchhiking should be very common and many passengers would be expected to accumulate (Hartl and Clark, 2007). The accumulation of passengers can be important, especially to the extent that genotype-by-environment ($G \times E$) interactions are common in tumor progression. With considerable $G \times E$ interactions, passenger mutations might be fuel for further change in the selective environment of the tumor.

In actuality, most passengers should not be neutral, but actually somewhat deleterious on average. Although most theory has treated passengers as neutral, a recent treatment models the evolution of tumors assuming deleterious passengers (McFarland *et al.*, 2014). This model found that cancer progression is often a tug-of-war between accumulation of drivers that promote tumor growth and accumulation of passengers that retard it (McFarland *et al.*, 2014). Under considerable parameter space, a critical threshold for tumor size exists. Below the threshold, tumors eventually go extinct. Above the threshold, a macroscopic malignant tumor is expected to form. The model also found an optimal range of mutation for tumor growth. With low a mutation rate, insufficient drivers evolve, and the critical size is not reacted. Too high a mutation rate leads to mutational meltdown. McFarland *et al.* (2014) note possible therapy applications based on this model, which are discussed in brief in the 'Evolutionary Applications for Cancer Therapy' part of this article. How can we distinguish drivers from passengers? Frequent occurrences of the same genetic changes in independent cases would be strong evidence that those changes arose due to selection favoring them (Sprouffske *et al.*, 2012). For this reason, evolutionary geneticists are particularly interested in convergent evolution (Tenaillon *et al.*, 2012). Similar logic can be used to find drivers in cancer evolution.

Evolutionary geneticists have developed a plethora of statistical tests designed to infer selection (Johnson, 2007; Hartl and Clark, 2007). Although such tests can be and are used to infer drivers in cancer progression, Sprouffske *et al.* (2012) note the statistical complications involved with these tests. Further development is necessary. Experimental evolution approaches are another possible way to distinguish drivers from passengers (Sprouffske *et al.*, 2012).

Metastasis

Metastasis occurs when cells from the primary tumor detach and form new tumors (metastases). Much of the mortality from cancer comes not from the primary tumor, but from the metastases that later appear. One estimate places the metastases contribution to cancer deaths at 90% (Mehlen and Puisieux, 2006). An evolutionary understanding of metastasis is thus a major part of studies of the evolution of cancer.

Metastasis inherently involves the evolutionary ecology processes of dispersal and colonization (Fidler, 2003; Merlo *et al.*, 2006; Talmadge and Fidler, 2010). In the late nineteenth century, a British surgeon named Stephen Paget proposed what would be called 'the seed and soil hypothesis' for cancer metastasis. Paget used the analogy of a plant shedding its seeds to explain his view of metastasis. He noted, "When a plant goes to seed, its seeds are carried in all directions, but they can only live and grow if they fall on congenial soil" (quoted in Talmadge and Fidler, 2010, p. 5650). With respect to metastasis, the seed and soil represent respectively propagules from the primary tumor and prospective locations and tissue types for metastases. Despite considerable controversy during the twentieth century, substantial evidence now supports a version of the seed and soil hypothesis (Fidler, 2003; Merlo *et al.*, 2006; Talmadge and Fidler, 2010).

What is known about the clonal relationship between tumor cells in primary tumors and metastatic sites? Marusyk and Polyak (2010) note conflicting evidence, with the extent to which metastases have diverged from the primary tumor being still controversial. Still, metastases often do resemble primary tumors. Weigelt *et al.* (2003) found that the gene expression profiles of primary breast tumors and metastases from the same patient usually clustered together, distinct from the tumors of other patients. Moreover, in some patients, the gene expression pattern in the primary tumor was practically indistinguishable from the metastases. Some evidence supports metastases occurring much earlier than previously thought during tumor progression, and that genetic differentiation may increase between the primary and metastatic tumors during subsequent evolution (Marusyk and Polyak, 2010).

Evolutionary Applications for Cancer Therapy

The potential applications of evolutionary biology to cancer therapies are many and varied. Increasingly, the evolutionary ecology approach of using other organisms to mediate cancer is being used in therapy (Pepper *et al.*, 2009). Dang *et al.* (2001) presented an interesting example of such bacterial therapy. They were motivated by the observation that growth of tumor often outpaces growth of blood supply leaving large parts of the tumor hypoxic. Anaerobic bacteria would be expected to thrive in such conditions. Dang *et al.* (2001) searched for bacteria that would not only grow under these conditions, but would selectively kill tumor cells in adjacent regions, They found such an agent in *Clostridium novyi*. After genetic modifications of this bacterium to reduce toxicity to the mouse host and other modifications, this bacterium in combination with traditional chemotherapy can result in dramatic regression of mouse tumors. Use of other organisms in therapy is complicated by the fact that these organisms themselves evolve. This evolution 'a double-edged sword': it can be advantageous considering that the organisms can coevolve measures against the defenses tumor cells may evolve, but this evolution can also have potential negative side effects (Pepper *et al.*, 2009). The evolutionary potential of these

biological agents need to be considered in the development of therapies.

Evolutionary quantitative genetics theory may provide insights into cancer therapy. For instance, consider therapy that involves giving patients combinations of drugs. When would such combination therapy be most likely to be more effective than single therapies? Evolutionary quantitative theory (e.g., Roff, 2006) would predict that such treatment would be most effective when the tumor's resistance to one drug comes at a cost of being less resistant to other drugs. The next most likely case for combination therapy to be effective is when resistance to one drug evolves with little or no cross-resistance. Combination therapy is not expected to be effective when the evolution resistance to one drug also results to resistance to other drugs (see also Pepper *et al.*, 2009).

Pepper *et al.* (2009) and others have suggested that we should think about therapies based on evolutionary rates. The McFarland *et al.* (2014) model of deleterious passengers has similar applications for treatment. Recall that this model predicted an optimal range for mutation rates. For most neoplasmas that have yet to become malignant, reducing mutation rates would limit progression. One way to reduce mutation rate would be to suppressing inflammation. Vaughan *et al.* (2005) has found that the use of aspirin and other NASIDs limit progression of Barrett's esophagus to malignant esophageal adenocarcinoma, presumably by limiting inflammation. In contrast, malignant tumors are already beyond the size threshold. Here, raising the mutation rate so as to generate a mutational meltdown might be the best strategy. McFarland *et al.* (2014) provide further details.

A related consideration is the number of cell division. Higher numbers of cell division could increase the risk of cancer. This supposition is consistent with the general increase incidence of cancer with age (Attolini and Michor, 2009). Wodarz and Komarova (2007) proposed that high rates of apoptosis increase cell turnover, thus generating more mutants and increasing the probability of cancer progression. They suggest limiting apoptosis might reduce cancer risk, but such a strategy has yet to be put into practice.

Finally, some cancer experts are considering adaptive therapy, a treatment strategy where the aim is to manage a stable tumor population rather than trying to kill as many cancer cells as possible (Gatenby *et al.*, 2009). Its use is still in development.

Outstanding Questions

1. The nonhuman animals that have been used as models for cancer research have evolved differences from humans in the way cell physiology and thus, cancer works. For instance, the role of telomerase differs in cancer progression in human and mice (reviewed in Hanahan and Weinberg, 2011). How well do animal models of cancer suffice? How can we use evolutionary principles and data to improve the use of the models?
2. Cancer biologists have postulated for a long time that only some cells – cancer stem cells – retain the potential to evolve into tumors (Cairns, 1975). The exact role of cancer stem cells remains unclear (Hanahan and Weinberg, 2011). The extent to which only some cells can retain the potential to form tumors affects several aspects, including the effective population size, of tumor evolution (Attolini and Michor, 2009).
3. What are the rates of cancer in other mammals, especially nonhuman primates? What are the genetic mechanisms that underlie the supposedly better cancer suppression mechanisms in very large animals, and can we exploit them to reduce cancer incidence and progression in humans?
4. Some evidence suggests the presence of tradeoffs between wound healing capacity and cancer suppression (Aktipis and Nesse, 2013). What is the nature of these tradeoffs? What genes are involved? How can we exploit this information to develop better therapies?

See also: Evolutionary Medicine II. Use of the Comparative Method and The Animal Model. Evolutionary Medicine III. Mismatch. Evolutionary Medicine IV. Evolution and Emergence of Novel Pathogens. Microbiome

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Evolutionary Medicine II. Use of the Comparative Method and The Animal Model

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Medical research tries to find treatments for human diseases, often by exploring the underpinnings of basic human biology, but most experiments are not carried out on humans or in human tissues. Instead, experimental work typically uses animal models: nonhuman animals that serve as proxies for human biology. Animal models allow scientists to conduct studies that would be impossible to complete in humans: experiments involving surgery, tissue manipulations, or breeding controls that would be unethical to try on people. Humans are also poor subjects for research into heredity because they have a long gestation period and take many years to grow to maturity. As a result, animal models have become powerful tools behind advances in all aspects of human biology, including physiology, functional anatomy, embryonic development, cell biology, genetics, and biochemistry (Rand, 2008).

Experimental studies in biology and medicine have been dominated by a short list of animal models, including the fruit fly (*Drosophila melanogaster*), nematode worm (*Caenorhabditis elegans*), zebrafish (*Danio rerio*), mouse (*Mus musculus*), and Norway rat (*Rattus norvegicus*). All became popular models because they have characteristics that make them convenient to use in a laboratory (Bolker, 2012). As a rule, they are small, robust, and easy to care for in captivity. They also grow to maturity quickly and breed frequently, making it possible to conduct experiments that span multiple generations over a relatively short period of time. Many model animals also have traits that make them particularly amenable to specific types of research: for example, the nematode *C. elegans* has a fixed developmental pattern that allows researchers to trace the fate of every cell in its body (Kenyon, 1988), and zebrafish are transparent as embryos, making it possible to use protein markers and fluorescent dyes to track cell movement and changes in gene expression *in vivo* (Gilbert, 2013). In addition, researchers have bred mutant varieties of these model animals or deliberately altered their genomes in order to track and determine the function of specific genes (Rubin, 1988; Jennings, 2011), neither of which would be possible in humans.

But while practical considerations were instrumental in making these species popular proxies for humans, it is their shared evolutionary history that makes correlations with human biology possible. Every model animal is a branch off the same family tree; although each has a unique evolutionary history, they also shared a common ancestor with humans at some point in the past (Dunn *et al.*, 2014). Common ancestry means they will share at least some aspects of their physiology with humans. Choosing a model animal should therefore include a consideration of its specific evolutionary history, to determine whether it will be a good analog for the human condition of interest. For some studies, fidelity between human and model characteristics is most desirable (Rand, 2008); in others, unique traits of the model species may be effective reflections of human tissues or disease states (Maher, 2009).

Deep Homology, Distant Relatives, and the Persistence of Ancestral Traits

Evolutionary relationships among species can be drawn as a series of nested branches called a phylogeny (**Figure 1**). Branches fork at speciation events, where two descendant groups are derived from a hypothetical common ancestor. Any traits that are unique to the hypothetical ancestor may be inherited by its descendant taxa, producing a group of species called a clade that share these derived characteristics, or synapomorphies (Harvey and Pagel, 1991; Brooks and McLennan, 1991).

This pattern of inheritance with modification underlies why many discoveries made in model animals are applicable to human biology: when humans and an animal model both inherit versions of a trait from a common ancestor, the traits are homologous and may retain similarities in genetic sequence, phenetic expression, and physiological function (Lauder, 1994). By manipulating these shared features in a

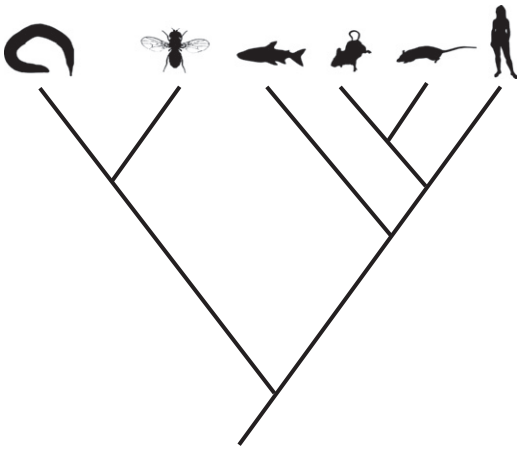

Figure 1 The phylogenetic relationships of the five most commonly used animal model species relative to humans. From left to right, species illustrated are *Caenorhabditis elegans* (roundworm), *Drosophila melanogaster* (fruit fly), *Danio rerio* (zebrafish), *Mus musculus* (mouse), *Rattus norvegicus* (rat), and *Homo sapiens* (human). Note that the laboratory mouse and rat are more closely related to one another than either is to humans. Although they are not particularly closely related, *C elegans* and *D. melanogaster* are still more closely related to one another than either is to any of the vertebrate animal models. Animal icons adapted from PhyloPic.org by Frank Förster (*C. elegans*), David Liao (*M. musculus*), Rebecca Groom (*R. norvegicus*) used via CC BY-SA 3.0, all others public domain.

model animal, we can obtain insights that are applicable to human biology.

A fruit fly, for example, looks nothing like a human. It is grossly different in size, body plan, internal anatomy, and developmental patterning: unsurprising, as molecular clock estimates suggest its lineage diverged from the lineage that includes humans more than 700 million years ago (Wray *et al.*, 1996; Wang *et al.*, 1999). But as an animal model, fruit flies have been instrumental in advancing our understanding of human heredity, gene expression, protein function, and the regulation of embryonic development (Rubin, 1988; Roberts, 2006) because flies and humans both inherited genes critical to cell survival and differentiation from an ancient common ancestor (Liebeskind *et al.*, 2011; Ramos *et al.*, 2012). Aspects of the genes that code for these traits have diverged over time, as humans and flies evolved significant differences in body plan (Gilbert, 2013). But their genes still have a deep homology (Bejerano *et al.*, 2004), and have retained enough functional similarities that experimental manipulations in flies can illuminate how the homologous human version works.

Closer Relationships, Greater Fidelity

Fruit flies and nematode worms have been excellent models for understanding genetics and development, but would be inappropriate for examining physiological systems such as the adaptive immune system – which is only found in vertebrates (Flajnik and Kasahara, 2010), or mammary glands – which are only found in mammals (Liem *et al.*, 2001). In cases like these, the best model animals share a more recent common ancestry with humans.

Species with recent common ancestry will be nested more deeply in a phylogenetic tree and share more synapomorphies than species that are more distantly related. For example, humans, mice, and zebrafish are all members of a clade descended from ancient bony fish. As such, they share a suite of derived characteristics that includes two pairs of lateral appendages and specialized tissues such as bone and enamel (Liem *et al.*, 2001; Hall and Witten, 2007). But humans and mice share a more recent common ancestry – they are both members of a clade that descended from early mammals – and as such they share additional derived characteristics that are not found in zebrafish: including hair, the ability to secrete milk, and facial morphology associated with chewing (Liem *et al.*, 2001). Closely related lineages share more derived characteristics, increasing the similarity of their anatomy, physiology, and their responses to disease and medical interventions.

This nested pattern of relationships and synapomorphies is why researchers use rodents as models in early-stage biomedical research, but switch to nonhuman primates as the final model animal when testing the efficacy of new drugs and medical treatments. Primates are far more difficult to maintain in the laboratory than mammalian models like rats and mice (King *et al.*, 1988; Shanks and Pyles, 2007), but their more recent common ancestry with humans also gives them more genetic and physiological similarities to humans. Physiological similarities between humans and their primate relatives make it possible to identify side effects that may not appear in more distantly related model species, or determine whether a treatment that worked in mice or rats remains effective in humans (Johnsen *et al.*, 2012).

Common Ancestry, Unique Histories

Many medical conditions are widespread among vertebrates: examples include cancer, infertility, obesity, and heart disease (Natterson-Horowitz and Bowers, 2012). The ubiquity of these conditions probably comes from the common ancestry of the clade – if the anatomy and physiology of a common ancestor left it susceptible to these conditions, its descendants can inherit that susceptibility as well as its other traits.

But although vertebrates suffer from similar illnesses, many treatments developed using model animals fail to translate to human biology (Shanks and Pyles, 2007; Seok *et al.*, 2013). While some failures can stem from methodological problems in experimental design, including small sample sizes, insufficient blinding, and inappropriate choices of model species (Pound *et al.*, 2004; van der Worp *et al.*, 2010), others may be a result of characteristics that are unique to the model species' lineage. The model animals still become ill. But aspects of their physiology can differ from humans, and these differences can affect their susceptibility to disease and their metabolic response to experimental treatments (Shanks and Pyles, 2007).

Evolution is ongoing, and after a speciation event each sister-lineage continues to accrue new characteristics. As a result, every model animal has traits unique to its own lineage, even while it shares other similarities with humans. Those differences make correlating model and human physiologies an imperfect process. For example, in recent years zebrafish have become a promising model animal for understanding bone formation (Spoorendonk *et al.*, 2008; Apschner *et al.*, 2011; Asharani *et al.*, 2012) – results from these studies are broadly applicable to mammals because the characteristics under investigation are conserved throughout vertebrates. But all vertebrate bone is not identical. Zebrafish, like other advanced teleost fish, have lost the ability to make osteocytes (Apschner *et al.*, 2011). Because osteocytes are the cells responsible for bone remodeling in other vertebrates, this teleost synapomorphy makes the zebrafish an inappropriate model for studies of bone remodeling, even as they remain a useful model for investigating more ancestral characteristics.

Evolutionary Mutants, Convergent Traits, and Unique Inventions

In some cases, model animals are chosen for study because they have traits that differ from normal human biology. For example, instead of using mutant strains of 'standard' model animals like flies or zebrafish, researchers may choose more unusual species whose normal phenotypes mimic human diseases. These evolutionary mutant models (Albertson *et al.*, 2009) can come from a broad range of taxa: examples include blind cavefish, which have naturally regressive eye loss and have been used as models for human retinal degeneration (Jeffery, 2001); and more recently turtles, whose phallus normally develops with an open groove for sperm transport, have served as a model for hypospadias, a congenital pathology

where the mammalian urethra fails to close along its midline (Larkins and Cohn, 2015). By examining normal developmental pathways in these model animals, researchers hope to determine how their traits are produced and ultimately develop a nuanced understanding of how differences in the regulatory regions of genes can affect phenotype (Albertson *et al.*, 2009).

Another tactic takes advantage of convergent evolution: choosing a model animal that independently evolved a trait similar to one found in humans. In this case, the model and human traits will not be identical, but they may share functional similarities that let the model's behavior provide insights about human function. For example, humans and songbirds independently evolved the ability to reproduce meaningful sounds by imitating adults; the process shares many functional similarities in both taxa including characteristic immature vocalizations ('babbling'), a dependence on social cues, and an interplay between vocal muscle activation and auditory feedback (Doupe and Kuhl, 1999; Knudsen, 2013). The zebra finch (*Taeniopygia guttata*) has been a particularly valuable model for exploring the neural underpinnings of this convergent system (Mello, 2014). There is nothing fundamentally different about the physiology of individual nerves in humans and birds. But songbirds have markedly different cerebrum anatomy than humans (Proctor and Lynch, 1993; Liem *et al.*, 2001), which means they comprise a second evolutionary experiment in neural circuitry for effective learning and song production. Researchers hope that examining the anatomical organization and neurochemical function of these circuits will unearth common organizational principals that can be applied to analogous regions in the human brain.

Sometimes a model animal has a unique trait that improves our ability to investigate a fundamental biochemical or physiological system. A classic example is the use of the squid genus *Loligo* for examining neuron function during the first half of the twentieth century. Squid have giant neurons in their mantle that control escape behaviors (Young, 1938). Mammals have no equivalent to these giant axons (Hill *et al.*, 2004), but the sensors available at the time were too large to fit inside a vertebrate neuron. Squid giant axons were easier to manipulate, and because they are much larger than vertebrate neurons – up to 1 mm in diameter – they could accommodate sensors to measure voltages inside their cell membrane. These experiments were instrumental to our understanding of how action potentials are produced (Hodgkin and Huxley, 1939), and were ultimately applicable to vertebrate neurons due to their common ancestry with squid (Dunn *et al.*, 2014). Although the morphology of squid and vertebrate neurons had diverged, the underlying cellular machinery for electrical conduction, inherited from a common ancestor (Liebeskind *et al.*, 2011), remained essentially the same.

The Challenge of Extrapolating from Models

Experiments in model animal systems have been integral to developing our understanding of scores of basic biological processes, but paradoxically, clinical treatments developed in animal models are sometimes ineffective in humans (Shanks and Pyles, 2007; Seok *et al.*, 2013). These failures occur often enough that some authors have suggested direct assays of human biomarkers could be a more useful method of predicting drug efficacy (Littman and Williams, 2005). Indeed, one challenge of extrapolating from any biological model, whether animal proxy or human genetic assay, is navigating the complex relationship between genetic differences and the morphological and physiological changes that cascade from them. Depending on their specific role in physiology or development, some genes simply drive more significant changes than others (Haag and True, 2001).

It is particularly important to remember that species sharing a high degree of morphological homology do not always share the same degree of physiological similarity (Burggren and Bemis, 1990). For example, although chimpanzees and humans are closely related, share many homologous traits, and can both be infected by HIV, chimpanzees are a poor model for studying the effect of the virus because they do not become ill the way humans do. Selecting the best animal model for a medical study is not simply a matter of getting as 'close' to humans as possible. An effective animal model for a human disease state will ideally replicate how the disease behaves in humans at some measureable level, whether genetic, or the development of symptoms, or the process of recovery (Pouladi *et al.*, 2013).

But any one animal model does not have to replicate every detail of the condition in question: it only has to accurately reflect the specific details that a study is interested in. In the case of HIV, cats turned out to be an effective model system for studying how the virus attacks the human immune system. Cats are more distantly related to humans than chimpanzees and have fewer morphological and physiological similarities, but the feline response to FIV, a virus in the same retroviral family as HIV, closely matched the human response to HIV.

Conclusions

Choosing a model animal for a study should not stop at practical considerations of cost and convenience. In medical research, the phylogenetic relationship of the model to humans is also important. Considering the evolutionary history of a potential model organism can help pinpoint which traits of interest may share similarities with humans, and which have diverged over time. The closeness of the relationship, the presence of homologous traits, and the unique traits bequeathed by the model's lineage are as critical to consider as the availability of mutant strains or the ease of breeding. Studies that aim to explain basic biological processes that are ancestral and maintained in many animals may be able to select from a much broader array of animal models than either studies focused on the progression of disease inside a closely related group of animals or studies that aim to identify effective treatments for a human disease.

It is also important to remember that closely related species are not always the best model for some aspects of human physiology. Researchers should look for a model species that is a good match for the 'specific traits of interest,' considering whether that species has retained those traits from a shared ancestor or evolved them independently. Different species are

likely to be better suited for modeling different aspects of a complex problem. Indeed, simply picking a species from a short list of 'standard' animal models may unnecessarily limit the types of questions we are capable of answering. We should be open to developing new animal models, each carefully chosen to effectively mirror a particular trait.

See also: Amniotes, Diversification of. Developmental-Genetic Toolkit for Evolutionary Developmental Biology. Gene Networks Driving Development, Conservation and Evolution of. Insects and Ecdysozoa, Diversification of. Parallel and Convergent Molecular Evolution. Phylogenetic Tree

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Evolutionary Medicine III. Mismatch

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Glossary

Antagonistic pleiotropy Concept where a trait has beneficial, fitness promoting effects in early life but may incur costs later in life.

Developmental plasticity Processes of potential adaptive value in which an organism uses environmental cues to tune its phenotypic development to enhance fitness in a particular environment.

Maternal constraint Maternal and utero-placental factors that operate to limit fetal growth in the mammal.

Introduction

The emerging research domain of evolutionary medicine is concerned with the application of evolutionary principles to understanding human health and disease. It infuses traditional medical thinking, which primarily centers on proximate explanations and reductionist approaches, with evolutionary concepts to offer ultimate explanations for why disease may occur, thus providing a more complete framework for understanding the origins of disease risk. Formalized as a discipline less than three decades ago, it has rapidly gained traction in recent years (Alcock, 2012). This reflects the growing appreciation of how it complements clinical/biomedical approaches, providing a better understanding of human disease vulnerability at the levels of both the individual and the population.

Evolutionary medicine aims to address why, despite millennia of evolutionary history for selection pressures to operate, the human body is still vulnerable to disease. Nesse and Stearns (2008) identified six pathways by which evolutionary processes can influence disease susceptibility in humans. We expanded on these by further incorporating several aspects of population genetics (Gluckman *et al.*, 2009; **Table 1**).

In this article we focus on two major pathways to disease risk: evolutionary mismatch, and life history-associated factors underlying developmental mismatch. As both pathways involve aspects of the 'mismatch' concept, it is important to recognize the nuanced differences between the two. The basis for evolutionary mismatch is the dramatic change in contemporary environments and lifestyles compared to those during which the majority of selective processes giving rise to the modern human genotype have operated. On the other hand, developmental mismatch is underpinned by the evolved processes of developmental plasticity that operate early in the life course and influence the mature phenotype and, hence, disease risk in a particular context. Central to both pathways is the key principle that selection operates to maintain and promote Darwinian fitness, and is essentially heedless of quality of health past the age of peak reproduction. It should also be noted that evolutionary and developmental histories are not causative of disease per se, but instead mediate disease risk.

Evolutionary Mismatch

Evolutionary mismatch arises when the environments in which contemporary populations now live have deviated from that experienced during most of our evolutionary history. While numerous species demonstrate environment-altering behaviors to sustain their fitness (a process known as niche construction), humans are unique for their capacity to dramatically and rapidly change their built, sociocultural and nutritional environments, and much of this change has occurred relatively recently in evolutionary terms, spurred by the Agricultural and Industrial Revolutions (Fogel, 2004). The processes of selection generally operate over timescales of multiple generations to millennia, gradually changing the allelic frequency within a population. Yet, the genetic substrate for selective processes to act upon is somewhat limited by the range of environments previously experienced by the lineage. The slow tempo at which selection operates has become far outpaced by the speed of environmental change, and this lag has left our bodies mismatched and unable to adequately cope with consequent deleterious effects on health (Gluckman and Hanson, 2006a).

Evolutionary Novelty

The nature of the environment that can give rise to evolutionary mismatch can be distinguished in two ways. The first is when an individual encounters a wholly novel exposure. This may be in the form of a toxicant such as asbestos, to which humans have had minimal exposure and thus lack evolved detoxification mechanisms. Widespread mining and industrial use of asbestos from the late nineteenth century led to chronic inhalation of asbestos in many individuals, hence resulting in an epidemic of pleural mesothelioma, a rare cancer of the cells that surround the lungs and line the chest wall.

Exposure to a novel environment is another example of evolutionary novelty leading to mismatch. For example, scurvy, a disease clinically characterized by inflamed and bleeding gums, anemia, and skin hemorrhages, results from lack of dietary ascorbic acid. Humans are among the few primates that have lost the ability to synthesize ascorbic acid due to a mutation in *GULO*, which encodes the enzyme responsible for the committed step in the biosynthetic pathway. It is thought that this was an initially neutral mutation that arose in a frugivorous cladal ancestor. However, it only has a detrimental impact upon exposure to a novel dietary environment lacking in vitamin C, such as that experienced by eighteenth-century sailors undertaking long voyages (Buklijas *et al.*, 2011).

Table 1 Eight pathways by which evolutionary processes can modulate disease vulnerability

Pathway	*Conceptual basis*	*Clinical examples*
Mismatch	Exposure to an environment of evolutionary novelty (in entirety, or in extent)	Myopia; metabolic mismatch
Life history-associated factors	Trade-offs and antagonistic pleiotropy	Developmental mismatch: poor early life nutrition leading to obesity and metabolic dysfunction in adulthood
Excessive defense mechanisms	Dysregulation of normally adaptive defense activity	Extreme pyrexia; fear
Human–pathogen coevolution	Rapid rate of microbial evolution relative to that of humans	Antibiotic resistance
Evolutionary constraints	Consequences of human evolutionary history giving rise to certain anatomical features	Bipedalism underpinning back pain; ancestral herbivorism requiring a now-defunct appendix that is prone to inflammation
Sexual selection	Competition between males, and possibly certain sexually dimorphic biological characteristics such as higher testosterone	Greater extrinsic and intrinsic mortality among males
Balancing selection	Heterozygote advantage enabling the persistence of an apparently deleterious allele	Sickle cell anemia
Demographic history	Population bottlenecks, founder effects	Modern Finnish population demonstrating differential susceptibility to a range of diseases compared to rest of Europe

Note: The authors note that these categories are not discrete, and that many diseases will draw on multiple pathways.

Juvenile-onset myopia refers to near-sightedness resulting from refractive error that occurs around age 8 to 14. Myopia has a genetic component: having a myopic parent increases a child's risk of developing the same condition, and recently a mutation responsible for its onset has been identified in members of an Israeli tribe (Mordechai *et al.*, 2011). However, there is clearly strong environmental and cultural contribution to its development in the wider population. Risk factors implicated in its etiology include urbanization, increased education, being indoors, reading and close-up work, and underuse of peripheral vision (You *et al.*, 2012; Goldschmidt and Jacobsen, 2014), all of which are components of evolutionarily novel built environments. This, in turn, has led to preventative strategies being evaluated which encourage young children to spend time outdoors during this critical period of ocular development (French *et al.*, 2013). It appears that some populations may be predisposed to development of myopia, but that the condition is only revealed upon exposure to a 'myopigenic' environment. For example, rates of myopia are relatively low among Indians residing in India, but high among Singaporean Indians exposed to multiple environmental risk factors (Morgan and Rose, 2005).

The mismatch concept has a broad scope and can illuminate upon aspects of both physiological and mental health. Humans are exceedingly social animals, but while we have evolved to live in simple societies in groups of 100–150 (Dunbar, 2003), the modern day societal structure is characterized by large social groups, complex social hierarchies and stratification, democratization enabling greater autonomy, and myriad interactions involving personal and nonpersonal social networks and communication methods. The confluence of these evolutionarily novel environmental attributes may exceed the evolved cognitive and emotional adaptive capacities of some individuals, contributing to mental health disorders. It is perhaps telling that forecasts by the World Health Organization of morbidity causes in 2030 have pinpointed depression as the second highest contributor worldwide, and as the largest cause in high-income countries (Mathers and Loncar, 2006). There are some provocative human data linking social environment with mental illness, suggesting that urbanicity is associated with increased amygdala responses to social stress (Lederbogen *et al.*, 2011). Other studies have demonstrated correlations between real-world social network size and amygdala volume (Bickart *et al.*, 2011), and online social network size with gray matter density in brain regions involved in social cognition (Kanai *et al.*, 2011).

Mismatch between the ages of reproductive maturation and of psychosocial competence can also raise the risk of mental health disorders. Adolescence refers to the period from the onset of puberty to that when social recognition as an adult is attained, and the post-pubertal phase of adolescence is essentially an evolutionarily novel social construct. While the age of pubertal onset in the Paleolithic is unknown it may have been around 7–13 years, with full reproductive capacity being reached at perhaps age 9–14 (Gluckman and Hanson, 2006b) given that in modern hunter-gatherer populations the age at menarche is lowest in populations with high extrinsic mortality (Walker *et al.*, 2006). Further, competence in parenthood and as an adult within the simple small-scale societies necessitates psychosocial maturity, and it is likely that the age at which this was reached would have evolved to coincide with that of reproductive capacity for maximum fitness advantage (Gluckman and Hanson, 2006b). The advent of agriculture and settlement, and the associated increases in childhood morbidity and undernutrition then delayed age at menarche, but this was again matched by the increased societal complexities brought about by population aggregation. Then, improved standards of living and healthcare

accompanying the enlightenment facilitated the modern secular decline in the age at menarche. Other pre- and postnatal factors such as being born small with rapid postnatal weight gain can accelerate puberty (Sloboda *et al.*, 2007).

However, as discussed earlier, the complexities of the social environment have only increased since, and exponentially so since the arrival of the information age. Further, neuroimaging data and functional studies demonstrate that it is not until the third decade of life that brain regions associated with impulse control and judgment become fully mature (Lebel and Beaulieu, 2011; Cauffman and Steinberg, 2000). It is unclear whether late brain maturation is a modern day phenomenon resulting from increased societal complexity, from societal reticence in treating adolescents as adults upon biological maturity, or whether it is simply an evolved aspect that has adverse consequences in the modern world. Indeed, changing cultural norms in contemporary Western societies have led to prolongation of the period before youth become accepted as adults. Evidence suggests that those who experienced earlier puberty are at greater risk of exhibiting acting-out behaviors, depression, substance abuse, and suicidal attempts (Michaud *et al.*, 2006; Kaltiala-Heino *et al.*, 2003). Thus, it may be that the asynchronous timing of biological and of psychosocial maturation has consequences reflected in greater morbidity and mortality among adolescents (Office of the Prime Minister's Science Advisory Committee, 2011).

Evolutionarily Novel Range of Exposures

The second way by which evolutionary mismatch can arise is when exposure to a particular aspect of the environment is beyond the range previously experienced by the lineage. An excess of nutrition in the modern affluent world, leading to metabolic mismatch and contributing to the epidemics of obesity and metabolic disorders, is an illustrative example of this. The relatively recent beginnings of agriculture, compared to the appearance of the earliest members of genus *Homo*, suggests that humans and their hominin ancestors lived the bulk of their existence as hunter-foragers, and it is this lifestyle that has served as the template on which human biology and metabolism have been molded. Estimates of the likely dietary composition that prevailed in the Paleolithic indicate that it differed markedly from that in modern day societies: it was likely to have been highly varied and not reliant on a few cereal grains as dietary staples; intake of lean protein, fiber, and micronutrients such as calcium and ascorbic acid was higher, while that of fat and sodium was lower (Eaton and Konner, 1985; Eaton *et al.*, 1988). The major and progressively escalating driver of these changes has been the development of agriculture, industrialization, and extensive processing of food, underpinned by rapid cultural changes and technological advances (Lucock *et al.*, 2014). The contemporary obesogenic environment is characterized by a hypercaloric diet of readily available, palatable food and drink high in refined carbohydrates, sugar, sodium, and evolutionarily novel *trans*-fats, and a lifestyle of increased sedentariness. These pronounced changes in our nutritional and energetic patterns may overwhelm our evolved metabolic physiology and adaptive capacity. Accordingly, susceptibility to nutrition-related disease phenotypes such as obesity and type 2 diabetes mellitus is increased (Gluckman and Hanson, 2006a; **Figure 1(a)**).

Although humans as a species are vulnerable to metabolic mismatch, a degree of variation in disease risk exists at the level of the individual or population. For example, type 2 diabetes risk appears to be ethnicity-specific; it is greater in South Asian, Chinese, and Black populations than in Caucasians throughout the entire range of BMIs. Indeed, at a BMI of 24, clinically defined as normal, there is a greater than fourfold incidence of diabetes among South Asians than in those of Caucasian descent (Chiu *et al.*, 2011). The genetic component explaining variance in risk of obesity and its complications is small (McCarthy, 2010; Speliotes *et al.*, 2010), and there is now increasing evidence implicating developmental factors as a key contributor (see section Developmental Mismatch).

Developmental Mismatch

Developmental Plasticity

Developmental plasticity refers to the evolved and ubiquitous ability to adjust phenotypic development in response to environmental cues experienced in the more plastic early stages of development (Bateson *et al.*, 2004). It is the basis by which multiple phenotypes may be generated from a single genotype. Developmental plasticity may be induced by cues that generally fall within the normal range of physiological and ecological conditions, such as quantitative and qualitative aspects of, in mammals, maternal nutrition, maternal stress, and predator risk. The processes underpinning adaptive developmental plasticity evolved to promote fitness; they confer potential adaptive benefit by enabling the organism to exploit external cues to tune its phenotype to better survive stresses and match its environment.

However, the inherent constraints and energetic costs of maintaining plasticity limit much plasticity to the early period in life, although there may be *trans*-generational legacies (Gluckman *et al.*, 2007a). Because of this constraint, responses may be made not just for immediate survival advantage concurrent with the environmental challenge, but also for anticipated conditions later in life for delayed adaptive advantage. The latter type of response has been termed predictive adaptive responses (PARs) (Gluckman *et al.*, 2005a). For example, a fetus may gain information about the nutritional characteristics of the environment into which it will be born based on the nature of nutrition received from its mother; suboptimal maternal nutrition may induce PARs and thus signaling the adoption of an energy conserving phenotype to best adapt to a poor postnatal nutritional environment. These do not necessarily lead to apparent changes in phenotype or adaptive advantage *in utero* or at birth, but later in life. The biology and conceptual framework underlying PARs has been reviewed in detail (Bateson *et al.*, 2014; Bateson and Gluckman, 2011; Hanson and Gluckman, 2014).

The anticipatory strategy of PARs is not inerrant. Erroneous predictions, for example due to inaccurate transmission of cues, or to significant changes in the environment subsequent to the establishment of PARs, can result in the organism

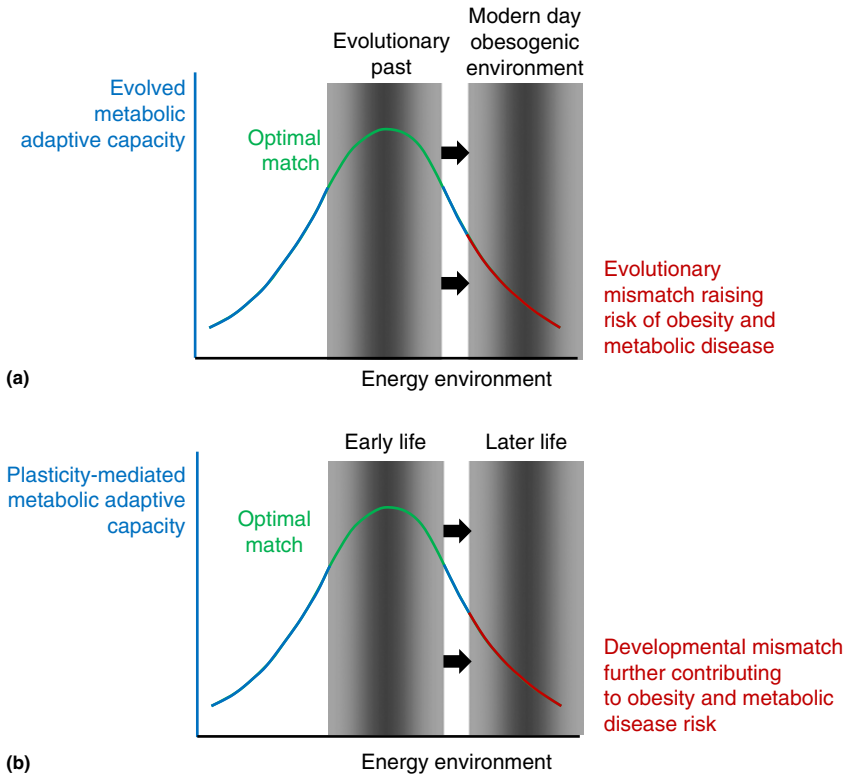

Figure 1 Our metabolic adaptive capacity as shaped by the energy environments experienced during our (a) evolutionary and (b) developmental histories can become mismatched to the modern day or later life planes of nutrition, leading to increased risk of obesity and its complications.

possessing a phenotype that is discordant with the mature environment. This leads to a situation of developmental mismatch wherein risk of chronic noncommunicable disease is increased. Since selection operates to maximize reproductive fitness and not health or longevity, and – at least with respect to most noncommunicable disease – the increased disease risk likely manifests after the period of peak reproduction has passed, inaccurate anticipatory responses will generally have little impact on reproductive success. This, therefore, provides little impetus for negative selection to operate, enabling the evolutionary retention of developmental plasticity and PARs in spite of the potentially deleterious later life consequences. Modeling work has also shown that even under conditions of environmental stochasticity, persistence of induced phenotypes over several generations offers adaptive advantage over both non- or fully plastic strategies (Jablonka *et al.*, 1995).

Another factor that may play a role in why PARs are conserved may relate to maternal care seen in mammals, birds, and some other taxa. In mammals, the cues of most relevance are prenatal, and forecast, for example, poor postnatal nutrition, even though the infant will undergo a protected period of postnatal maternal care before becoming autonomous. To induce the physiology appropriate to autonomous life in poorly nourished circumstances may be disadvantageous while being suckled. Indeed, it is well established experimentally and clinically that signals of poor intrauterine nutrition lead to long-term induction of insulin resistance with its survival value in postweaning poor nutritional circumstances (Duque-Guimarães and Ozanne, 2013). However, to be insulin resistant during the period of high-fat milk feeding would reduce adipose deposition and thus make the growing individual more likely to be unable to buffer the energetic demands of the rapidly growing brain after weaning under conditions of malnutrition or infection (Kuzawa, 2010). Such data explain why children born with growth retardation only become insulin resistant at about the age of 3 years (Mericq *et al.*, 2005), which is coincident with likely past weaning behaviors.

Developmental Origins of Obesity and Its Complications

Although the literature is dotted with sporadic reports as far back as the 1930s hinting at the role of early life pathways in disease risk, it was not until David Barker and associates' meticulous work on a large UK cohort in the 1980s, showing that low birth weight correlated with increased risk of death from ischemic heart disease in middle-age, that the role of development as a determinant of later life disease risk gained sustained attention (Barker *et al.*, 1989; Gluckman and Buklijas, 2014). Articulations of this phenomenon into a viable conceptual framework (Gluckman *et al.*, 2005a,b), together with progress in epigenetic biology (Gluckman and Buklijas, 2014; Hanson and Gluckman, 2014), helped give wider credence to the idea that events occurring at the very earliest stages of life could have lifelong health sequelae. There is now a substantial corpus of research from domains spanning epidemiology through to clinical medicine and animal experimental work, showing that risk of noncommunicable disease

in later life is modulated by early life events. By positioning developmental factors as a key determinant of disease risk, this framework, formalized as the developmental origins of health and disease (DOHaD) (Gluckman and Hanson, 2006c), has significantly advanced our understanding of human morbidity in the context of modern day environments.

Much of the DOHaD literature has focused on how developmental influences have shaped risk of adiposity, cardiovascular, and metabolic diseases, including insulin resistance, type 2 diabetes, and hypertension. Experimental work using rodent models has demonstrated that a maternal low-protein diet during pregnancy induces hypertension, dysregulations in vascular endothelial function and lipid metabolism, and a preference for high-fat food in adult offspring (Langley-Evans *et al.*, 2006). Fetal exposure to maternal undernutrition influences intermediary metabolism and appetite control, leading to obesity, insulin resistance, decreased physical activity, hyperphagia, and dietary fat preference in adulthood (Vickers *et al.*, 2000, 2003). Crucially, increasing the degree of developmental mismatch through a high-fat postnatal diet amplifies these phenotypic effects. Hence, in this model, fetally undernourished offspring appear to have adopted an integrated, energy conserving phenotype to cope with an anticipated low postnatal plane of nutrition. This metabolic physiology is rendered inappropriate upon exposure to an obesogenic environment to which the offspring are developmentally mismatched, thus leading to increased disease vulnerability. An equivalent situation in humans has been well studied using cohorts of descendants of famine survivors. For example, offspring of women who were pregnant during the wartime famine of 1944–45 in the Netherlands were at higher risk of coronary heart disease, dyslipidemia, glucose intolerance, and (in women) adiposity at age 50–58 compared to unexposed individuals (Roseboom *et al.*, 2006). Dietary preference for fat has also been observed (Lussana *et al.*, 2008). *In utero* exposure to the Chinese famine of 1959–61 was associated with hyperglycemia and clinical indicators of metabolic syndrome (Li *et al.*, 2010, 2011), while exposure to the Nigerian civil war famine of 1967–70 during fetal life and infancy increased risk of hypertension and impaired glucose tolerance at age 40 (Hult *et al.*, 2010).

While famine serves as a regrettable natural experiment to investigate DOHaD in humans, they are relatively rare and extreme. Indeed, recent work shows that developmental induction of long-term consequences can occur within quite normative exposures *in utero* which do not lead to changes in birth weight (Gale *et al.*, 2006; Godfrey *et al.*, 2011), emphasizing the subtle and likely adaptive nature of plasticity. In humans, there are multiple situations by which developmental mismatch can arise that may affect much of the population and thus have profound public health implications. A major pathway, particularly relevant to developing countries experiencing economic prosperity, is the rapid transition to an obesogenic environment of nutritional plenitude and associated decreases in energy expenditure. Relatedly, increased urbanization, rural-to-urban migration, and emigration to more prosperous countries may leave the individual – who has likely experienced a low plane of nutrition early in life – ill equipped to handle the nutritionally overloaded environment encountered (Ebrahim *et al.*, 2010; Shan *et al.*, 2011). Cultural factors may also contribute. Decreasing family size in many countries has led to corresponding increases in proportion of first-borns who, owing to the processes of maternal constraint that limit fetal growth and operate to a greater extent in primiparous pregnancies, are more likely to be born smaller than later-born siblings, be more insulin resistant in later childhood, and more adipose in adulthood (Ayyavoo *et al.*, 2013; Reynolds *et al.*, 2010). The introduction of formula feeding as an alternative to breastmilk is another factor that has been linked to increased obesity risk (Jwa *et al.*, 2014; Harder *et al.*, 2005).

Extensive epidemiological data have shown that there is a clear gradation in the relationship between early life cues and later disease risk (Rich-Edwards *et al.*, 2005; Harder *et al.*, 2007; Osmond *et al.*, 1993). This indicates that plasticity operates across the normal range of exposures, and that normative developmental experiences can still be associated with adverse health outcomes. Thus it would be fallacious to dichotomize populations into low versus at-risk, as disease risk arguably applies across the entire population. It should also be noted that individuals experiencing nutritional developmental mismatch are also subject to evolutionary metabolic mismatch; in this way, the effects of the former compound those of the latter (**Figure 1(b)**).

Molecular Basis of DOHaD

There is strong evidence from investigations in animals, and emerging evidence in humans, that epigenetic mechanisms play a key role in mediating the processes of developmental plasticity. Epigenetic mechanisms are the molecular processes that establish and maintain mitotically heritable patterns of gene expression in a DNA sequence-independent manner. Key mechanisms include DNA methylation, covalent modifications of histone protein tails that induce chromatin reconfiguration, and the gene expression-regulating activity of noncoding RNA. In the previously described rat model of fetal undernourishment, decreased expression of *Ppara*, encoding a fatty acid oxidation regulator, in offspring hepatic tissue was concordant with hypermethylation at its promoter region. Importantly, neonatal administration of leptin to these pups normalized epigenetic and gene expression changes and completely prevented development of the primed phenotype (Vickers *et al.*, 2005; Gluckman *et al.*, 2007b). Similarly, in a model of maternal protein-restriction in which offspring become hypertensive as adults and show dysregulations in lipid metabolism and vascular endothelial function, increased expression of *Ppara* and *GR* was observed together hypomethylation at the promoter regions (Lillycrop *et al.*, 2005). Furthermore, offspring from rat dams that received folic acid supplementation were spared the metabolic dysfunction phenotype, and methylation and gene expression levels were restored to those of controls (Lillycrop *et al.*, 2005).

The famine cohort studies have provided support for the tractability of epigenetic marks in response to early life events in humans. Differential methylation at *IGF2*, a maternally imprinted gene central to growth and development, and at several candidate genes involved in metabolic and cardiovascular disease was seen in gestationally exposed individuals

in their sixth decade of life (Heijmans *et al.*, 2008; Tobi *et al.*, 2009). The effect sizes were small, but these studies clearly demonstrate the temporal stability of the molecular memory arising from a transitory environmental exposure. The recognition that the epigenome may serve as a molecular transducer of information from environment to genome has prompted the establishment of multiple prospective cohort studies investigating the epigenetic underpinnings of developmentally influenced disease risk in normal populations (Ng *et al.*, 2012; Soh *et al.*, 2013; Saffery *et al.*, 2012). Early data have already linked the epigenome to later phenotypic variation of clinical relevance. For example, degree of methylation at specific sites of the *RXRA* promoter measured at birth correlated with adiposity of the child at age 6–9 years and explained a significant component of the variance in BMI at that age (Godfrey *et al.*, 2011). Methylation levels were further inversely associated with maternal carbohydrate intake in early pregnancy, demonstrating that even subtle changes in the nature of nutrition received *in utero* affects epigenetic regulation.

Implications for Human Health

We have described how disease risk is influenced by both the evolutionary and developmental histories of the individual, and thus a greater understanding of human health and disease from these perspectives has considerable value in devising interventional strategies. In the case of evolutionary mismatch, given the inherent temporal limitations of our genetic repertoire in adapting to environmental novelties, it seems likely that our recourse is to change the environment, and/or adapt our behaviors to the changed environment. With respect to nutrition, some studies on native peoples have supported the notion that dietary modifications in favor of varied and healthful foods may have beneficial effects. For example, diabetic Australian Aboriginal people who maintained a traditional hunter-gatherer diet and lifestyle for 7 weeks showed substantial improvements in several metabolic parameters (O'Dea, 1984), while observational studies have shown that rates of obesity and type 2 diabetes among Pima Indians living in remote mountainous regions of Mexico are markedly lower than for those residing in the United States (Schulz *et al.*, 2006). Nevertheless, despite the recent popularity of the so-called 'Paleolithic diet' attempting to emulate the presumed diet of our preagricultural hunter-gatherer ancestors, there are few well-controlled studies of its long-term efficacy, and there is unlikely to be a single diet that is appropriate for all individuals due to variations in our metabolic physiology as influenced by our genetic and developmental backgrounds.

In light of the unabated rise in rates of overweight, obesity, and associated chronic diseases in both high and low-middle income countries (Ng *et al.*, 2014; International Diabetes Federation, 2013), it is becoming evident that voluntary lifestyle modifications in adulthood, which have been the mainstay of public health strategies targeting obesity, are ineffective. Alternative strategies based on recent scientific advances underscoring the role of development in disease risk have been well canvassed (Gluckman *et al.*, 2011) – these recognize the value of exploiting the period early in the life course when physiological set-points for metabolism, appetite and satiety are being established. Exciting research is now accruing from the field of developmental epigenetics showing that risk markers of adiposity (Godfrey *et al.*, 2011; Perkins *et al.*, 2012) and of metabolic syndrome (Yoo *et al.*, 2014) may be measurable at birth. This suggests the potential for the use of prognostic biomarkers to identify at-risk infants for early intervention, although it is imperative that such studies incorporate within- and between-cohort replications to validate findings. Ample experimental evidence that developmentally primed phenotypes can be reversed by endocrinological, dietary, and pharmacological means (Vickers and Sloboda, 2012) offers proof of concept that a poor start to life need not have immutably deleterious effects. What is clear is that the focus should be on optimizing an individual's diet and health, starting from as early as the preconceptual period via maternal well-being, through to the neonatal life, infancy and childhood.

See also: Basic Science and Evolutionary Biology. Developmental Plasticity and Phenotypic Evolution. Epigenetic Inheritance. Human Life Histories, Evolution and. Life History Trade-offs. Maternal Effects

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Evolutionary Medicine IV. Evolution and Emergence of Novel Pathogens

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Glossary

Coalescent theory A retrospective, mathematical framework for relating genetic variation to historical evolutionary and demographic processes.

***De novo* phenotypes** New traits, which are present for the first time in a population.

Glycoprotein A molecule that consists of a carbohydrate and a protein bound together.

Influenza hemagglutinin One of the surface glycoproteins found on influenza viruses. Its primary function is for binding the virus to sialic acid binding sites.

Microparasites Parasites that can complete their life cycle within a single host and can be transmitted directly to other hosts of the same species, for example, influenza, HIV, and Ebola.

Phylodynamic models Theoretical population genetic models relating the complex demographics of pathogens to the structure of their phylogenetic trees.

Sialic acid Nitrogen or oxygen substituted derivatives of the nine-carbon monosaccharide, neuraminic acid.

Social contact networks A social structure made up of individuals and the contacts between them, which are often visualized as graphs.

Introduction

The evolutionary history of humans is characterized by dynamic shifts in population density and the structure of our social contact networks. Agriculture, the advent of City-States, European expansionism, and modern globalization have all had profound effects on the ecology of humans (Mcmichael, 2004). Our changing dispersal, demographic, and contact patterns have impacted both commensal and pathogenic organisms. Fruit flies in the genus *Drosophila*, numerous Yeast species, *Escherichia coli* and other human commensals tracked our expansion throughout the world (Keller, 2007; Pamer, 2007). However, pathogenic species followed too. In the 1700s and 1800s, smallpox and measles – spread to the Americas by European explorers – decimated indigenous populations (Mcmichael, 2004; Cliff *et al.*, 1993). At the turn of the last century, the 1918 flu killed between 20 and 40 million people worldwide in little over a year (Noymer and Garenne, 2000). Troop movement during WWI played a critical role in the emergence and spread of that virus (Oxford *et al.*, 2002). Today, we are beset by the human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) epidemic, the threat of pandemic avian influenza, hepatitis C, Ebola, tuberculosis, and myriad neglected tropical diseases (Mcmichael, 2004; Morse, 1995; Worobey *et al.*, 2008; Hotez, 2014). The critical factors uniting the spread of commensal and pathogenic species, the emergence of new diseases, and the rapid spread of pandemic strains are the increasing density and connectivity of our populations, coupled with the evolutionary lability of many parasites, for example, bacteria and viruses (Scarpino *et al.*, 2015; Galvani and May, 2005; Pourbohloul *et al.*, 2009; Stoddard *et al.*, 2009).

Modern travel patterns, heterogeneity in population density, variable immunity, and changing proximities to wild and domesticated animals interact to drive complex patterns of disease transmission and emergence (Galvani and May, 2005). SARS and SO-H1N1 spread rapidly around the globe, but because of heterogeneity in contact patterns and immunity, these diseases were not the disasters predicted early in the epidemics (Pourbohloul *et al.*, 2009; Meyers *et al.*, 2005). A similar situation also occurred during the 2014–15 Ebola outbreak in West Africa, where models with more realistic human contact patterns predicted yielded more accurate predictions (Scarpino *et al.*, 2015). The threat of wildlife disease and its spillover to humans and our food is also becoming an increasing concern (Weiss and McMichae, 2004; Frenzen, 2004; Chua *et al.*, 1999; Daszak, 2000). Changes in hunting and agricultural practices alter contact patterns between wildlife and domesticated animals (Daszak, 2000; Köndgen *et al.*, 2008). The result has been both the spillover of highly virulent diseases, such as Nipah virus and Ebola, and recombination between human and animal strains of influenza (Weiss and McMichae, 2004; Chua *et al.*, 1999; Daszak, 2000).

The emergence of HIV in central Africa illustrates both the importance of evolution and changing ecology on the epidemic potential of novel pathogens. The European reorganization of central Africa – from a dispersed collection of villages to a system with large urban areas – fundamentally changed human social contact patterns in the region (**Figure 1**; Hance, 1970; Worobey *et al.*, 2008). Recent genetic evidence suggests that in some of these emerging cities, HIV epidemics were raging by the late 1950s or early 1960s (Worobey *et al.*, 2008). Using historical census data (Hance, 1970), the author compared the rates of population growth, urbanization, and HIV infection – as reconstructed from HIV genomic data by Worobey *et al.* (2008) – in the Democratic Republic of the Congo (DRC), the Republic of Congo, the Central African Republic, and Gabon. Strikingly, the rate of increase in HIV infections lags the rate of urban growth, while the rate of population growth remains essentially constant (**Figure 2**). This result highlights the importance of both changing host ecology, in this case the urbanization of central Africa, and pathogen evolution in the emergence of new diseases (Schrag and Wiener, 1995; Antia *et al.*, 2003; Weiss and McMichae, 2004; Holmes, 2009). In the next section, the author discusses

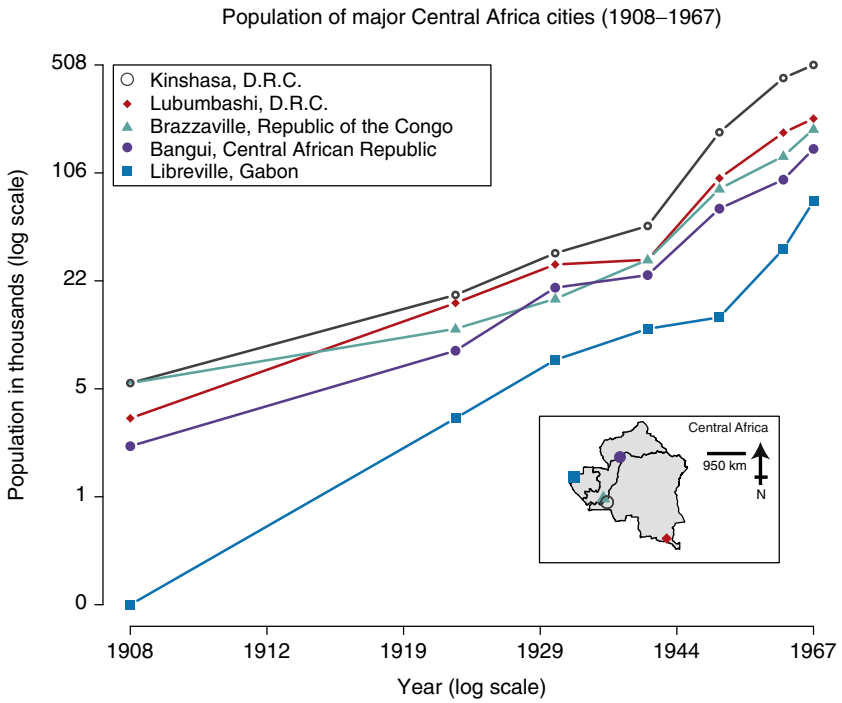

Figure 1 Between 1900 and 1970, the population size of the five largest central African cities grew by over 100-fold. This dramatic increase in size was due mostly to urbanization, where individuals migrated from rural areas to the cities, as opposed to population growth.

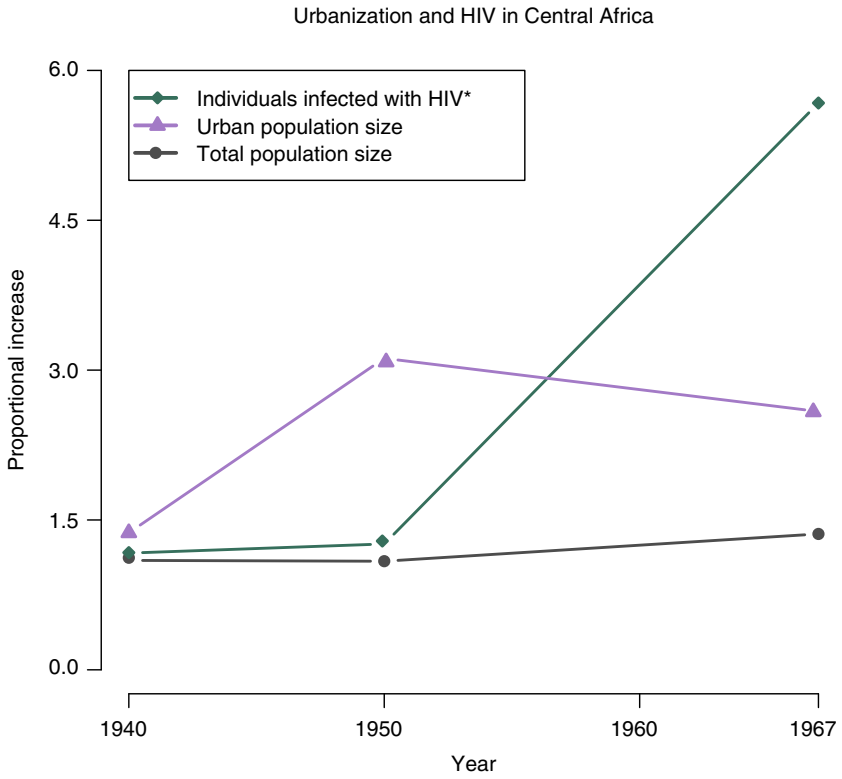

Figure 2 Estimates from genomic data suggest that HIV was already spreading rapidly in central Africa by the 1960s. As predicted by theory from epidemiology, there is evidence for a role of urbanization in the spread of HIV. The figure plots the proportional increase in growth rate by decade for individuals infected with HIV (green), urban population size (purple), and total population size (black). Note how the HIV epidemic lags urbanization, while the population growth rate remains essentially constant.

modern methods for studying the dynamics of infectious diseases using genomic data and then in the following sections the author illustrates the role of these new methods in studying emerging infectious diseases.

The Phylodynamics of Emerging Infectious Diseases

Pathogen host shifts are responsible for outbreaks of severe disease in wildlife, livestock, and human populations (Antia *et al.*, 2003; Dobson and Foufopoulos, 2001; Morens *et al.*, 2004; Daszak *et al.*, 2000b; Altizer *et al.*, 2003). Despite the biological importance of such host shifts, many gaps remain in our understanding of how and why they occur. Because many pathogens – especially RNA viruses – mutate so rapidly, their evolutionary and ecological processes are inextricably linked (Antia *et al.*, 2003; Koelle *et al.*, 2009; Pybus and Rambaut, 2009; Levin, 1999). Therefore, studying epidemics requires models able to connect evolution to ecology. The emerging field of phylodynamics seeks to leverage the genetic variation of pathogens to investigate their complex, epidemiological dynamics through the use of mathematical transmission models (Grenfell, 2004; Holmes and Grenfell, 2009; Volz *et al.*, 2009). Linking these models with the genetic sequence data – now routinely collected during disease outbreaks – provides an unprecedented opportunity to advance our scientific understanding of how evolution affects epidemics and pathogen establishment (Antia *et al.*, 2003; Holmes and Grenfell, 2009; Pybus and Rambaut, 2009; Leventhal *et al.*, 2015; Woolhouse *et al.*, 2005).

What are the drivers of pathogen emergence and reemergence? How are microparasites able to cause epidemics in novel hosts? These questions have been the focus of epidemiology since its inception and remain of immediate importance for wildlife, human, and livestock populations (Anderson and May, 1992; Daszak *et al.*, 2000a). It has become clear that answering these questions requires an understanding of how rapid evolution contributes to epidemic potential (Antia *et al.*, 2003; Pepin *et al.*, 2010; Pybus and Rambaut, 2009; Leventhal *et al.*, 2015; Woolhouse *et al.*, 2005). For emerging infectious diseases, the key evolutionary event is often the generation of *de novo* phenotypes in pathogens (Altizer *et al.*, 2003; Woolhouse *et al.*, 2005; Parrish *et al.*, 2008). These phenotypes might include a strain with increased transmissibility, or immune escape variants. Because of the large population sizes and high mutation rates of many viruses, it is hypothesized that selection on *de novo* variation may be a critical factor during viral adaptation to novel hosts and coevolution with existing hosts (Crill *et al.*, 2000; Eshelman *et al.*, 2010; Pybus and Rambaut, 2009; Altizer *et al.*, 2003; Woolhouse *et al.*, 2005; Parrish *et al.*, 2008).

Since its inception, the field of phylodynamics has seen significant advances in theoretical population genetic models relating the complex demographics of pathogens to the structure of their phylogenetic trees (Koelle *et al.*, 2006; Norström *et al.*, 2012; Volz *et al.*, 2009). These include mathematical models describing how deterministic epidemic dynamics shape neutral genetic variation (Volz, 2012), models describing how rates of coalescence can be related to epidemiological model structures (Koelle and Rasmussen, 2012), and powerful statistical models to estimate the parameters of those mathematical models (Rasmussen *et al.*, 2011). However, the existing mathematical models are insufficient for investigating the role of *de novo* evolution in epidemics. The emergence of novel pathogen phenotypes, such as a strain with higher transmissibility or increased virulence, during an outbreak necessitates a dynamic model structure. Although capable of incorporating selection and allowing for stochasticity, the current models have a static structure and are thus unable to account for newly arising phenotypic variants (Koelle and Rasmussen, 2012; Rasmussen *et al.*, 2011; Volz, 2012).

A Short Primer on the Coalescent

The primary tool for modern population genetic inference is coalescent theory, which provides a retrospective, mathematical framework for relating genetic variation to historical evolutionary processes (Wakeley, 2008). Coalescent theory permits the study of the evolutionary history of a population by sampling individuals in the present (Drummond *et al.*, 2005; Wakeley, 2004, 2008). Consider a population in which individuals are related by a shared ancestry rooted at their 'most recent common ancestor (MRCA).' Going forward in time and starting from the MRCA, the population diverges with lineages forming and dying. Looking backwards, lineages fuse, reducing in number until only a single lineage remains; the coalescent is a quantitative, probabilistic framework for determining when lineages join, or 'coalesce,' backwards in time (Wakeley, 2008; Fu and Li, 1999). Because the coalescent considers neutral genetic variation, all pairs of existing lineages are equally likely to coalesce (Wakeley, 2008; Kingman, 1982a,b). The result is a genealogy tracing the current individuals backwards in time to the MRCA. The parameters of a coalescent model describe this stochastic, genealogical process. The rate that these lineages are born and die is also a function of the nonneutral evolutionary forces acting on the population and demographic processes (Wakeley, 2008). Therefore, selection, demography, and other evolutionary processes will leave signatures in the shape of genealogies (Wakeley, 2008; Drummond *et al.*, 2005; Parsch *et al.*, 2001; Nei and Takahata, 1993). The expected coalescent time and the rate of coalescent are both highly sensitive to changes in ecological and evolutionary dynamics. As a result, coalescent theory can be used to extract information about phenotypic evolution from the genetic variability of populations.

The Coalescent and Infectious Diseases

Applying coalescent theory to the study of infectious disease dynamics presents a number of challenges (Frost *et al.*, 2015). First, sequences are typically sampled serially, as opposed to the classical application of coalescent theory where sequences are collected from a single time-point (Koelle and Rasmussen, 2012; Volz, 2012; Stadler *et al.*, 2012). Second, unlike many traditional applications, often a large fraction of infected individuals are sampled (Volz, 2012). Third, complex population dynamics emerge from an epidemic process. In two recent papers, Volz *et al.* (2009) and Volz (2012) derived the coalescent for structured pathogens undergoing complex population dynamics. The models in (Volz, 2012; Volz *et al.*, 2009) allowed for: (1) the nonlinear growth rate of pathogen populations during

epidemics, (2) birth and transmission rates that change during an epidemic and are not always proportional to population size, and (3) the changing variance in the number of transmissions per infected individual. Using the novel coalescent framework developed by Volz *et al.* (2009), Rasmussen *et al.* (2011) demonstrated that with a model for the rate of coalescence, it was possible to infer historical epidemiological patterns from simulated sequence data. The statistical methods developed by Rasmussen *et al.* (2011) are Bayesian particle filter methods, which, once an equation exists for the rate of coalescence, can approximate the likelihood of a model given a genealogy.

Selection acting on genetic variants arising from *de novo* phenotypic evolution adds an additional layer of complexity to the coalescent process (Frost *et al.*, 2015). This form of evolution will result in new disease model compartments that arise stochastically. Therefore, modeling selection on *de novo* variation requires a dynamic model structure. The Volz *et al.* (2009) and Volz (2012) coalescent models can have arbitrary structure, but critically this structure must be static during the course of evolution. Consider a mutation increasing the transmissibility of a pathogen. As this mutation spreads, the transmission dynamics change. This effect can be easily visualized in the transmission tree, where hosts are connected if one host's pathogens seeds infection in an uninfected host (**Figure 3**). Because two pathogen lineages cannot coalesce unless they come from a shared host, changes in the transmission tree will affect the rate of coalescence for pathogens.

How Does Selection for Increased Transmissibility Affect Influenza Phylogenies?

In 2004, an influenza outbreak in greyhound dogs had a case fatality rate close to 40% (Crawford, 2005). Sequencing of viral isolates determined that the virus responsible for this outbreak, influenza A/H3N8, arose from a recent spillover from horse populations (Crawford, 2005). Subsequent phylogenetic analysis provided early evidence for viral evolution during adaptation to a novel host. Molecular changes in the A/H3N8 hemagglutinin gene, encoding the viral surface glycoprotein, indicated evolution of increased transmissibility in canines (Crawford, 2005).

Over the period of 4 months in 2011, nearly 200 New England harbor seals died of pneumonia caused by an avian influenza, also of the influenza A/H3N8 subtype (Anthony *et al.*, 2012). The H3N8 strain had acquired a mutation that increased its ability to transmit between mammalian hosts (Anthony *et al.*, 2012). Because influenza infects the gastrointestinal tract of birds and the upper respiratory track in mammals, transmissibility had been a barrier to species jumping. Variation in infection location is primarily caused by differences in the sialic acid binding site of influenza infecting avian and mammalian hosts (Fergusson *et al.*, 2003; Smith *et al.*, 2009; Suzuki *et al.*, 2000). However, reassortment of influenza strains with different cell-type specificity and evolution in hosts with mixed sialic acid types, such as pigs, can facilitate spillover (Fergusson *et al.*, 2003; Smith *et al.*, 2009; Suzuki *et al.*, 2000). Sequence data from the harbor seal outbreak suggests this as a mechanism for increased transmissibility (Anthony *et al.*, 2012).

Using Phylodyanmics to Study Disease Reporting During the 2014 Ebola Outbreak

Infectious disease surveillance data can be unreliable during unfolding crises in which resources are limited and public health authorities have poor access to affected communities. During the 2014–15 Ebola outbreak in West Africa, surveillance efforts primarily detected cases that were treated in healthcare facilities, and may have missed a sizable fraction of infections (Meltzer *et al.*, 2014; WHO Ebola Virus Response Team, 2014). Initially, the CDC estimated for every Ebola case reported in Sierra Leone and Liberia, as many as 2.5 times as many cases went unreported (Meltzer *et al.*, 2014). Accurate outbreak projections and assessments of intervention strategies depend on reliable estimates of underreporting rates. However, underreporting can be a very dynamic process, potentially varying in time, space, and/or with outbreak size, and driven by intrinsic properties of the pathogen, human behavior, diagnostic practices, and the healthcare infrastructure.

The primary difficulty the public health community faced in estimating underreporting is the limited data commonly available during an outbreak, namely confirmed and suspected cases and mortalities. From these data alone, one cannot estimate the rate of underreporting without making strong modeling assumptions and, even with such assumptions, we often lack statistical power to make precise estimates.

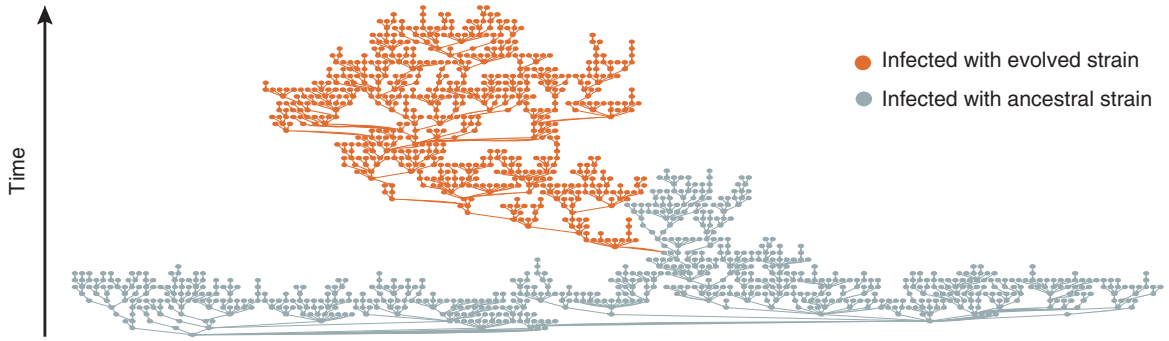

Figure 3 The results from a computer simulated emerging infectious disease outbreak. Hosts are represented as circles, with lines connecting individuals who infected each other. Time starts at the bottom of the plot. Approximately one-third of the way through the outbreak, a mutation occurred that increased the transmissibility of the pathogen. Individuals infected with this new strain are colored in orange.

However, underreporting can cause a mismatch between incidence estimated from case data and incidence reconstructed from genetic data using phylodynamic methods. For example, if there is a constant level of underreporting, case count data will reflect lower transmission rates and lead to underestimation proportional to the underreporting rate. However, the extent of genetic variation among viral sequences taken from the same set of cases will reflect the true, larger population size of circulating viruses, and lead to estimates closer to the true incidence. This is true even if viral sequences are only collected from reported individuals, assuming reported and unreported individuals are mixing with each other. In a phylodynamic analysis of Ebola virus genome sequences, Scarpino *et al.* (2015) estimated that underreporting of cases may be between 0–70%, with the most likely value being 17%.

Beyond underreporting, leveraging phylodynamic methods during emerging infectious disease outbreaks can potentially address a range of important, but historically challenging, questions. These include, but are certainly not limited to, the potential for evolution to alter the virulence or transmissibility of the pathogen, the role of subclinical or asymptomatic infections in transmission, the importance of cross-border transmission, and the relative role of various transmission routes in sustaining an outbreak. During the next pandemic, or emerging infectious disease outbreak, deploying phylodynamic methods and next-generation sequencing to improve surveillance and decision-making will be essential.

Conclusions

In this article we have seen the emergence of novel pathogens is a complex process and one affected by host ecology and behavior, as well as, pathogen evolution (Leventhal *et al.*, 2015; Woolhouse *et al.*, 2005). For some diseases, such as influenza, it seems clear that the most important determinant is pathogen evolution. However, for others, such as SARS, Ebola, and Middle East respiratory syndrome corona virus host factors seem to play a larger role in regulating spread. Although a pathogen's often rapid rate of evolution can contribute to its epidemic potential, these high rates also facilitate the application of phylodynamic methods to the study of emerging infectious diseases. Future work in the rapidly expanding field of evolution and the emergence of novel pathogens will undoubtedly provide numerous scientific insights, but hopefully, better prepare us for the next pandemic.

See also: Coalescent and Models of Identity by Descent. Pathogen Epidemiology. Predation and Parasitism. RNA Viruses, Evolution of

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Evolvability, Quantitative Genetics of

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Introduction

The concept of evolvability came late to evolutionary biology. This may be a symptom of the much-discussed blackboxing of development and the genotype–phenotype relation during the modern synthesis (Gould, 2002; Amundson, 2005). A core assumption of the synthesis and the adaptationist program was that selection is always provisioned with sufficient genetic variation to operate. Evolvability was taken for granted, a given premise, and was not itself a focus of research. This started to change in the 1970s and 1980s driven by a renewed interest in evolutionary constraints. Concepts such as allometry and heterochrony were used to study the structuring of variation (e.g., Gould, 1977). The emergence of evolutionary quantitative genetics provided statistical tools for studying patterns of genetic variation in natural populations, and researchers began testing the premise of 'unlimited' genetic variation. The blackboxing of the genotype–phenotype map was still in place, however, as mutation rates and allelic effects were treated as fixed, non-evolving parameters. The real breakthrough in the study of evolvability came with the emergence of evolutionary developmental biology in the 1990s. Almost from the start 'evodevo' researchers were talking about evolvability and many see the study of evolvability as the key contribution of evodevo to evolutionary biology (e.g., Von Dassow and Munro, 1999; Hendrikse *et al.*, 2007).

Today, the term evolvability is routinely used as a label for much research in evodevo, evolutionary quantitative genetics, and other fields in evolutionary biology. While an ISI search on 1 July 2014 turned up 1 hit for 'Evolvability' before 1990 (and that 1 is from computer science), and 70 for 1990–99, it gave 785 hits for 2000–09, and 728 for 2010–14.

Definitions and Origins of the Concept

The term evolvability was practically never used before 1990. It is interesting that one of the first uses was by Richard Dawkins, who in an article entitled 'The evolution of evolvability' starts out by saying that "A title like [this] ought to be anathema to a dyedin-the-wool, radical neo-Darwinian like me!" (Dawkins, 1988, p. 201). This reflects the attitude that evolutionary biology is about what happens when the conditions for evolution by selection are fulfilled and not about what makes these conditions in the first place. It is not clear what role Dawkins' article had in establishing interest in evolvability, but together with a chapter in Dawkins (1996) it may have helped ease acceptance of the concept among mainstream evolutionary biologists. Arnold *et al.* (1989) and Alberch (1991) are possible entry points for Dawkins' ideas to the communities that later embraced the term 'Evolvability.'

Even if the term 'Evolvability' was not used, interest in the origin and evolution of variation date back to Waddington and other critics of the neo-Darwinian blackboxing of development, and to an increasing interest in evolutionary constraints. Olson and Miller (1958) initiated a 'morphological integration' research program focused on the origin of patterns of variation and their relation to functional interrelationships between traits. Riedl's (1978) influential *Order in Living Organisms* argued that organisms were structured to facilitate variation along functional lines. In the 1980s, many theorists discussed evolvability under other labels. Layzer (1980) and Conrad (1983), and also Waddington (1957), discussed 'adaptability,' Wagner (1984) 'adaptive versatility,' and Campbell (1987) 'evolutionary drivers.' Lewontin's (1978) Scientific American article on 'Adaptation' had a prescient discussion on the prerequisites for adaptive evolution, which he suggested depended on the two properties of 'continuity' and 'quasi-independence.' The former referring to the standard neo-Darwinian assumption that small changes in a trait must lead to small changes in fitness and the second to the possibility of changing traits without messing up other traits.

Interest in the ability of complex systems to change had also emerged in computer science. Kauffman (1990, p. 135) explicitly defined evolvability in this context as the ability to 'successively accumulate useful variations.' His 1993 book *The Origins of Order* played a key role in raising interest in evolvability among biologists and philosophers alike (Kauffman, 1993).

It is hard to pinpoint the exact emergence of the evolvability concept within evolutionary developmental biology. Perhaps the focal idea in evodevo is to study how development structures variation so as to provide evolvability. Theoretically this is conceptualized with the genotype–phenotype map. Alberch (1991) is among the first to state this explicitly, but elements of the idea can be found, for example, in Waddington (1957), Lewontin (1974), Riedl (1978), and Raff and Kaufman (1983). The influential review by Wagner and Altenberg (1996) firmly establishes the genotype–phenotype map at the focus of evolvability studies. They start by defining evolvability as "… the ability of random variations to sometimes produce improvement" (p. 967), and then narrow this to a property of the genotype–phenotype map, the 'genetic system,' when they write: "Evolvability is the genome's ability to produce adaptive variants when acted upon by the genetic system" (p. 970). They then focus on 'modularity,' the variational (quasi-)independence of traits, as the key prerequisite of evolvability. In their view, the main problem in maintaining evolvability of a complex organism is to avoid everything being tangled together in 'unbounded pleiotropy.' The evodevo solution to this is that evolution is driven by changes in *cis*-regulatory modules with restricted spatio-temporal expression. Early versions of this idea were expressed by Jacob (1977) and Dickinson (1990) and it was firmly established by the second half of the 1990s (reviewed in Stern, 2000; Davidson, 2001; Carroll, 2008).

Many specific definitions of evolvability have been given, see Love (2003), Schlichting and Murren (2004), Hansen (2006), Pigliucci (2008), Brookfield (2009), Pavlicev and Wagner (2012), Kopp and Matuszewski (2014) for examples,

reviews, and discussions of these. Most of these put emphasis on the ability for adaptive evolution, but Schlichting and Murren (2004) explicitly want to include capacity for non-adaptive evolution.

Evolvability in Quantitative Genetics

The term evolvability was introduced to quantitative genetics by Houle (1992), who operationalized it as the expected response to selection. Houle criticized the standard use of heritability as a measure of evolutionary potential, and suggested instead the use of mean-scaled additive variation as a comparative measure of 'evolvability.' Houle (1992) worked with the standard deviation divided by the mean (the coefficient of variation), but the variance divided by the mean squared is a more direct measure of evolvability because the change in the trait mean per generation under directional selection measured by a selection gradient β is

$$\Delta\bar{z} = V_A\beta$$

where V_A is the additive genetic variance. Hence, if evolvability is to be measured as the response to selection per strength of selection, the evolvability becomes

$$e = \frac{\Delta\bar{z}}{\beta} = V_A$$

Standardizing this with the mean yields the mean-scaled evolvability,

$$e_\mu = \frac{V_A}{\bar{z}^2}$$

as indeed suggested by Houle (1992) for traits under directional selection. Hoffmann and Parsons (1997) and Hansen *et al.* (2003a, 2011) have argued that e_μ is usually the best measure of evolvability for quantitative traits, but with the caveat that mean-scaled measures should not be used for traits on ordinal, interval, or proportional scales. See also Garcia-Gonzalez *et al.* (2012) for common errors in using these measures. The e_μ measure is practically equivalent to the additive variance on log scale.

Table 1 summarizes the results from literature compilations of univariate evolvability and heritability estimates. One insight is that evolvabilities of complex traits such as life histories, behaviors, and 3D size measures can be an order of magnitude higher than the evolvabilities of simpler traits such as linear morphological measurements. Still, even an evolvability of a tenth of a percent, about the median for linear morphological measurement, can support rapid evolution. With this evolvability, a trait under unit selection (mean-scaled selection gradient of one) would double in 694 generations, and with 1% evolvability, as is common for complex traits, the trait could double in 70 generations. Hence, most univariate evolvabilities are large enough to propel essentially instantaneous adaptation on geological time scales.

Table 1 also summarizes mean-scaled mutational variances. These are obtained from mutation-accumulation experiments (Halligan and Keightley, 2009), and reflect the amount of additive genetic variance that arises in each generation by new mutations. These estimates are no more than 2–3 orders of magnitude below corresponding measures of standing variation, which implies that standing evolvabilities can be replaced over a few hundred generations (Houle, 1998), and the supply of mutations does not appear very limiting for the evolvability of univariate traits. Life-history traits tend to have more than an order of magnitude higher mutational evolvability than morphological traits. The high evolvability of life history and other complex traits may therefore be caused by their higher mutability. Houle (1998) suggested that this is due to a larger mutational target size, as more genes may have an effect on more complex traits.

Table 1 Median evolvabilities (mean-scaled additive variances), e_μ, and heritabilities, h^2, for univariate traits

Trait group	e_μ	h^2	*Source*	n
All traits	0.25%	0.33	H92	842
	0.26%	0.29	HPH11	960
	1.28%[a]	–	GG12	35
Life history	0.80%	0.20	H92	131
	0.95%	0.16	HPH11	149
	1.42%	–	GG12	10
Morphological	0.15%	0.39	H92	545
Linear morphological	0.09%	0.35	HPH11	394
	0.14%	–	GG12	7
Weight and volume	0.94%	0.27	HPH11	125
	1.13%	–	GG12	10
Morphological counts	0.21%	0.40	HPH11	50
Shape	0.37%	0.29	HPH11	42
Growth	1.00%	0.28	H92	163
Physiological	0.49%	0.12	HPH11	89
	2.60%	–	GG12	3
Behavior	1.93%	0.26	HPH11	44
	2.60%	–	GG12	4
Developmental stability	0.05%	0.03	HPH11	28
Mutation evolvability				
All traits	0.0032%	0.0023	HML96	57 (56)
Life history	0.0190%	0.0012	HML96	17 (16)
Life history	0.0257%	0.0012	HK09	34 (19)
Morphological	0.00072%	0.0032	HML96	26
Growth	0.0025%	0.0023	HML96	10

[a]This higher total is influenced by a lower proportion of linear traits in this study. Based on published reviews of additive or mutational genetic variances from which median evolvabilities could be computed: H92 is Houle (1992), HML96 is Table 1 from Houle *et al.* (1996), HK09 is Table 1 from Halligan and Keightley (2009) not including virus, HPH11 is Hansen *et al.* (2011), GG12 are error-corrected estimates from appendix 1 in Garcia-Gonzalez *et al.* (2012). Trait categories follows source except for Garcia-Gonzalez *et al.* (2012) that were grouped according to the criteria in Hansen *et al.* (2011). The h^2 for mutational traits is relative to the environmental variance in the lab experiment. Sample sizes in parentheses are for heritabilities if different from evolvabilities.

Evolvability and Heritability

The classic and still most commonly used measure of evolutionary potential in quantitative genetics is the narrow-sense

heritability,

$$h^2 = \frac{V_A}{V_P}$$

where V_P is the total phenotypic variance in the population. Under certain assumptions this determines the slope of the parent–offspring regression (Lynch and Walsh, 1998). The heritability is related to evolutionary potential because it quantifies parent–offspring similarity, a core element of evolution by natural selection. Also, the 'breeder's equation,'

$$\Delta\bar{z} = h^2 S$$

where S is the selection differential, suggests that heritability is a useful measure of evolvability in Houle's sense of predicting the response to selection if selection is measured by a differential and not by a gradient. The two formulations are mathematically equivalent because

$$\Delta\bar{z} = h^2 S = \left(\frac{V_A}{V_P}\right) S = V_A \left(\frac{S}{V_P}\right) = V_A \beta$$

since the selection gradient equals the selection differential divided by the phenotypic variance. Mathematical equivalence does not equal biological equivalence, however, and the two formulations make radically different assumptions as to what variables can be treated as independent biological entities.

Houle (1992) argued that most variation in heritabilities is due to variation in the environmental component of the phenotypic variance and less to variation in additive variances. A consequence of this was an overturning of the established generalization that life-history traits and fitness components had little additive genetic variance while morphological traits had more. Life-history traits indeed have lower heritabilities than morphological traits (**Table 1**), but this is due to higher levels of environmental variance and not to lower levels of additive variance.

Hansen *et al.* (2011) showed that heritabilities and (mean-scaled) additive genetic variances are almost completely uncorrelated with each other. This puzzling result comes from the fact that the additive genetic variance tends to covary with other components of variance. It can be shown theoretically that levels of additive genetic variance scale positively with levels of dominance and epistatic variance, and there is a strong empirical correlation between genetic and environmental variances. Traits that are sensitive to genetic perturbances also tend to be sensitive to environmental perturbances. When heritabilities are used to measure additive genetic variance we are in effect using a measuring stick (the phenotypic variance) that is strongly correlated with what we want to measure. The effect of this 'rubber measure' is to remove essentially all correlation between the two.

In itself this observation does not establish the correct measure of 'evolvability,' but it does show that heritabilities cannot be used interchangeably with additive genetic variances that are either mean standardized or unstandardized.

Evolvability for Multivariate Traits

Evolutionary quantitative genetics describe the evolutionary response in a trait vector, $\mathbf{z}$, as

$$\Delta\bar{\mathbf{z}} = \mathbf{G}\boldsymbol{\beta}$$

where $\boldsymbol{\beta}$ is the selection gradient, a vector consisting of partial regression coefficients of relative fitness on the traits, and $\mathbf{G}$ is the additive genetic variance matrix with variances along the diagonal and covariances on the off diagonals (e.g., Lande and Arnold, 1983). The possibility of studying genetic constraints through the G-matrix was part of the attraction of evolutionary quantitative genetics (e.g., Cheverud, 1984; Arnold, 1992). The G-matrix quantifies the role of genetic covariance in impeding or channeling evolutionary change. The response to selection may deviate from the direction of the selection gradient toward directions with more additive genetic variation (higher evolvability).

Strictly speaking, this equation describes evolution on a planar fitness surface (linear directional selection). This is usually a good approximation for a few generations, but over time nonlinearities in the fitness landscape will change the gradient, as the population evolves into more or less steep parts of the landscape.

For example, if the population evolves on a conical fitness landscape then the selection gradient will get more shallow and eventually approach zero as the summit is reached. This means that the summit of a single-peaked fitness landscape will always be reached, even if in a roundabout way, as long as there is positive evolvability in all directions (i.e., $\mathbf{G}$ is of full rank). With alternative peaks, however, patterns of evolvability may influence which optimum is reached.

Hansen and Houle (2008) proposed to measure evolvability in (vector) direction $\mathbf{x}$ as the length of the projection on $\mathbf{x}$ of the response to a (unit) gradient in direction $\mathbf{x}$. This yields an evolvability, $e(\mathbf{x}) = \mathbf{x}^{\mathrm{T}}\mathbf{G}\mathbf{x}$, where T denotes transpose and $\mathbf{x}$ is normalized to unit length. This measure is equivalent to the additive genetic variance in direction $\mathbf{x}$ (Lin and Allaire, 1977), and also proportional to the increase in fitness (Agrawal and Stinchcombe, 2009; Chevin, 2013).

Several related measures focus on how genetic covariances affect evolvability. Agrawal and Stinchcombe (2009) suggested comparing evolvabilities, $e(\mathbf{x})$, with and without genetic covariances set to zero, and Kirkpatrick (2009) presented a decomposition of the response into a correlation matrix and single trait evolvabilities. Marroig *et al.* (2009) proposed to use evolutionary flexibility, which is measured with the cosine of the angle between a selection gradient and the response it induces. A population that tracks closer to the direction of selection is more 'flexible.'

These measures do not capture constraints from stabilizing selection on other characters. Hansen *et al.* (2003b) proposed to study selective constraints with 'conditional evolvability' defined as the evolvability of a character when other defined characters are kept invariant. They showed that this equals the additive genetic variance of the residuals from a regression on the constraining variables (the conditional variance). Hansen and Houle (2008) showed that the conditional evolvability along a (vector) direction $\mathbf{x}$ when the traits are constrained to

evolve only in this direction is $c(\mathbf{x}) = (\mathbf{x}^T\mathbf{G}^{-1}\mathbf{x})^{-1}$, with $\mathbf{x}$ normalized to unit length.

Does Evolvability Affect Evolution?

If univariate evolvabilities are not limiting on geological time scales as indicated by **Table 1**, we expect trait evolution to be governed by selection and adaptation, and genetic constraints to be unimportant. This view approached a consensus, but has come under scrutiny. Hansen and Houle (2004, 2008), Blows and Hoffmann (2005), Kirkpatrick (2009, 2010), Gomulkiewicz and Houle (2009), and Walsh and Blows (2009) are among those who have raised questions. The arguments are concerned with multivariate constraints. Even if individual traits appear evolvable, the variation they display may be tangled up with other characters or based on alleles with generally deleterious effects on fitness. Conditional evolvabilities may be much smaller than unconditional evolvabilities.

Starting with Schluter (1996), comparing the direction of maximal evolvability (aka genetic line of least resistance) with direction of species divergence has been a standard test for genetic constraints. This approach suffers from methodological difficulties, and has not yielded conclusive results. More recent tests based on direct measures of evolvability seem to support a correlation between evolvability and rates and directions of change, but this is far from conclusive (Bolstad *et al.*, 2014).

Lee and Gelembiuk (2008) argue that invasive species often have high evolvability and are able to rapidly adapt to novel environments. There is also evidence that species in stressful environments or with wide ranges are more evolvable (Hoffmann and Parsons, 1997; Hoffmann *et al.*, 2003; Kellermann *et al.*, 2012). Evolvability may be an important predictor of the ability of species to survive climate change (Hoffmann and Sgro, 2011; Hoffmann *et al.*, 2013).

Determinants of Evolvability

Most research on evolvability is qualitative and usually related to the structure of the genotype–phenotype map. "Evolvability is the ability of the genetic system to produce and maintain adaptive genetic variants" (Hansen, 2006, p. 129), and the question is what properties the 'genetic system' needs to achieve this. The following is a list of such properties. Raff (1996), Gerhart and Kirschner (1997), Budd (2006), and Hansen (2006) are general reviews from this point of view.

Determinants of Evolvability: Continuity

Continuity of the genotype–phenotype-fitness relation was one of Lewontin's (1978) criteria for evolution to be possible. The argument, which goes back to Fisher (1930) and even to Darwin's gradualism, is that evolvability requires improvements in small steps. It must always be possible to make small changes in phenotype with small changes in genotype, and there must be paths of fitness improvements with small steps. Dawkins (1996) gives an elegant discussion of this point.

Determinants of Evolvability: Modularity

Lewontin's (1978) other requirement was 'quasi-independence,' which means that it must be possible to change a character without destroying the adaptations of other characters. Wagner and Altenberg (1996) identified the combinatorial explosion of interactions between parts as the primary problem of complex adaptation, and argued that the solution must be a modular structuring of the genotype–phenotype map that restricted pleiotropic interactions to happen within and not between modules. This idea connected well with observations of developmental modularity where many organs after their initiation are internally regulated and able to develop independently of the rest of the organism (e.g., Raff, 1996). In evolutionary developmental biology, modularity almost appears as a synonym of evolvability. This is manifest both in the idea of evolution being driven by changes in *cis*-regulatory 'modules' (Carroll, 2008), and in the treatment of organs and gene networks as autonomous units, as for example, seen in the studies of butterfly eyespots and their patterning gene networks (Brakefield and Roskam, 2006).

Modularity is often measured as a lack of pleiotropic links between modules. Variational independence may also be achieved with compensatory pleiotropic effects, however, a degree of pleiotropy may even increase conditional evolvability (Hansen, 2003; Pavlicev and Hansen, 2011).

Determinants of Evolvability: Coordination and Integration

If mutations affect traits in a coordinated manner they are more likely to fulfill an adaptive need and less likely to produce maladaptive side effects. For example, left–right symmetries in bilaterians increase the probability that mutations will affect paired appendages in a coordinated manner (Dawkins, 1996). The flip side is that asymmetries become harder to evolve. Integration increases evolvability in some directions at the cost of other directions. Olson and Miller (1958), Riedl (1978), Cheverud (1984), Jones *et al.* (2007), and Pavlicev *et al.* (2011) are among those who have argued organisms are structured or integrated so as to produce coordinated variation that reflects functional integration.

Determinants of Evolvability: Robustness

Genetic robustness is the ability to maintain an appropriate phenotype when experiencing mutation or other genetic changes. Since evolvability depends on the ability to express variation, the immediate expectation is that canalization and robustness will decrease evolvability. 'Hidden' variation may accumulate in robust systems, however, the expressed variation in mutation-selection balance is not necessarily reduced (as in the 'Haldane–Muller principle'). Robust systems may also be able to boost evolvability by releasing hidden variation if they become decanalized (Hermisson and Wagner, 2004). Rutherford and Lindquist (1998) suggested that the ability to decanalize in periods of stress may exist as an adaptation for evolvability if the stress is caused by a changing environment.

They further presented evidence that the heat-shock protein Hsp90 may act as a 'capacitor' to release variation under stress. Although the evolution of capacitors is possible (Masel, 2005), the release of hidden variation under disturbance is a generic property of genetic systems (Hermisson and Wagner, 2004), which makes it hard to assess whether observed 'capacitors' are adaptations for evolvability.

A positive relation between robustness and evolvability has been found in some genetic systems (reviewed in Wagner, 2005). For example, models of secondary RNA-folding configurations has the property that the more robust configurations are also those with the largest set of neighboring configurations, and through neutral drift among equivalent genotypes that make the same configuration it can be easier to evolve to a new configuration from a robust configuration (Wagner, 2008).

Determinants of Evolvability: Mutability

Mutation is a necessary prerequisite for long-term evolvability. Although levels of standing additive variance in mutation-selection balance depend nonlinearly on strength of selection, population size, number of loci, epistasis, pleiotropy, mutation rate, and mutational effect sizes, and is thus hard to predict, the estimates in **Table 1** indicate that the supply of new variation through mutation is unlikely to severely constrain the evolvability of quantitative traits. Similarly, estimates of molecular rates of mutation including base-pair substitutions, as well as duplications, deletions, and transpositions of larger bits of DNA are often surprisingly high in complex organisms (Lynch, 2007).

Duplication and subsequent divergence through subfunctionalization or neofunctionalization is an important mechanism of gene and genome evolution (Force *et al.*, 1999; Lynch, 2007). It is also important on the morphological level as in the classical model of how segmentation can give rise to segments with new specialized functions (e.g., Raff, 1996). Duplication and also co-option of genes and structures for new functions is made possible by the modularity and robustness of the genetic and developmental systems.

If evolution is mutation limited, we would expect population size to be an important determinant of evolvability. This does not seem to be the case except in very small populations (Willi *et al.*, 2006).

Determinants of Evolvability: Recombination

The realization that recombination of Mendelian genes generates new variants and provides a potential for a population to evolve far outside its initial range of variation dates back to the beginning of the twentieth century and was instrumental in establishing natural selection as a force capable of producing large differences between species. Since then, the origin and maintenance of sex and recombination has been a major topic of research (Williams, 1975; Bell, 1982). Sexual reproduction allows the bringing together of advantageous mutations that happen in different individuals and give massive boosts to evolvability when evolution is mutation limited (as in small populations). It also allows the purging of deleterious variants and is instrumental in maintaining adaptation in small populations.

Determinants of Evolvability: Epistasis

Short-term evolvability depends on additive genetic variance and covariance, but nonlinearities in the genotype–phenotype map will soon start to change gene effects and consequently patterns of variation (**Figure 1**). Epistasis, in the sense of a dependence of gene effects on genetic background, describes such nonlinearities, and if there are systematic patterns of gene interaction, systematic changes in evolvability may ensue (Rice, 1998; Hansen, 2006, 2015). In particular, Carter *et al.* (2005) have shown that positive directional epistasis, where allele substitutions with positive effects on the trait tend to elevate the effects of other positive allele substitutions (including mutations), will elevate evolvability under directional selection, whereas negative directional epistasis, where the effects of other positive substitutions are decreased, will lead to canalization and reduced evolvability. Hence, it is not epistasis per se that is important, but the patterns of gene interaction. The traditional epistatic variance components from statistical genetics are uninformative about (relevant) patterns of epistasis, and thus uninformative about evolvability (Hansen, 2013).

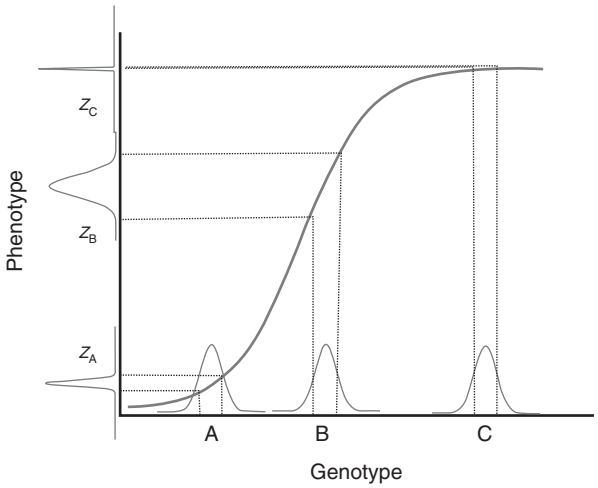

Figure 1 The genotype–phenotype map, epistasis, and the evolution of evolvability. Similar amounts of 'molecular' genetic variation is shown around three positions (A, B, C) in genotype space on the *x*-axis, but these are mapped to very different levels of expressed (additive genetic) variation on the phenotype axis. Evolution from left to right in genotype space will first increase evolvability due to positive curvature in the genotype–phenotype map from A to B and then 'canalize' due to negative curvature from B to C (e.g., Rice, 1998). Positive curvature will be expressed as positive directional epistasis because gene effects increase when the genetic background change from A to B, and negative curvature will be expressed as negative directional epistasis. Hence, directional epistasis makes the genetic variance evolve (Carter *et al.*, 2005). Modified from Hansen, T. F., 2015. Measuring gene interaction. In: Moore, J.H., Williams, S.M., (Eds.), Epistasis: Methods and Protocols. New York, NY: Humana Press, pp. 115–143.

The Evolution of Evolvability

Gould (2002, p. 1270) presented the 'paradox of evolvability' in terms of the question, How can something evolve that is not of immediate use? This is not much of a paradox as stated, because there is no doubt evolvability will evolve when the genetic system evolves (Pigliucci, 2008), but the paradox illustrates skepticism toward individual-level adaptations for evolvability. Many find it more plausible that evolvability is a group- or species-level adaptation (e.g., Waddington, 1957; Gerhart and Kirschner, 1997; and even Dawkins, 1996). Others favor nonadaptive evolution as a side effect of selection on the evolvable trait (Hansen, 2011), canalizing selection on variation or robustness (Layzer, 1980; Le Rouzic *et al.*, 2013), or more general organizational changes in the genetic system (Maynard Smith and Szathmáry, 1995; Budd, 2006). Yet others favor neutral evolution of evolvability due to 'systems drift' (True and Haag, 2001; Wagner, 2005), or due to weak selection against changes in genome architecture in finite populations (Lynch, 2005, 2007). A form of individual-level adaptation for evolvability is also possible in that it may benefit an individual to make variable offspring to allow selection to pick the best adapted, as exemplified by the tangled-bank hypothesis for maintenance of sex (Ghiselin, 1974; Bell, 1982). Unfortunately, much work on the evolution of evolvability does not separate evolution and adaptation (Gardner and Zuidema, 2003; Hansen, 2011).

See also: Developmental Biases on Morphological Evolvability. Robustness and Evolvability in Molecular Evolution

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F

Founder Speciation

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Introduction

When colonizing new areas, species may discover new niches, be subjected to a novel selection regime, and evolve new traits that differentiate them from their ancestors (Thomas *et al.*, 2001; Gillespie and Roderick, 2002; Kawecki, 2008; Sexton *et al.*, 2009). If the colonizing population is small, its genetic composition may only contain a subset of the genetic variance from the parental population. Through further sub-sampling in subsequent generations, these *founder events* might cause a significant reduction of the effective population size. The loss of genetic variation and the rescaling of allele frequencies that occurs throughout the genome during the colonization process is known as a *founder effect* (Templeton, 1980; Coyne, 1994).

Founder effects can have three consequences for the evolutionary potential of a population. If the amount and nature of the remaining genetic variation of the founders is low and the bottleneck catastrophically reduces the levels of genetic variance, the founder effect might lead to extinction of the population (Frankham, 1995, 1998). A second possibility is that the new population will suffer from inbreeding depression. Natural selection acts more expediently on larger populations, so founder events might constrain the evolution of founder populations simply because there will be a lag period before new mutations accumulate for selection to act on (Hoffmann *et al.*, 2003; Willi *et al.*, 2006; Killermann *et al.*, 2006; Hill *et al.*, 2006). Finally, high degrees of inbreeding can reallocate the amount of additive genetic variance and lead to trait decanalization, thereby overcoming genetic constraints that could not otherwise be overcome in a larger population (Cheverud and Routman, 1996; Turelli and Barton, 2006). One of the mechanisms by which this can occur is when inbreeding uncovers and perpetuates combinations of alleles that would be selected against, or simply remain cryptic, in large populations (Carson, 1990; Gibson and Dworkin, 2004; Schlichting, 2008). Occurrences of the first two scenarios – extinction and inbreeding depression – have been extensively documented (Hedrick and Kalinowski, 2000; Pekkala *et al.*, 2014). The prevalence of the third possible outcome remains debated. While it has been shown that inbreeding can lead to significant changes in the phenotypic average and an increase in the additive component of the total phenotypic variance for a variety of morphological, physiological, sexual, and life history traits (Goodnight, 1987; Lopez-Fanjul and Villaverde, 1989; Garcia *et al.*, 1994), it is unclear how much of this increase in phenotypic variance is really heritable and thus relevant to evolution (Bryant *et al.*, 1986; Lopez-Fanjul and Villaverde, 1989; Garcia *et al.*, 1994; Wade *et al.*, 1996; Whitlock and Fowler, 1999).

Founder events may also lead to changes in traits potentially involved in reproductive isolation and generate reproductive isolating mechanisms. This process, termed *founder speciation*, hypothesizes that small population size could foster the evolution of reproductive isolation, and ultimately result in new species (Templeton, 1980). Support for founder speciation is ambiguous. Theoretical treatments have suggested founder speciation is likely in some cases and unlikely in others. Even though the majority of founder speciation models are unrealistic and all models of founder speciation are regarded as unfeasible (Charlesworth, 1997; Coyne and Orr, 2004), a subset of models does work, yet remains largely untested (Slatkin, 1997; Uyeda *et al.*, 2009).

Laboratory experiments have also found support both for and against founder speciation. As a result, one camp of evolutionary biologists argues that founder effects might be the driving force in evolutionary biology and the most prevalent form of speciation (Templeton, 2008). Others have declared the topic solved, stating that founder effects play no role in the evolution of new species (Charlesworth, 1997; Coyne and Orr, 2004). The relative importance of the process likely resides between these two views. It is, therefore, important to evaluate if founder speciation is possible in actual biological systems. The outstanding question is thus more empirical than theoretical. Here, evidence both for and against founder speciation is compiled and its implications investigated.

Theoretical Support

Founder speciation has been proposed to explain how island and high-altitude endemic species might have originated from small parental populations (Vermeij, 1987; Paulay and Meyer, 2002). The main support for the founder speciation hypothesis comes from theoretical efforts that have modeled how quickly allele frequency changes induced by genetic drift can result in reproductive isolation (Gavrilets and Hastings, 1996).

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Ernst Mayr proposed the first model of founder speciation (Mayr, 1942, 1954; Provine, 1989; Coyne, 1994). His verbal proposition stated that genetic drift could affect the level of genetic variance in a system in such a way that it would determine how selection could act, thus making founder effects important in the origin of new species. The premise of the model is quite simple: given that epistasis is rampant across the genome, the selective value of an allele involved in reproductive isolation is contingent on the genetic backgrounds present in the ancestral population. When such a population undergoes a population size decrease, a fast change of the genetic environment might occur, simply because the allele frequencies of the founding population might have changed. Those changes might lead to changes at other loci similar to a chain reaction (i.e., genetic revolution). Finally, changes across the genome might lead to a new state of equilibrium which in turn might give rise to a population that is reproductively isolated from the parental population. This model, known as *genetic revolutions* was inspired by "the conspicuous difference of most peripherally isolated populations of species" (Mayr, 1954). Mayr's idea was controversial among geneticists (e.g., Haldane, 1964 reviewed in Provine, 2004) and fell out of favor because of its vagueness, and the lack of evidence supporting any of the components for the process (Lewontin, 1965; Lande, 1980; reviewed in Provine, 2004; Nei, 2005).

Mayr's genetic revolutions model may not be a prevalent mode of speciation, but it is an appealing hypothesis because it relies on two simple, testable ideas. First, alleles with the potential to cause reproductive isolation must be segregating within species. Thorough scans for within-species incompatibilities have discovered them in several taxa (Charlesworth and Laporte, 1998; Reed and Markow, 2004; Good *et al.*, 2008; Cutter, 2012; Corbett-Detig *et al.*, 2013). Second, the effect of such an allele must be contingent on the genetic background (Good *et al.*, 2008; Matute *et al.*, 2014). The results from several studies suggest that background-specific effects are not only real but also pervasive, even for such extreme phenotypes as hybrid sterility and inviability. Thus, as occurs with most (if not all) phenotypes (Gibson and Dworkin, 2004; Chari and Dworkin, 2013), the genetic background can modify the penetrance of alleles involved in reproductive isolation.

A second model proposed by Hampton Carson, dubbed *founder-flush speciation*, requires not only a founder event but also the colonization of a new habitat (Carson, 1968, 1971, 1975; Carson and Templeton, 1984). In this model, the founder event reduces the effective population size, leading to changes in allele frequencies along the genome. Recombination in the newly arisen population will generate new allelic combinations that might not have survived in the ancestral population. Natural selection by the new ecological environment then locks together new allelic combinations, which might lead to the origin of new reproductive barriers. Carson was heavily influenced by the spectacularly diverse Hawaiian *Drosophila* and proposed that these species might have arisen through several rounds of founder-flush speciation (Carson and Kaneshiro, 1976; Mueller-Dombois *et al.*, 1981). Even though these *Drosophila* island endemics are frequently cited as the prime examples of founder speciation (Kaneshiro, 1976; Carson and Kaneshiro, 1976; Carson, 1982; DeSalle and Templeton, 1988; Hollocher and Williamson, 1996), the connection between island colonization and the origin of new reproductive isolating mechanisms remains to be explored.

Despite founder speciation being disregarded by some due to its presumed slow speed of action, there are theoretical arguments suggesting drift can promote rapid divergence (Nei, 1976; Nei *et al.*, 1983; Slatkin, 1996). Theoretical models have shown that drift can promote rapid divergence in sexually selected traits and that the time required for inducing reproductive isolation is relatively short if inbreeding is strong (Lande, 1981; Wu, 1985; Uyeda *et al.*, 2009). These simulations also suggest that the evolution of sexual isolation by sexual selection is stochastic in small populations. In groups of large animals (i.e., vertebrates), the effective population size might commonly be small and stochastic changes might play a role in the divergence of mating preferences and reproductive isolation.

Theoretical Challenges

Theories of founder speciation have been strongly scrutinized since their inception (Barton and Charlesworth, 1984; Barton, 1989; Gavrilets and Hastings, 1996; Charlesworth, 1997; Slatkin, 1997). The main, and most cited, criticism is that founder speciation requires very specific conditions to take place, thus rendering it unlikely to be an important mechanism of speciation. Some simulations have found that reproductive isolation can evolve through drift, though other models have found that drift can constrain the evolution of the same types of isolating barriers if starting conditions are slightly different (i.e., Charlesworth and Smith, 1982; Uyeda *et al.*, 2009). Several authors have proposed that models of founder speciation are unrealistic because of two general principles in population genetics. First, reproductive isolation is more likely to evolve through gradual evolution than through large abrupt changes like founder events (Barton and Charlesworth, 1984; Barton, 1989; Coyne and Orr, 2004). Second, most models require genetic drift to act for a long time, rather than just in the founding generation, in order to generate new species (Rice and Hostert, 1993; Gavrilets and Hastings, 1996; Gavrilets, 2003; Futuyma, 2006). The ensuing prolonged inbreeding will result in founder populations with low genetic variability and limited capacity to respond to selection, which in turn might hamper their establishment on new environments (Willi *et al.*, 2006; Kawecki, 2008; Kawecki *et al.*, 2012).

A second group of critiques of founder speciation is aiming at the fact that all the original formulations argued that large populations were not likely to split into new species because large populations supposedly had little potential to evolve new traits (Mayr, 1954; Carson and Templeton, 1984; Provine, 1989). Both, theoretical models and experimental results have shown that large populations do not lack evolutionary potential, and if anything, they have a more pronounced response to selection; it is in small populations where drift, or its interaction with selection, plays a larger role in shaping genetic composition (Hill and Caballero, 1992; Kawecki, 2008; Jones *et al.*, 2014).

A final criticism of founder speciation hypotheses is that, even if drift could drive the evolution of reproductive

isolation, the process would be extremely slow and, more often than not, natural selection would be the faster-acting and therefore the prevalent force within the evolving system (reviewed in Coyne and Orr, 2004). The conclusions of simulation efforts (Lande, 1981; Wu, 1985; Uyeda *et al.*, 2009) and the evidence from experimental evolution experiments (see below) have suggested that founder speciation can occur quickly and that ignoring the process because of its supposed glacial pace is short-sighted.

Experimental Evidence

While founder speciation is a longstanding theory, few direct tests have been made of either its validity or prevalence. Evidence for it can be divided into two categories: experimental evolution and natural assessments.

Evidence I: Experimental Evolution

The premise of testing founder speciation with experimental evolution is simple: if founder speciation proceeds quickly and a species has the potential to originate through founder effects, then applying strong inbreeding in laboratory conditions should give rise to reproductive isolation from the original population.

The first such experiments were the results of accidental bottlenecks. An accidental test was performed in *Nereis acuminata*. A single population of this marine polychaete underwent serial bottlenecks following collection and maintenance in the laboratory (Weinberg *et al.*, 1992). Even though the authors observed reproductive isolation between the founded population and the putative parental population, the results might signal cryptic population stratification: the 'founder population' could have been just part of a population of *N. acuminata* that at the time of collection was already reproductively isolated from the putative parental population (Rodriguez-Trelles *et al.*, 1996).

A second type of experiment addressed the effects of bottlenecks in a more controlled manner. In particular, several flush and crash experiments have tested the evolution of reproductive isolation. Large populations of flies (*Drosophila* and *Musca*) were subjected to extreme levels of inbreeding (e.g., cages with hundreds or thousands of individuals were reduced to a single mated female) and levels of reproductive isolation were measured after several such crashes (reviewed in Rice and Hostert, 1993 and Powell, 1997). The rationale behind these experiments is that extreme cases of inbreeding might create the conditions for genetic drift to induce the evolution of reproductive isolation in founder populations (Carson, 1971). These experiments, carried out in at least five different species, demonstrate that reproductive isolation can evolve after two steps: a strong founder event (crash and inbreeding) and a subsequent population expansion (flush; Powell, 1978; Dodd and Powell, 1985; Ringo *et al.*, 1985, 1986; Dodd, 1989; Bryant and Meffert, 1993 but see Galiana *et al.*, 1993; Moya *et al.*, 1986). A second general pattern is that the increased reproductive isolation detected between the parental population and the inbred/flushed populations is usually asymmetric and it is almost always in the form of stronger female choice from the parental population discriminating against males from the inbred/flushed populations (Powell, 1978).

All these studies, however, have two common, yet understandable, caveats. The first one is that all the experiments have been carried out in flies, and it is unclear to what extent the results from flies can be expanded to other organisms. The second is that, in all of these experiments, the number of replicates has always been low (<50 replicates) and consequently, they have little power to determine how frequently founder events lead to the origin of reproductive isolation.

The study of founder speciation using experimental evolution has been formally criticized on three major grounds (Rice and Hostert, 1993; Coyne, 1994; Mooers *et al.*, 1999). First, founder-crash experiments usually involve newly collected lines from different geographic localities. Cryptic population stratification among collected individuals might result in differential adaptation to laboratory conditions with reproductive isolation arising as a by-product (Ödeen and Florin, 2000; Rundle, 2003).

Second, the biological parameters that crash experiments depend on are likely to be unrealistic. The most commonly used scheme involves crossing a single pair of flies in a brother–sister mating at every generation, resulting in extreme inbreeding. These conditions likely do not reflect the true nature of a founder effect, though it remains largely unknown how founder population size affects the evolution of reproductive isolation (Powell, 1978, but see Galiana *et al.*, 1993). Only one experimental approach has addressed this question by using multiple pairs of flies instead of one brother–sister pair to start every generation. Galiana *et al.* (1993) used *D. melanogaster* to assess the likelihood of founder speciation under different inbreeding schemes. Experimental populations were started with different numbers of founders, and the resulting populations were assessed for their levels of behavioral isolation toward the ancestral population. Populations with fewer founding individuals tended to be more reproductively isolated from the parental population than those with more founders. This conclusion remains provocative, but larger experiments with more statistical power will be required to establish whether the size of the founder population is correlated with the likelihood of founder speciation. Regardless of the actual population sizes achieved after inbreeding founding a natural founder event, these experiments showed that reproductive isolation can evolve as a result of severe reductions in population size, and also demonstrated the feasibility of studying founder effects (and the origin of reproductive isolation) in laboratory settings.

A third and final criticism of the crash and flush experiments is the unstable nature of the resulting reproductive isolation (Powell, 1978; Ringo *et al.*, 1985; Moya *et al.*, 1986 but see Dodd and Powell, 1985). The strength of reproductive isolation attained after experimental bottlenecks waxes and wanes over time in the vast majority of reports (Coyne and Orr, 2004). Once the experimental scheme is relaxed, reproductive isolation does not persist in the crashed populations. This is not a surprising result, as it is predicted by models of reproductive isolation driven by drift (Uyeda *et al.*, 2009). The unstable nature of reproductive isolation in experimental settings suggests that although founder effects can give rise to

reproductive isolation, other forces such as sexual selection, natural selection through reinforcement, or genetic assimilation might be necessary to stabilize the trait.

Criticism of founder speciation reduced enthusiasm for the process, and its study dwindled over the last decades of the twenty-first century. However, a new generation of theoretical and comparative studies has revitalized interest in founder speciation (Templeton, 2014). One of the most comprehensive studies on this subject has been performed in *Anolis* lizards. Schoener and Schoener (1983) were interested in extinction and experimentally introduced *Anolis* lizards (*Anolis sagrei*) in pairs of islands that were previously stratified by habitat. A few years later, they also introduced *Anolis carolinensis* in a subset of the islands. Much to Schoener and Schoener' surprise, the populations persisted in all but the smallest islands, and in some cases, they even attained a population size of hundreds of individuals. These controlled introductions constitute a replicated experiment of the effect of new habitats on small founding populations. Years after the introduction, *A. sagrei* and *A. carolinensis* showed morphological changes that persisted for many generations (Losos *et al.*, 1997, 2001; Kolbe *et al.*, 2012). These morphological changes indicate that the joint action of founder effects and natural selection can be a powerful driver of trait evolution of not only flies, but also anoles.

In another recent study, Matute (2013) used 1000 parallel populations of *Drosophila yakuba* that underwent a strong bottleneck to study whether founder effects could lead to reproductive isolation between the inbred lines and the parental population of *D. yakuba*. As expected, the most common outcome of this extreme inbreeding was extinction of the experimental population. Nonetheless, in a small proportion of cases, founder effects led to a significant level of premating reproductive isolation toward the parental stock. This experiment showed that both the mean behavioral isolation and its variance within lines were much larger in inbred populations than in populations that did not undergo a bottleneck. Finally, Matute also carried out artificial selection experiments on the inbred populations to determine whether the high phenotypic variance in reproductive isolation was caused by large genetic variance. In this study, the increase in phenotypic variance did not confer an increase in the response to selection for reproductive isolation in artificial selection experiments, indicating that the increased phenotypic variance is not caused by increases in additive genetic variance.

These two recent studies have two implications. First, they add to the body of evidence that suggest that founder effects might be important for evolution in two different biological systems but under a particular demographic regime (i.e., very strong bottlenecks). Second, they show that the interest in the experimental study of founder effects has not vanished and has the potential to be rekindled.

Evidence II: Natural Variation

An alternative approach to study the role of founder speciation has been to assess insular or isolated populations and look for evidence that they experienced a population bottleneck in the past. Three different methods have been applied to this end: range comparisons in sister species, phylogenetic methods, and demographic methods. The first approach in order of historical appearance is range comparisons in sister species. These studies have tried to determine the importance of founder effects by using pairwise comparisons between the range sizes of sister species to infer the relative frequency of founder, vicariant, and sympatric speciation (Lynch, 1989; Friesen and Anderson, 1997; Losos and Glor, 2003). If two sister species show very dissimilar geographic ranges, then it is considered likely that speciation occurred through founder effects; species with neighboring ranges of similar size are thought to have diverged via vicariance, and those with overlapping ranges potentially diverged in sympatry. This method, however, should be taken with reservations because it assumes that current geographic ranges reflect the ancestral ranges when speciation occurred, an assumption that remains largely untested (Losos and Glor, 2003; Richards and Carstens, 2007). Lynch (1989) applied this method to 66 frog species pairs, and 10 speciation events were best explained by founder speciation. Chesser and Zink (1994) evaluated geographic ranges of 20 pairs of North American species and inferred that two of them might have occurred through founder speciation. In line with the results from experimental evolution studies, both surveys of natural diversity suggested that small populations can indeed give rise to new species but that the relative occurrence of founder speciation is not as common as vicariant speciation.

A second family of comparative analyses has used phylogenetic models to investigate the role of geography in speciation (Barraclough and Vogler, 2000; Barraclough and Nee, 2001). These efforts are also based on analyzing the geography of sister clades but additionally incorporate phylogenetic uncertainty by studying all nodes in a species-level phylogeny. The premise, as in pairwise comparisons, is to use current species range to establish whether speciation occurred through colonization of a new and small habitat, or by the splitting of an ancestral habitat in two equivalent partitions. Again, these approaches find support for founder speciation in a small proportion of cases. This phylogenetic framework is superior to the use of pairs of species alone but still has its caveats. First, range asymmetry may result from the shape of the landscape, for example when a mountain range is located near the edge of a continent (e.g., the Andes). Second, as was a concern with the previous method, geographical ranges may have changed since speciation, a particular concern for areas with no historical record of species distributions (Barraclough and Vogler, 2000, Warren *et al.*, 2014).

Finally, a third approach has used a coalescent framework (i.e., isolation–migration models) to estimate a proxy of the effective population sizes during speciation. Estimation of ancestral population size is probably the most used approach (Baker and Moeed, 1987; Vincek *et al.*, 1997; Walsh *et al.*, 2005; Yeung *et al.*, 2011; Lane and Shine, 2011; Yoshida *et al.*, 2014). These demographic methods, however, are fraught with problems that limit the interpretation of the role of founder events (Becquet and Przeworski, 2009). Notably, changes in population structure over time may affect coalescent rate estimates and, as a consequence, influence effective population size estimates. To overcome these issues, a series of population genetics approaches have been developed to calculate changes

in the effective population size over time using whole genome data (Li and Durbin, 2011; Sheehan *et al.*, 2013). Even if such demographic approaches detect a significant reduction in past effective population size, it does not reveal anything about either its effect on the origin of reproductive isolation or the presence of a founder effect. Problems interpreting these results thus limit the possible application of purely demographic studies to establish the likelihood and importance of founder speciation.

Conclusions

The importance of founder speciation in the evolution of new species remains a contentious issue in evolutionary biology (Templeton, 2008). Theoretical studies have proposed that founder speciation is possible in certain biological contexts. Experimental evolution studies have shown that bottlenecks can lead to changes in reproductive isolation in the same manner that inbreeding affects other phenotypic traits: a displacement of the mean phenotypic value and a substantial increase in the between-population phenotypic variance (Powell, 1997). Comparative and phylogenetic analyses have also lent support for the evolution of new species after habitat shifts and founder events. The combination of these results has changed the focus of study from whether founder speciation is possible (it is) to the biological conditions that affect its efficacy and frequency. The current evidence suggests that founder speciation might be rare. Rarity, however, does not equal unimportance, and in cases such as the colonization of new environments, founder speciation might be more important than is currently appreciated. Only an integrated framework of comparative phylogenetics, population genetics, and experimental evolution will be able to disentangle the relative importance of founder speciation and its alternatives.

See also: Genetic Drift, Models of Random. Speciation, Geography of. Species Concepts and Speciation

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Fungal Evolution: Aquatic–Terrestrial Transitions

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Glossary

Arbuscular mycorrhizal symbiosis Symbiotic association between a plant and a fungus of the phylum Glomeromycota. Nutrient transport occurs through a structure called arbuscule inside the plant cells.
Cryptogamic Formed by bryophytes (mosses, liverworts, and hornworts), ferns, horsetails, lichens, and algae.
Dikarya Clade containing Ascomycota and Basidiomycota.
Dikaryon A type of hypha that is formed after sexual reproduction in some fungi, in which each cell has two unfused nuclei.
Environmental DNA Genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material (Thomsen and Willerslev, 2015).
Hypha A long branching filamentous structure with rigid walls that may be reinforced by perforated cross-wall septa. Hyphae form a network called a mycelium.
Mating types Molecular mechanisms that regulate compatibility in sexually reproducing organisms, often referred to by numbers, letters, or simply '+' and '−.'
Pectinase enzymes Collective term for a group of enzymes that are able to break down or to transform pectins contained in the primary cell walls of terrestrial plants.
Perithecium A small flask-shaped fruiting body in some ascomycete fungi containing the asci (in which the spores are produced).
Rhizoids Hair-like filaments in Fungi and some plants.
Thallus Vegetative body of a fungus (or alga, lichen, bryophyte).
Yeasts Single-celled Fungi.
Zoospores Spores which are self-propelled by means of flagella.

Fungi and Fungal-Like Microoorganisms

Abundant and cosmopolitan, fungi are a major component of terrestrial ecosystems. Phylogenetic analyses of multiple nuclear genes have challenged the traditional division of Fungi (Eumycota) into four main groups – Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota – and a major reclassification of this kingdom has been proposed (Hibbett *et al.*, 2007; **Figure 1**). The most notable changes concern the Chytridiomycota and Zygomycota. Both have been shown to be polyphyletic; Chytridiomycota has been redefined and Zygomycota is no longer a recognized group. In addition, Microsporidia, unicellular parasites, are now included in the Fungi, and several 'basal' genera are not grouped in higher taxa (Blair, 2009). This classification is currently under revision. Cryptomycota (Jones *et al.*, 2011; not shown in **Figure 1**) is a newly recognized group, which has been united with the Microsporidia, based on phylogenomics and shared genomic traits (James *et al.*, 2013). The resolution of branching order in the deepest parts of kingdom Fungi remains uncertain and instead of converging on a single solution, recent studies of early Fungi have produced conflicting phylogenies (Sekimoto *et al.*, 2011).

In addition, some lineages conventionally associated with Fungi based on morphological similarities and similar ways of life (e.g., oomycetes, slime molds) have been reclassified into separate eukaryotic supergroups (Blair, 2009). The oomycetes and hyphochytrids, together with some marine flagellates, form part of the clade defined by Cavalier-Smith and Chao (2006) as the phylum 'Pseudofungi,' which is a sister to the photosynthetic chromistan (golden-brown) algae and belongs to the stramenopiles lineage that otherwise includes brown algae (Beakes *et al.*, 2012).

An Aquatic Origin for the Fungal Lineage

Molecular data shows that the oomycetes (fungus-like organisms no longer included in Fungi) have their evolutionary roots in the sea and that a number of predominantly marine taxa appear to diverge before the two main terrestrial lineages of oomycetes (Saprolegniales and Peronosporales; **Figure 2**; Beakes *et al.*, 2012). Interestingly the changes in thallus morphology that occurred during oomycete evolution, as summarized by Beakes *et al.* (2015), appear similar to the morphological sequence in Fungi. However, while evolutionary transitions from marine to freshwater or terrestrial environments can be traced for oomycetes, transitions to land in the Fungi are less clear:

1. The question of whether the Fungi are marine in origin needs more investigation. Kis-Papo (2005) reported the difficulty encountered in defining marine fungi – the definition is based on ecological and physiological requirements and not on taxonomic relationships – and suggested that they probably had diverse terrestrial origins, subsequently adapting convergently to similar marine ecological niches. In contrast, a study by Le Calvez *et al.* (2009), based on an inventory of fungal diversity in deep-sea hydrothermal environments, concluded that the emergence and initial diversification of Fungi occurred in a marine environment even if the data are not fully conclusive. This study reported an unexpected diversity in three phyla (Chytridiomycota, Ascomycota, and Basidiomycota) and also a distinctive yet previously unknown ancient Chytridiomycota lineage. To explore the diversity of marine fungi, Richards *et al.* (2012) took an environmental

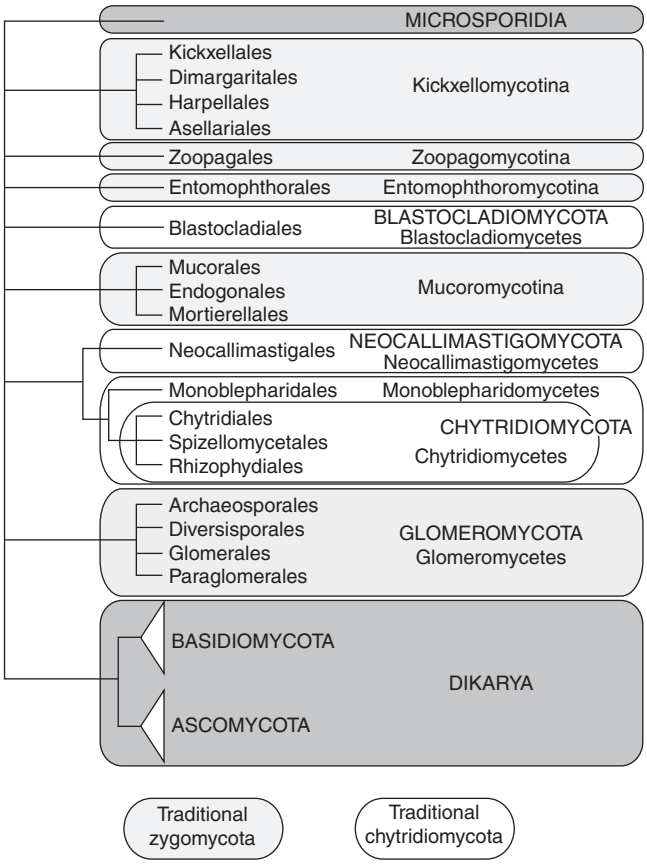

Figure 1 Phylogeny and classification of Fungi (currently under revision) (courtesy of David Hibbett; reproduced from Hibbett, D.S., Binder, M., Bischoff, J.F., *et al.*, 2007. A higher-level phylogenetic classification of the fungi. Mycological Research 111, 509–547, with permission).

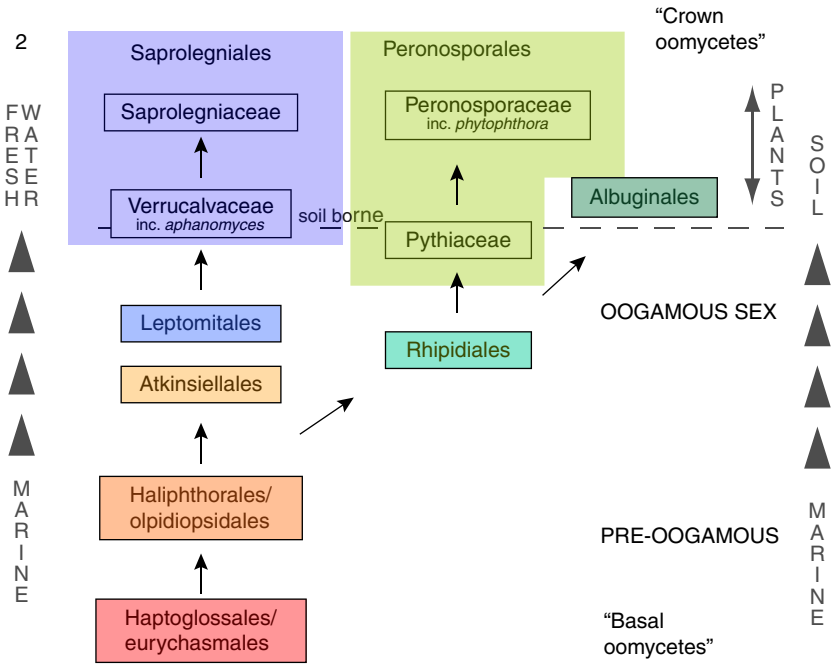

Figure 2 A schematic summary of the possible phylogenetic relationships between the main oomycete orders and families, based on current molecular data and their ecology (courtesy of Gordon Beakes; reproduced from Beakes, G.W., Glockling, S.L., Sekimoto, S., 2012. The evolutionary phylogeny of the oomycete "fungi". Protoplasma 249, 3–19, with permission from Springer).

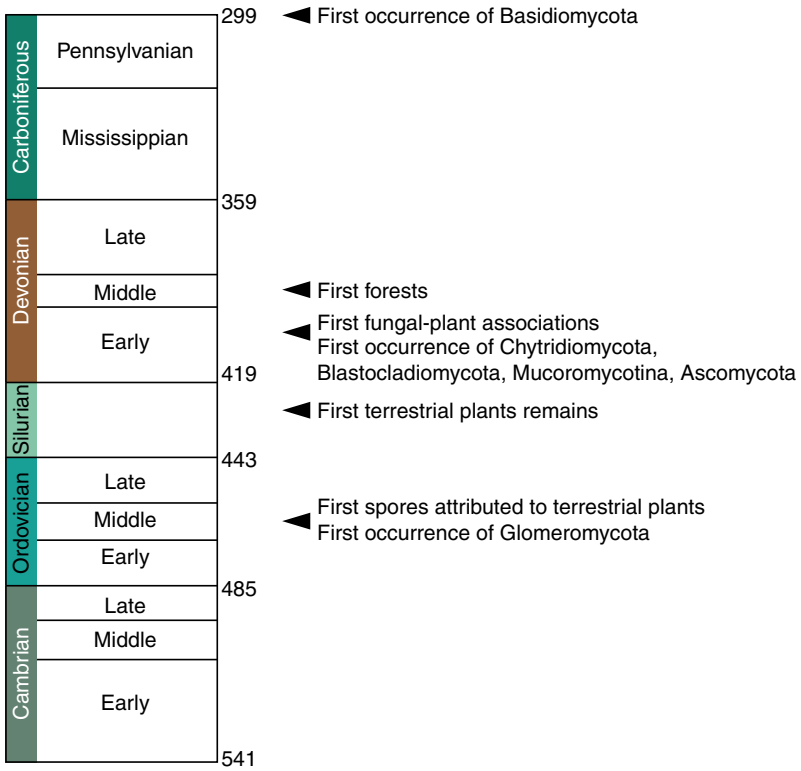

Figure 3 Earliest occurrences of Fungi and plants in Paleozoic times. Ages in millions of years are taken from the International Chronographic Chart of the International Commission on Stratigraphy, 2014.

DNA approach for phylogenetic analyses. They found that fungal sequences detected in marine environments encompass a large diversity of forms and lineages, including chytrids, filamentous hyphal types, and multicellular forms. However, the majority of the sequences clustered with the more-derived Ascomycota and Basidiomycota yeasts. An investigation of the genome of diverse Fungi from marine environments could help to make more precise phylogenetic hypotheses and answer the question of marine ancestry.

2. An aquatic (freshwater) or semi-aquatic origin of Chytridiomycota seems likely, followed by the diversification of the major phyla of Fungi in terrestrial environments. Several lines of evidence point to this hypothesis:

- The phylogeny by James *et al.* (2006), using data from six gene regions and nearly 200 species, shows that the early-diverging fungal lineages consist of a grade of zoosporic fungi, suggesting that the earliest fungi were primarily aquatic and lacked aerial spore dispersal. Subsequent losses of motile spores, which occurred at least four times, parallel the evolution of new mechanisms of spore dispersal, such as aerial dispersal in mycelial groups (e.g., Ascomycota and Basidiomycota) and eversion of the polar tube (an extrusion apparatus that evaginates during spore .discharge) in the Microsporidia.
- Recently, using a phylogeny based on 40 fungal genomes, Chang *et al.* (2015) showed that pectinases, enzymes for degrading plant cell walls, duplicated in an ancestral fungus that probably still lived in freshwater in association with streptophyte algae (= charophytes). As a result the authors considered that early terrestrial fungi might have evolved in semi-aquatic microbial slime, with the ancestors of the Zygomyceta tracking arthropods or other animals onto land, while the ancestors of the Dikarya followed plants.
- Extant Glomeromycota live as obligate symbionts of bryophytes, vascular plants, and cyanobacteria. This raised the possibility that terrestrial members of the Glomeromycota living symbiotically with cyanobacteria or algae in semi-aquatic and humid habitats later became the symbiotic partners of early land plants (Schüßler, 2002). Alternatively a transition to land as plant partners has also been proposed (Selosse and Le Tacon, 1998).
- Research on the origin of the genes acting in the fungal symbiotic pathway (arbuscular mycorrhizal symbiosis involving Glomeromycota) also focuses on algal lineages, such as charophytes, that are related to land plants. A stepwise evolution of the plant symbiotic 'toolkit' (i.e., the set of genes required for symbiosis) in algal ancestors, with several components predating the first land plants, has been recently proposed (Delaux *et al.*, 2012, 2013). Elements of this 'toolkit' may, therefore, have facilitated the interactions between aquatic charophyte-like ancestral algae and diverse symbiotic microorganisms, later being recruited and further developed for mycorrhizal evolution on land.

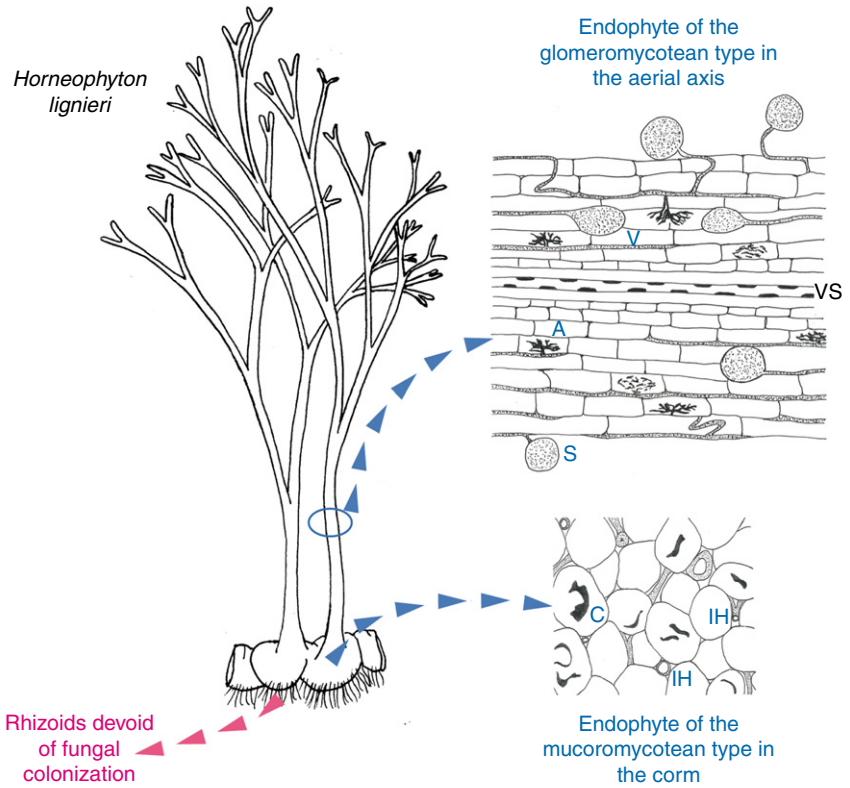

Figure 4 Diagram illustrating dual colonization by endophytes in the Glomeromycota and Mucoromycotina of the fossil plant *Horneophyton lignieri* from the 407 Myr-old Rhynie Chert. S, spore; A, arbuscule-like structure; V, vesicle; VS, vascular strand; C, coil-like structure; IH, intercellular hyphae (reproduced from Strullu-Derrien, C., Kenrick, P., Pressel, S., *et al.*, 2014. Fungal associations in *Horneophyton lignieri* from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant–fungus symbioses. New Phytologist 203, 964–979, with permission from John Wiley and Sons).

Aquatic to Terrestrial Environments – Fossil Evidence of Fungi

Molecular clock studies have produced a range of time estimates for the origin and diversification of Fungi. As a result the molecular timetree shows that, while some phylogenetic uncertainty exists, major lineages of Fungi likely originated in the Neoproterozoic (1000–541 Ma), which is much earlier than the evidence provided by the fossil record (Blair, 2009; Berbee and Taylor, 2010). Microfossils in rocks of the mid Ordovician Period (ca 460–470 Ma) provide the earliest evidence of both fossil plants (earliest spores) and fossil fungi (Glomeromycota) (Rubinstein *et al.*, 2010; Redecker *et al.*, 2000; **Figure 3**), but no direct links between these organisms from that period have been proven. Fungal fossils become more common in the early Devonian (around 400 Ma) (**Figure 3**). The earliest direct evidence of Fungi associated with plant or plant remains is based on organisms fossilized in situ in the 407 million year old Rhynie Chert (Trewin and Rice, 2004). This fossil site is characterized by the occurrence of both aquatic and terrestrial environments. Among the Fungi, zoosporic species, in particular chytrids, were some of the most diverse elements. Rhynie chert Chytridiomycota have been documented inside algae, various plant, or fungal spores and plant tissues. Blastocladiomycota, previously considered a group of Chytridiomycota (**Figure 1**), also occurred associated with plant organs (see Table 1 in Strullu-Derrien *et al.*, 2015). These fungi colonized aquatic freshwater and wet terrestrial environments, and developed either as saprophytes or parasites.

Associations of the arbuscular mycorrhizal type (involving Glomeromycota) have also been described in several plants from the Rhynie chert (e.g., Remy *et al.*, 1994; Taylor *et al.*, 2005; Krings *et al.*, 2007; Strullu-Derrien *et al.*, 2014). Recently a dual colonization involving Glomeromycota and Mucoromycotina (**Figure 1**) was described in one of these plants, indicating that early fungal symbioses were more diverse than assumed hitherto, and overturning the long-held paradigm that the early mycorrhizal partners were exclusively Glomeromycota (Strullu-Derrien *et al.*, 2014; **Figure 4**). In contrast to chytrids in aquatic and wet habitats, symbiotic mutualistic associations with other fungal groups developed with plants living in terrestrial environments (Strullu-Derrien *et al.*, 2014; Taylor *et al.*, 2015). In the early terrestrial environments, Glomeromycota (and possibly also Mucoromycotina) appear to have developed as obligate symbionts of plants, a role that they still occupy today (see above the hypothesis concerning the transition from freshwater to terrestrial concerning Glomeromycota). In the Mucoromycotina, however, the earliest symbionts may have also been saprotrophic.

One fungus from the Rhynie chert has been attributed to Ascomycota (Taylor *et al.*, 1999). This fossil consists of

perithecia immersed within the aerial axes of one of the plants, however it does not fit comfortably within an extant taxonomic group (Berbee and Taylor, 2010) and its habitat (freshwater/terrestrial) has not been explored. During the early phases of land colonization by plants, root systems evolved into a broad range of complex multicellular organs specialized in anchorage and nutrient acquisition, and the size of the plants increased. Unlike the relationships between Fungi and early plants, the history of those involving trees is still not documented (**Figure 3**). One structural feature that has been used to identify fossil Basidiomycota in the absence of sexual reproductive organs is the clamp connection, a distinctive structure formed by growing hyphal cells. The first accepted Basidiomycota based on this feature occurs within a structurally preserved fern from the Carboniferous (ca 330 Ma) (Krings *et al.*, 2011), 80 Ma after the earliest putative occurrence of Ascomycota. Ancestors of all modern groups of Fungi were likely present by the end of the Carboniferous (**Figure 3**) however little is currently known about the transition to land for the most derived groups (i.e., Ascomycota and Basidiomycota).

Evolution of Traits from Primitive to More-Derived Fungi

Several traits should be considered in deciphering the aquatic (freshwater)–terrestrial transition in Fungi. We have chosen to focus on three: aquatic traits, hyphal habit, and sexual reproduction, which illustrate how Fungi evolved from zoosporic ancestors living in aquatic environments to highly adapted terrestrial groups (i.e., Ascomycota and Basidiomycota).

Aquatic Traits (Motile Spores, Non-Hyphal Growth form, Reproduction)

Chytrids represent an ancestral form of Fungi with motile spores, mostly non-hyphal growth form, and predominantly asexual reproduction. Chytrids reproduce through the production of motile spores (zoospores), typically propelled by a single, posteriorly directed flagellum. They are mostly aquatic (freshwater) or, if terrestrial, rely on water for dispersal or infection (Webster and Weber, 2007). Chytridiomycota are mostly non-hyphal Fungi; the simplest form of thallus is a sac-like, multinucleate cell with no appendages or branches. More sophisticated forms have branching rhizoids attached to the thallus body, which may be itself simple or branched (Griffin, 1996). Members of Chytridiomycota are predominantly asexual, with dehiscence and discharge of thin-walled zoospores from sporangial openings. Ultimately zoospores withdraw their flagellum into their body, deposit a cell wall, and develop into a sessile thallus. Asexually produced thick-walled resting sporangia may form (Powell, 2009). Sexual reproduction of Chytridiomycota is speculative, based on only a few studies, but sexual reproduction may be difficult to recognize or observe rather than truly absent. A thick-walled resting sporangium is likely to be the site of meiosis. In Blastocladiomycota, a clade of flagellated Fungi for which the phylogenetic position is not fully resolved (**Figure 1**), the resting sporangia form in water but can resist drying and will germinate with meiosis, releasing haploid zoospores when water is added. The same may be true for resting sporangia of Chytridiomycota (Miller and Dylewsky, 1981; Berbee, personal comm.). Fungi such as chytrids with motile spores are wholly or partly aquatic and require water for dispersal. This trait is lost in terrestrial species, which disperse aerially. In addition, aquatic Fungi are mostly non-hyphal, whereas hyphae developed in more-derived terrestrial groups. Finally, the mode of reproduction (see below) appears to have changed in correlation with the aquatic–terrestrial transition.

Hyphal Habit

The evolution of filamentous hyphae underlies an astounding diversity of fungal form and function, however little is known about diversification among hyphae. For example, recent work from Dee *et al.* (2015) demonstrated multiple independent origins of the hyphal habit by studying the cellular structure and evolutionary origins of the hyphal filamentous form in the Monoblepharidomycetes (Chytridiomycota) (**Figure 1**). Most members of this clade are found in freshwater environments. Hyphal Monoblepharidomycetes are an early-diverging fungal lineage, a clade sister to the genera without hyphae. Reconstruction of ancestral states indicates that hyphae arose independently within this lineage and in at least two other lineages (Ascomycota and Basidiomycota).

Most of the Fungi develop hyphae, which can be siphons (coenocytic hyphae), and which do not form multicellular structures or septate (multicellular) hyphae. In a recent article on the evolution of multicellularity, Niklas (2014) discussed the advantages of developing siphonous and multicellular body plans. The siphonous body plan might have conferred a selective advantage by permitting a single cell to achieve a large size and thus explore nutritionally variable landscapes (e.g., Sekimoto *et al.*, 2011; Niklas, 2014). Achieving a larger size could also have allowed more independent functioning in the face of a hostile environment likely to damage the hyphae, increasing the chance that some portion of an organism survived. The evolution of multicellularity requires the acquisition of a mechanism to partition cytoplasm into uni- or multinucleated cellular units and a mechanism for coordinating cytokinesis with cell wall deposition (Niklas, 2014). However the relationship between cell size, nuclear number, and nuclear division and cell division in fungi is different from plants or animals. In fungi, cytokinesis, partitioning cytoplasm, and cell wall deposition are linked in the dikaryon, but not in the monokaryon where cells can be multinucleate and septa form in response to cells size, not nuclear number. Multicellularity has several advantages. It allows the encapsulation of nuclei into separate but cytoplasmically connected cells (i.e., the establishment of physiological boundaries permitting isolation or coordination of metabolic processes). Also, it might evolve as a life-history strategy to allow an organism to explore space and persist through time until an optimal window of opportunity for reproduction arises (Niklas, 2014). Hyphae of Fungi that do exhibit complex developmental pathways form septa at regular intervals, though the septa usually have a central pore (Moore *et al.*, 2011)

allowing intercellular communication. The Ascomycota and Basidiomycota represent major derived lineages, which evolved from a common ancestor with septate hyphae (multicellularity).

The evolution of hyphae may have coincided with the rise of larger, multicellular food sources over half a billion years ago, when Chytridiomycota diverged from the other fungi. Data provided by Dee *et al.* (2015) suggest that both siphonous hyphae and septate hyphae arose independently from a single-cell predecessor living in freshwater environment. Early soils were formed by cryptogamic ground covers (Edwards and Kenrick, 2015) dominated by communities of cyanobacteria, basal eukaryotes of various sorts as well as small bryophyte-like organisms. Fungi likely moved from water to land to colonize these plants. Ground covers changed to biomass-rich covered mature soils as eukaryote organisms (plants and animals) diversified, providing new ecological niches for the evolution of Fungi.

Sexual Reproduction = An Ancestral Character in Terrestrial Fungi

A sexual cycle is considered to be ancestral in Fungi. Members of most of the traditional 'Zygomycota,' except Glomeromycota (**Figure 1**), share a sexual mode of reproduction through the fusion or conjugation of two hyphal branches (gametangia) to form a zygote, which undergoes meiosis and eventually germinates into a zygosporangium, which produces spores. Gametangia arise from hyphae of a single mycelium or from different but sexually compatible mycelia (Moore *et al.*, 2011). Ascomycota and Basidiomycota also reproduce sexually. In most Ascomycota, cytoplasmic fusion leads to dikaryons that form meiotic spores within a characteristic sac-like structure, the ascus. In Basidiomycota, dikaryons also form, but meiotic spores are produced on reproductive structures called basidia. A recent article of the evolution of fungal sexual reproduction (mainly focusing on a particular group of closely aligned species in the Basidiomycota) by Heitman *et al.* (2013) led the authors to suggest that unisexual reproduction could have been ancestral: perhaps sex was first a selfing mode of reproduction, generating diversity and eliminating deleterious mutations. The more complex versions of sexual reproduction, involving mating types and sexes, appeared later. Ascomycota usually have two mating types while Basidiomycota can have thousands of different mating types (Casselton, 2002). The sexual mode of reproduction characterizes the terrestrial Fungi, but sexual reproduction is known for so few members of the aquatic Chytridiomycota as to raise the possibility of its loss in some clades of this phylum. However this should not be overstated due to missing data.

When early plants colonized land, they evolved organ systems with multiple adaptations to rooting functions and physiological adaptations to growth in soils, gradually colonizing different types of environments. The development of larger plants was a key prerequisite for feeding in larger animals but also for the diversification of Fungi, which were able to evolve a wider range of enzyme activities (e.g., for wood decomposition). The combination of paleontological and genomic approaches is providing insights into the coevolution of plants and Fungi over >400 Ma, and how this has shaped their respective evolution and the geochemistry of earth, ocean, and atmosphere (Selosse *et al.*, 2015). Several key areas (e.g., relevance of the fossil record for improved timetree calibrations, better knowledge of fungal interactions in extant aquatic environments) would benefit from further cross-disciplinary developments to increase our knowledge on the origin and evolution of the fungal lineage.

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See also: Land Animals, Origins of. Seedless Land Plants, Evolution and Diversification of. Symbiosis, Introduction to

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G

Gene Interactions in Evolution

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Glossary

Additive genetic variance Defined formally as the covariance between average effects and average excesses, from a practical perspective it is the component of the variance among individuals that is heritable and can contribute to a response to selection.

Average effect The effect that an allele has on the phenotype measured as a deviation from the population mean with the rest of the genome randomly drawn from the population.

Epistasis Interactions among alleles at two or more loci resulting in a phenotype that is different from what would be expected from the loci acting independently.

Epistatic variance Variance among individuals in a population that can be attributed to interactions among loci.

Local average effect The metapopulation extension of the average effects. The average effect that an allele has on the phenotype in a particular deme measured as a deviation from the metapopulation mean with the rest of the genome randomly drawn from a particular subpopulation.

Metapopulation A set of subpopulations connected by a low level of migration. A population of populations.

Physiological genetic effects Also known as functional or biological genetic effects. Genetic effects, including the main effects of loci, and interactions within and among loci measured for individuals without reference to the population in which it is measured. Physiological genetic effects are constant and unaffected by changes in allele frequency in a population.

Statistical genetic effects Genetic effects, including the main effects of loci and interactions within and among loci measured in the context of a particular population. Statistical genetic effects are a function of the population they are measured in and will change as allele frequencies change.

Statistical and Physiological Gene Interactions

Epistasis is an interaction among loci such that the effect of a particular genotype at one locus on the expression of a trait is dependent on the genotype at other loci (Whitlock *et al.*, 1995). An excellent example of this is found in the genetics of the coat color of Labrador retrievers (Kaelin and Barsh, 2013; **Figure 1**). In these dogs there is a 'B' locus that codes for coat color, such that B_1B_1 and B_1B_2 individuals have black coats, whereas B_2B_2 individuals have dark brown or 'chocolate' coats. However, this locus is modified by a second 'E' locus that has two alleles, one of which, the E_2 allele, prevents the deposition of melanin in the hairs. Thus, E_1E_1 and E_1E_2 individuals are black or brown depending on the genotype at the B locus, whereas E_2E_2 individuals are yellow regardless of the genotype at the B locus. Gene interactions also include dominance, which both the B locus and the E locus show. Dominance occurs when the heterozygote (e.g., B_1B_2 genotype) is not intermediate between the two homozygotes (B_1B_1 and B_2B_2 genotypes). In the case of the B locus the B_1B_1 and B_1B_2 have the same phenotype, that is different from the phenotype expressed by the B_2B_2 genotype, and the B_1 allele is said to be dominant over the B_2 allele. These examples of epistasis and dominance, where the effect of specific genotypes on the phenotype is measured against a constant scale, are often termed 'physiological' (Cheverud and Routman, 1995), 'functional' (Hansen and Wagner, 2001), or 'biological' (Moore and Williams, 2005) epistasis and dominance to distinguish them from 'statistical' gene interactions.

In quantitative genetics the phenotype, or value of traits of interest, are measured on individuals. However, the measure of interest is not the phenotype of the individual, but the variance among individuals in phenotype, V_P. The reason for the focus on the variance in phenotypes is that evolution in general, and selection in particular can only act if there is phenotypic variation in a population. Ignoring genotype by environment interactions, in standard quantitative genetics this phenotypic variance is broken up into underlying causal components. First the phenotypic variance can be divided into genetic and environmental components:

$$V_P = V_G + V_E$$

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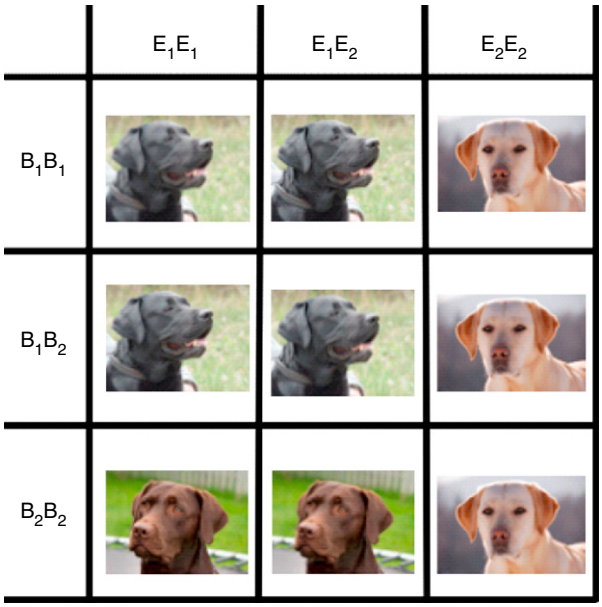

Figure 1 The genetics of coat color in Labrador Retrievers. B_1 is dominant over B_2, thus B_1B_1 and B_1B_2 individuals are black, and B_2B_2 are chocolate colored. E is epistatic with the B locus, and E_2E_2 individuals are yellow regardless of genotype at the B locus.

	A_1A_1	A_1A_2	A_2A_2	$p_A=p_B=0.5$ genotypic mean	$p_A=p_B=0.75$ genotypic mean
B_1B_1	1	0	−1	0	0.5
B_1B_2	0	0	0	0	0
B_2B_2	−1	0	1	0	−0.5
$p_A=p_B=0.5$ genotypic mean	0	0	0		
$p_A=p_B=0.75$ genotypic mean	0.5	0	−0.5		

Figure 2 Physiological and statistical epistasis example using additive-by-additive epistasis. Reproduced from Goodnight, C.J., 2014. Long term selection experiments: Epistasis and the response to selection. In: Moore, J.H., Williams, S.M. (Eds.), Epistasis: Methods and Protocals. New York, NY: Springer Science and Business Media, LLC, pp. 1–18.

where V_G is the variance in phenotype due to differences in genotype, and V_E is the variance in phenotype due to environmental influences. It is useful to further divide the genetic variance component:

$$V_G = V_A + V_D + V_{EP} + V_E$$

where V_A is the additive genetic variance, V_D is the dominance variance, and V_{EP} is the epistatic variance. The additive genetic variance, V_A, is the component of phenotypic variance that is most important for adaptive evolution. Although the formal definition is more complicated (Fisher, 1930), a practical definition of additive genetic variance is the portion of the phenotypic variance that is due to genetic effects that can be passed from parent to offspring (Falconer and Mackay, 1996). The importance of the additive genetic variance lies in the fact that it is the component that can contribute to a response to selection. The dominance variance V_D is the component of variance that can be attributed to within locus interactions (dominance), and V_{EP} is the component of variance that can be attributed to between locus interactions. The epistatic component of variance is typically further divided into specific epistatic interactions, for example, for a two locus two-allele system there are eight independent genetic variance components: additive at each locus, dominance at each locus, additive-by-additive epistasis, additive-by-dominance and dominance-by-additive epistasis, and dominance-by-dominance epistasis (Goodnight, 2000a).

The important distinction between physiological genetic effects and statistical genetic effects is that physiological genetic effects are a function of the organism's genotype that is independent of the population in which it is measured. In contrast statistical genetic effects are a function of both the organisms and the allele frequencies of the population in which they are measured (Cheverud and Routman, 1995).

Consider the simple example of additive-by-additive epistasis. In Figure 2 the values of 1, 0, and −1 in the three-by-three matrix are the physiological genetic effects. These are constants (i.e., they are fixed properties of the genotypes), and do not change regardless of the allele frequencies at the interacting loci. In the margins are the statistical genetic effects. These are the effects of one locus taken as a weighted average of the genetic backgrounds in which it is found. In this system, when the allele frequencies at both loci equals 0.5 the marginal values for both loci are all zero. Because there is no dominance in this example, the additive genetic variance is simply the variance in these marginal effects, thus the additive genetic variance is also zero, and the population is unable to respond to selection. In contrast when the allele frequencies are changed to 0.75 for both loci the weighted average for the genotypic effects are now nonzero, and the additive genetic variance is nonzero (0.187 5) (Goodnight, 2014).

Gene Interactions within Populations

Physiological dominance and epistasis are considered to be very common, if not nearly universal. The reason for this is that enzymes work in biochemical pathways where the different enzymes are the product of different loci. As a result any changes in the rate of flux through one enzyme will inevitably have effects on other enzymes in the pathway. These effects will be expressed as physiological epistasis. However, it has generally been observed that statistical epistasis tends to be a smaller component of the phenotypic variance than might be expected based on the ubiquity of physiological epistasis (Mackay, 2014). The most likely explanation for this is that statistical epistasis is maximized when all of the interacting

loci are at intermediate allele frequencies. It is reasoned that most loci are dominated by one or a few alleles at high frequencies with other, rare, alleles being at much lower frequencies (Cheverud and Routman, 1995). These are situations where the majority of genetic variance will be additive and able to contribute to a response to selection. Statistical epistasis will not be present unless there is physiological epistasis, however, the opposite need not be true. Nevertheless, genetic variance components attributable to gene interaction are frequently common enough that they must be taken into account (Roff and Emerson, 2006).

Statistical Gene Interactions and Drift

The effects of statistical gene interactions are best understood for genetic drift (e.g., Goodnight, 2006; Barton and Turelli, 2004). In an infinitely large population with no selection, allele frequencies will not change, and as a consequence the genetic variance components will remain constant. When population sizes are finite allele frequencies will change randomly due to genetic drift, with smaller populations being more strongly affected than larger populations. The accepted metric for measuring the effects of drift is the probability that two alleles are identical by descent. This is measured by Wright's inbreeding coefficient, f. In an idealized population of random mating diploid hermaphrodites the inbreeding coefficient increases in small populations:

$$f_{(t+1)} = \left(\frac{1}{2N}\right) + \left(1 - \frac{1}{2N}\right) f_{(t)}$$

where $f_{(t)}$ is the inbreeding coefficient at generation t, and N is the population size (Hedrick, 2005). This basic formula can be modified for more realistic population structures, such as separate sexes, and unequal sex ratios. More importantly for studying gene interactions, it can be modified to accommodate multiple loci and used to investigate the effects of drift on the conversion of epistatic and dominance variance to additive genetic variance (Goodnight, 1995, 2000b; **Figure 3**).

This is perhaps the most important effect of gene interactions on evolution within small populations. That is, in additive theory, a population bottleneck or period of small population size leads to a decline in the additive genetic variance. In contrast, in systems with gene interaction it is frequently the case that the additive genetic variance, and thus the ability for a population to respond to selection increases following a population bottleneck. This increase in additive genetic variance following a population bottleneck has been experimentally observed on multiple occasions (Van Buskirk and Willi, 2006).

The increase in additive genetic variance due to genetic drift warrants further discussion. In populations in linkage equilibrium the additive genetic variance is twice the variance in average effects, where the average effect of an allele is the effect of an allele on the phenotype, averaged over all possible genotypes and measured as a deviation from the population mean (Falconer and Mackay, 1996). Based on this simple definition it is apparent that there are only a few ways that the additive genetic variance can increase. It can be shown that the increase in the additive genetic variance due to changes in allele frequency when there is gene interaction is always associated with changes in the local average effects of alleles. The local average effect of an allele is defined as the effect that an allele measured in a specific subpopulation has on the phenotype taken as a deviation from the metapopulation mean (Goodnight, 1995, 2000b). Whenever there is an increase in the additive genetic variance it means that the local average effects of alleles are changing. How this occurs can be seen in **Figure 2** above. Consider a single large population with the A and B locus segregating. From this population found two new populations, one with a genotype of $A_1A_2B_1B_1$, the second with a genotype of $A_1A_2B_2B_2$. In the first population, ignoring the B locus, the A_1A_1 genotype has a genotypic value of 1, and in the second population it has a value of -1. The resulting local average effects of the A_1 allele are $+0.5$ in the first population and -0.5 in the second population. Although this is a contrived example, it demonstrates that increases in additive genetic variance are associated with changes in these local average effects, and that the increase in additive genetic variance is due to the effects of the alleles 'spreading' as the interlocus interactions become statistically

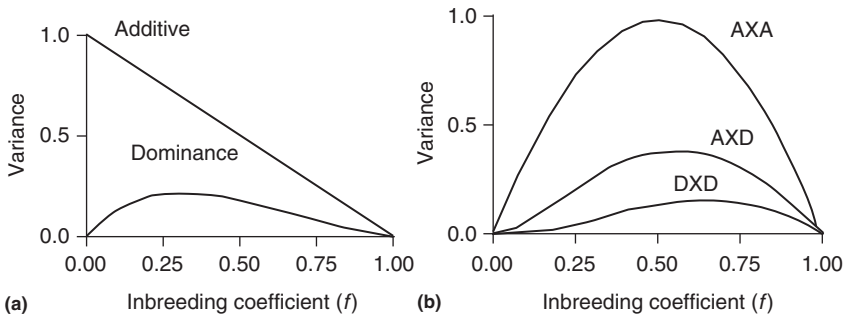

Figure 3 The additive genetic variance as a function of Wright's inbreeding coefficient. (a) Single locus genetic effects. (b) Two locus genetic effects. For additive variance the additive genetic variance declines linearly with inbreeding coefficient, whereas for variance components involving interactions the additive genetic variance is maximized at an intermediate inbreeding coefficient. Additive: single locus additive genetic variance; Dominance: Single locus dominance variance; AXA: Additive-by-additive epistatic variance; AXD: Additive-by-dominance and dominance-by-additive epistatic genetic variance; DXD: Dominance-by-dominance epistatic genetic variance. Reproduced from Goodnight, C.J., 2006. Genetics and evolution in structured populations. In: Fox, C.W., Wolf, J.B. (Eds.), Evolutionary Genetics: Concepts and Case Studies. Oxford, NY: Oxford University Press, pp. 80–100.

expressed as direct effects. Figure 4 uses a more sophisticated model and shows the expected variance in local average effects for different forms of genetic effects.

The increase in additive genetic variance coupled with the increased variance in local average effects is also associated with a decline in statistical epistatic variance within populations (Goodnight, 1988). The net result is that, due to finite population sizes, nonadditive genetic variance will frequently not be present as a measurable variance component within populations (Hill *et al.*, 2008), nevertheless it will be present between populations in the form of variance in local average effects (Goodnight, 2000b, de Brito *et al.*, 2005). Interestingly, changes in local average effects can also change over time, and under some circumstances this change can be driven by selection (Goodnight, 2004a). The result is that selection can generate, rather than consume, genetic variance. More importantly, it is possible that the adaptive value of alleles at a locus will switch over the course of selection, such that an allele that confers high fitness early in the adaptation process may be eliminated by selection later when, due to interactions with other loci, it is converted to a low fitness allele (Goodnight, 2014).

It is also important to note that the differentiation for local average effects and the differentiation for population means need not be related. When there is only additive variance, genetic drift will cause populations to differentiate for the population means, but there will be no differentiation for local average effects. In contrast, many of the interactions involving dominance have at most a small effect on the population mean, but may have substantial effects on differentiation for average effects (Goodnight, 2004b). One result of this is that populations, such as cryptic species, that are very similar in appearance, may nevertheless be highly differentiated for local average effects, and as a consequence reproductively isolated. In contrast, highly morphologically differentiated populations, such as some of the Hawaiian plant species groups, may be highly morphologically differentiated, but nevertheless not differentiated for local average effects, and as a result interfertile (Robichaux *et al.*, 1990).

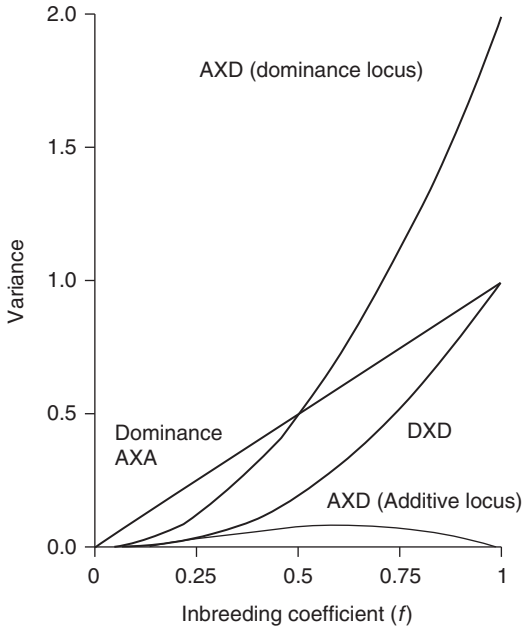

Figure 4 The variance in local average effects. The variance in local average effects for additive variance (not shown) is zero for all values of inbreeding coefficients. This means that for additive effects, regardless of how differentiated a population is the effect of an allele relative to other alleles will always be a constant. For genetic interactions the variance is always nonzero, meaning that the relative phenotypic effects of alleles will be different in different populations. Reproduced from Goodnight, C.J., 2006. Genetics and evolution in structured populations. In: Fox, C.W., Wolf, J.B. (Eds.), Evolutionary Genetics: Concepts and Case Studies. Oxford, NY: Oxford University Press, pp. 80–100.

Molecular Genetics and Physiological Epistasis

In a molecular genetic framework, interactions such as 'diminishing returns epistasis' (Maclean *et al.*, 2010), 'sign epistasis' (Weinreich *et al.*, 2005), and 'synergistic and antagonistic epistasis' (Desai *et al.*, 2007) are often discussed. These terms do not have direct parallels in the quantitative genetic framework described here, since they are referring to physiological epistasis, and the fitness effects of individual alleles rather than the statistical epistasis of the quantitative genetic variance component framework. In general, these interactions involve the order and timing of mutations in a gene network with an arbitrary number of loci, and the allelic effects are made in reference to a standard genetic background, usually the 'wild type' for the species or strain being studied (Kryazhimskiy *et al.*, 2011). The inherent polygenic nature of this approach is a distinct advantage for molecular genetic models that examine the effects of epistatic mutations at multiple loci that can accumulate over time. This molecular genetic concept and the quantitative genetic concept of epistasis are tied together through changes in variance components and changes in local average effects. For example, diminishing returns epistasis is a gene interaction in which mutations have their greatest impact on fitness when they are the sole mutation in a metabolic pathway, whereas if they are introduced into a pathway that already has mutations in it their impact will be reduced (Maclean *et al.*, 2010). From a quantitative genetic perspective this is caused by changes in local average effects that are contingent on the number of prior mutations.

Hansen (2013) gives two locus examples of 'positive,' 'negative,' and 'sign' epistasis (**Figure 5**) that are directly decomposable into quantitative genetic variance components. Positive and negative epistasis are equivalent to Desai *et al.* (2007) synergistic and antagonistic epistasis. Using the regression methods of Goodnight (2000a) positive epistasis can be shown to be a mixture of additive variance, additive-by-additive, additive-by-dominance, and dominance-by-dominance epistasis, although the relative proportions of the different components depend on the genotype frequencies. Similarly negative epistasis and sign epistasis can be shown to be mixtures of additive variance and additive-by-additive epistasis, again with the relative proportions of the two variance components dependent on gene frequency.

The examples given by Hansen (2013) are for two loci; however, the concepts of positive, negative, and sign epistasis will typically involve an arbitrary number of loci. In principle

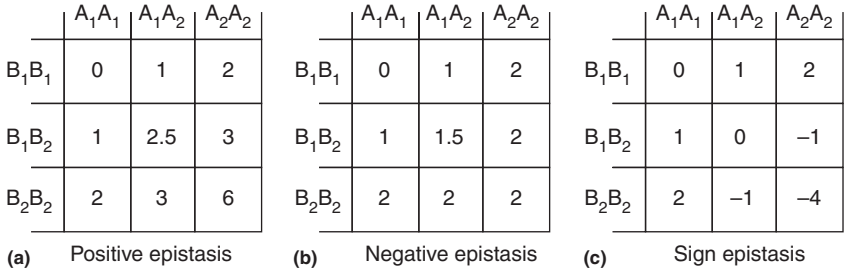

Figure 5 Examples of positive epistasis (a), negative epistasis (b), and sign epistasis (c) (Reproduced from Hansen, T.F., 2013. Why epistasis is important for selection and adaptation. Evolution 67, 3501–3511). These examples use physiological epistasis, which may be more appropriate for molecular approaches. For evolutionary studies they can be converted to quantitative genetic components. These can be found using the regression methods of Goodnight (2000a), and they will be a function of the genotype frequencies. In the case of a population in linkage equilibrium and a allele frequency of 0.5 at both loci, the positive epistasis (a) is 89% additive variance, 3.6% additive-by-additive epistasis, 3.6% additive-by-dominance epistasis, and 3.6% dominance-by-dominance epistasis. Negative epistasis (b) is 80% additive variance and 20% additive-by-additive variance, and sign epistasis (c) is 50% additive variance and 50% additive-by-additive epistasis.

this conversion from the molecular/physiological genetic approach to the quantitative genetic approach can be done for any degree of epistasis. The reality is that, for statistical reasons, there two-locus interactions will typically be adequate.

As Hansen (2013) points out, the big difference between the physiological genetic approach and the quantitative genetic approach is that with the molecular genetics approach the effects of selection on identified alleles can be followed. On the other hand, to study the effects of selection on the phenotype, some form of reckoning similar to that used in quantitative genetics needs to be done. On the other hand the quantitative genetic approach is well suited to studying the effects of selection on phenotypic change. Importantly, although the author cannot prove it, it appears that any two locus quantitative genetic system that has the same mean and gives the same variance component values as Hansen (2013) examples will also predict the same phenotypic change, although not necessarily the same allele frequency change.

Summary

Gene interaction, or physiological dominance and epistasis, is a necessary, but not sufficient, prerequisite for statistical gene interactions. Statistical genetic effects are variance components that are attributable to the underlying physiological genetic effects. Because selection requires heritable variation to be effective, it is the statistical rather than the physiological genetic effects that are important in evolution. The additive genetic variance is of primary interest in evolutionary change; however, statistical variance components due to dominance and epistasis are important because they can contribute to the additive genetic variance, and this contribution changes as allele frequencies change. Between demes this increase in additive genetic variance is associated with the shifting of the local average effects of alleles, causing populations to diverge for their effect on alleles, such that an allele that is good in one population may perform poorly in another population.

See also: Multivariate Quantitative Genetics. Quantitative Genetic Variation. Systems in Evolutionary Systems Biology

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Gene Networks Driving Development, Conservation and Evolution of

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Glossary

***Cis*-regulatory module (CRM)** A stretch of usually several hundred base pairs of DNA with multiple binding sites for TFs. CRMs are often located within several thousand base pairs on the gene locus or within introns. The bound TFs regulate expression of the target gene. CRMs are sometimes referred to as enhancers or *cis*-regulatory elements.

EvoDevo Short form of evolution of development. The field of biology that compares the development of different taxa to understand the basis of morphological diversity, ancestral features and the relationships between organisms.

Gene regulatory network (GRN) A network of regulatory interactions between genes. The regulatory interactions can include any of the known mechanism of gene regulation; including transcription factor regulation, signaling pathways, microRNA regulation, and other post translational mechanisms. Nodes within the GRN are genes, and edges drawn between them are the regulatory interactions.

Subcircuit A subset of usually several genes (nodes) from a GRN. Subcircuits can be considered as functional modules operating in a GRN.

Transcription factor (TF) A class of protein that binds to a DNA with sequence specificity to regulate expression of a target gene.

The Birth of Evolutionary Developmental Biology

Evolutionary developmental biology (EvoDevo), the study of the evolution of the developmental mechanisms underlying organismal morphological diversity, is a vigorous and growing field of research. The union of these areas seems so natural that it's hard to imagine that historically, evolutionary, and developmental biology were quite separate fields of investigation (e.g., Carroll, 2008; Raff, 1996, 2000). Early developmental biologists were interested in understanding subjects including how cells obtained their identity, how cells could impart properties onto other cells and how communities of cells were established (Carroll *et al.*, 2004). These studies, for instance, generated an understanding of morphogen gradients and organizing centers. Developmental biologists, primarily using the fruit fly, *Drosophila melanogaster*, as a model organism, also began to understand the genetic underpinnings of development. For instance, experiments performed in *Drosophila* revealed mutations that affected the position of appendages along the body (e.g., Lewis, 1978). Thus legs would form in the location of antennae, and two pairs of wings would from instead of one pair and one balancer pair. These experiments provided enormous insight to developmental biology, as they showed for instance that there could be genetic switches, i.e., gene products that could turn off or on an entire program or module inappropriately. Most famous of these gene mutations were to the *hox*, or homeotic genes (e.g., Gilbert, 2000). These genes are clustered along the chromosome and are expressed in overlapping domains along the anterior to posterior axis of the *Drosophila* embryo. Mutations in these genes lead to misregulation of this axis and inappropriate positional identity. Although these findings provided extraordinary insight into developmental mechanisms, it was assumed that such mechanisms and their genetic underpinnings were exclusive to *Drosophila*, or very closely related species. Hence, nothing much could be inferred about evolutionary processes. The strong assumption that distantly related organisms, and certainly species in different phyla, were constructed using different genes, indeed, that organisms were different because they used different genes, lead to the belief that little could be understood from comparative developmental genetics. The paradigm-shifting discovery in the mid-1980s that mice also had orthologs of these same homeotic genes was an enormous breakthrough (McGinnis *et al.*, 1984). The implication of this finding was that mice and *Drosophila* shared genes with sequences that were predicted to have arisen from a common gene present in their ancestor. Furthermore, the *hox* gene orthologs in mouse were also found to be clustered in the genome, expressed in overlapping domains along the anterior posterior axis, and most significantly, when mutated led to defects in axial skeletal patterning.(e.g., Graham *et al.*, 1989; McGinnis *et al.*, 1984). This initial discovery of similar uses of orthologous *hox* genes in highly disparate taxa was quickly extended to many more genes, for example, *pax6* orthologs were expressed in, and needed for the function of eyes, similarly *tinman* orthologs, for hearts, and *distalless* to extend appendages (Halder *et al.*, 1995; Olson, 2006; Panganiban *et al.*, 1997).

The Focus on Regulatory Evolution

These early findings led to the blossoming of the EvoDevo field. The burst of sequencing of genomes from across a diversity of animal taxa confirmed the initial findings that different animals have very similar sets of genes (e.g., Koonin, 2009; Putnam *et al.*, 2007; Srivastava *et al.*, 2010). Certainly expansions and taxon specific genes have evolved in specific lineages, but it has become quite clear that to understand how animals have evolved, we must understand how orthologous genes are used in different ways to achieve the diversity of developmental plans.

Before considering how gene function evolves, it is useful to think about types or categories of genes and how their

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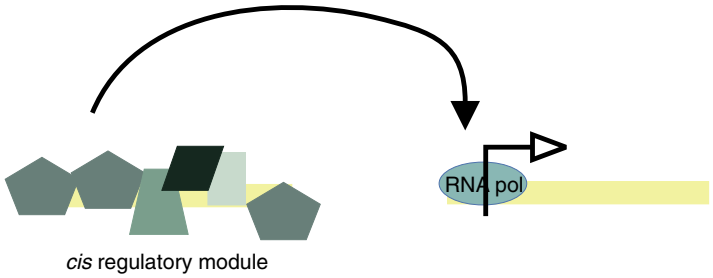

Figure 1 Enhanceosome on a *cis*-regulatory module (CRM) regulates gene transcription. Transcription factors proteins (depicted as green polygons) bound to a CRM. When bound these proteins will cause transcription of the target gene (arrow). Transcription factors can also act as repressors to block transcription of the target gene.

evolution might affect development. This has been discussed very elegantly by Carroll *et al.* (2004). Many genes in the genome function to control a variety of universal cellular processes and metabolism. All animals, and in many cases all life, will use the same gene orthologs for these critical processes. These genes therefore tend to be highly conserved and hence uninformative about the mechanisms of animal diversity evolution. There are also those genes that are expressed in particular cell types, for example, myosin in muscle cells, various neurotransmitters in neurons, hemoglobin in red blood cells. These genes also tend to be highly conserved among animals. Although as more taxa are sampled, and especially those at the base of the metazoa, there are variations in gene sequence and uses, which provide insight into how these genes evolved their function (e.g., Bridgham *et al.*, 2010). Finally, there are the subset of genes that are regulatory in nature, i.e., their function primarily is to direct the expression of other genes. These are mostly genes encoding transcription factors and signaling pathway components. There are other classes of regulatory genes, particularly microRNAs and other noncoding RNAs, but as yet very little is understood about how these might contribute to the evolution of development. Regulatory genes are expressed in different cells types and at different times during development. Most animals start life as a fertilized egg, which will repeatedly divide to produce many cells, each specified to the correct type. These cell types are the product of the sets of genes expressed in each cell, thus, for example, a neuron expresses a very different sets of genes than does a red blood cell. It is the job of regulatory genes to regulate the expression of other genes and thereby to determine cell type specific gene expression. Regulatory genes have therefore been very aptly termed the genetic toolkit for development (Carroll *et al.*, 2004), as these are the genes that are used to make the cell types and hence to construct the form of the embryo.

Before we can understand how development evolves, we must understand how new cell types evolve and how these cell types are positioned in the correct place in time and three-dimensional space in the embryo. A great deal of EvoDevo has therefore focused on the evolution of the function of regulatory genes (e.g., Wray, 2007). Before considering how these genes evolve, it is helpful to understood more clearly how they function during development. The functional role of a transcription factor is to bind DNA with some sequence specificity. Thus each transcription factor has a stretch of often only 6–13 base pairs of sequence to which the protein will bind. These sequences are often degenerate so that there are many variants within this sequence. Therefore within the genome there could be many thousands of loci where each protein could bind. Other transcription factors may bind nearby, to their own preferred binding site, and multiple copies of the same protein could also bind to preferred sites nearby. Thus, there forms a collection of proteins bound to a 300–800 base pairs stretch of DNA called the *cis*-regulatory module (CRM) (**Figure 1**); the collective binding of each of these proteins form a stable complex termed the enhanceosome (reviewed, (Arnosti, 2002). The transcription factors bound to the CRM activate the transcription of genes by helping to stabilize the complement of proteins that will recruit polymerase to the transcription start site or by influencing the chromatin state. Transcription factors may also act as repressors of gene expression. In these cases the bound protein will disrupt the recruitment of RNA polymerase or promote closed chromatin. In this way transcription factors can be conceived of as a binary switch; when bound in the right combination with other proteins they will work to either turn on or turn off transcription of a single gene. Signaling pathways initiate with a ligand, which are either membrane bound or diffusible. The ligand will bind a receptor to establish a signaling cascade, the outcome of which is to activate or repress the transcription of a target gene. Therefore signaling pathways regulate gene expression in neighboring cells, while transcription factors act cell autonomously.

Given the importance of these regulatory genes, they constitute a surprisingly small fraction of the total genes in the genome. For instance, in an extensive analysis of transcripts from the sea urchin, less than 10% are predicted to be transcription factors (Tu *et al.*, 2012). The genes therefore must be used multiple times, often in multiple cell types and at many different times during development. Additionally, in each context, each factor also regulates the expression of many other genes. New technologies permit an unbiased probing to reveal where transcription factors are bound to the DNA at any one time (Johnson *et al.*, 2007). These indicate that a given transcription factor may have thousands of target genes (e.g., Zeitlinger *et al.*, 2007). Transcription factors are therefore highly pleiotropic. Changing the function, for example, the binding properties of any one transcription factor, will potentially affect the expression of many thousands of other genes. Such large scale changes are presumed to most often be extremely deleterious and therefore unlikely to occur with great frequency. Therefore, the biochemical functions of transcription factors are likely to be highly conserved. For these

reasons it has been argued that a more likely common source of variation is of the *cis*-regulatory targets of these factors (Wray, 2007). This allows one target gene to change, and can also lead to more subtle effects that can pass the filter of natural selection. Increasingly, however, it is being discovered that transcription factors can also evolve. Critical to this type of evolution is the ability to evolve in ways that reduce pleiotropic effects, i.e., that only subsets of their target genes will change (Cheatle Jarvela *et al.*, 2014; Lynch and Wagner, 2008; Pick and Heffer, 2012). As yet, however, very little is known about how such protein level evolution changes the structure of GRNs but this is likely to be an important future direction in the field.

From Gene Regulation to Gene Regulatory Networks

A gene regulatory network (GRN) is a model that seeks to describe the entire set of regulatory interactions that occur in any given cell lineage or territory during development (Davidson, 2006; Davidson and Erwin, 2006). Instead of considering just one or even several regulatory factors at a time, GRNs include all of the gene regulatory events together into a network of interactions. Each node in the GRN is the CRM that regulates each gene at that time and place. The edges, or interactions, are the transcription factors that regulate the target gene at this regulatory module. GRNs, therefore, provide a systems-level explanation of development. As discussed above, changes in these regulatory interactions are a significant source of evolutionary change in development. The goal of GRN evolutionary studies is to understand how these changes alter the topology of the GRN and the effect that this has on development.

Ideally a GRN will start with regulatory molecules in the egg and end with a description of the genes expressed in the differentiated cell. In the example of **Figure 2**, a maternal factor may be inherited asymmetrically into cell lineage 'A' and will specifically activate zygotic transcription of the transcription factors A and B. Once these initial factors are expressed, they now coordinately activate yet more factors, in this example, transcription factors C, D, and E. This regulatory hierarchy will continue as combinations of factors expressed in any cell activate yet more transcription factors. Eventually, in this example, the factors expressed in cell type 'A' are sufficient to activate the expression of differentiation genes, i.e., those classes of genes that execute the final function of the cell. The set of expressed genes is termed the regulatory state of this cell, which is changing over developmental time. The other class of regulatory genes shown in this figure is the signaling pathway genes; in this example a signaling ligand is activated by transcription factors D and E. The ligand will bind its receptor in cell 'B.' The activated receptor will activate a transcription factor W through a signaling cascade. W in this example is a repressor and thus represses the expression of factor C. Consequently the factors downstream of C are also extinguished. W additionally represses V, which is also a repressor, and therefore W functions indirectly to activate the expression of factors Y and Z. The function of signaling here therefore is twofold, to repress the expression of genes in common with type 'A' and to also direct expression of specific factors that will direct it to cell type B. The regulatory state of cell 'B' therefore become W, Y, and Z which will activate expression of a distinct set of differentiation genes, R and S.

Figure 2 represents a basic, simplified version of the types of GRNs that have been determined experimentally (e.g., Aguilar-Hidalgo *et al.*, 2013; Chan *et al.*, 2009; Davidson, 2006; Davidson *et al.*, 2002; Kueh and Rothenberg, 2012; Morley *et al.*, 2009). Advances in technologies are making it increasingly possible to generate expansive data sets of all of the interactions that comprise a developmental GRN. RNA-Seq technologies associated with various cell type sorting approaches permit rapid assessment of the regulatory states in a particular cell type over time (Pepke *et al.*, 2009). These can be

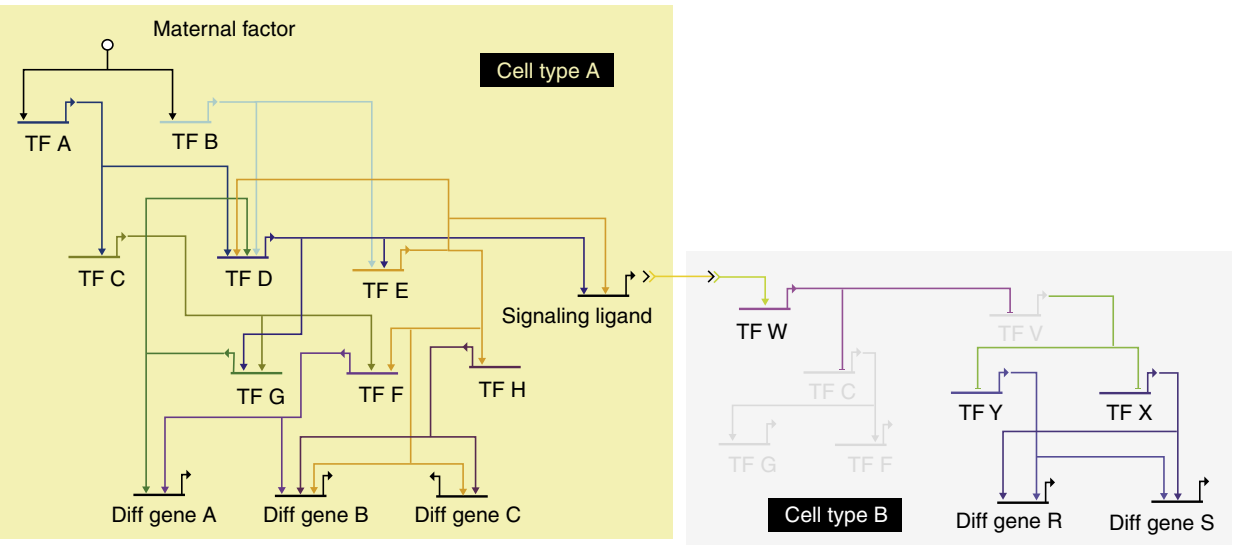

Figure 2 An stylized example of GRN. Each horizontal line represents the CRM for the regulated gene. Lines emanating from a gene represents transcription, with arrows indicating a positive regulation on the target gene and bars a repression event. TF- transcription factor, Diff Gene – terminal differentiation gene. GRN was drawn using drawn in Biotapestry (Longabaugh, 2012).

coupled to knockdown or other perturbation strategies to assay the change in expression profiles under perturbed conditions. These data can be used to fairly rapidly build a picture of the epistatic interactions between genes. Ideally the GRN should include the direct regulatory connections, i.e., that each interaction shown should be the binding of the factor to the CRM of the target gene. Here again new technologies are making this increasingly feasible. ChIP-sequencing, for example, is a high throughput method for identifying the regions of chromatin bound by a factor of interest (Johnson *et al.*, 2007; Pepke *et al.*, 2009). These types of technologies are expanding the number of taxa for which GRNs can be determined and therefore comparative analyses on GRN topologies will become a more accessible approach for studying evolution (Fischer and Smith, 2012).

When considering an entire GRN, as in **Figure 2**, types of subcircuits of several interacting nodes stand out. For example, **Figure 3** highlights a subcircuit of a group of three genes from **Figure 2** that are interconnected by positive feedback. Thus, in this example transcription factor D regulates E and G, and in turn D is regulated by G and E. Another type of commonly observed subcircuit is a feedforward loop. For example, again highlighted from **Figure 2**, factor E regulates H and the differentiation genes B and C. H also regulates differentiation genes B and C. These subcircuit topologies are thought to confer very particular dynamic functions. (Alon, 2007b), which are simply the result of the kinetics that occur naturally as genes are transcribed, translated, and bind to regulatory DNA (e.g., Ben-Tabou de-Leon and Davidson, 2009). Therefore, developmental GRN function can depend on the topology of the subcircuits of interacting factors. For instance, in the example of positive feedback, as one gene in the subcircuit is expressed, all genes will be transcribed and remain on due to this feedback. Thus a function of such a positive feedback loop in development is thought to be to establish a maintained memory of a transient input (Shoval and Alon, 2010; Alon, 2007a). A feedforward subcircuit is thought to function as a filter for transient inputs; the differentiation genes in this example will not be expressed until transcription factor E has driven the expression of transcription factor H, which in turn must be translated and processed before differentiation gene expression is permitted.

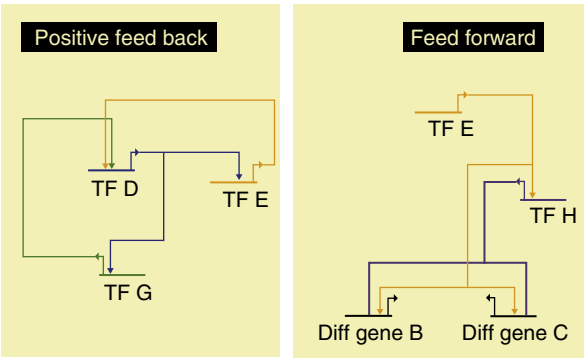

Figure 3 Two types of subcircuit commonly observed in GRNs, (a) Feedback loop and (b) Feedforward loop. Both subcircuits are found in **Figure 2** and highlighted here to show concept of modular subcircuit.

The developmental function of feed forward is therefore to ensure that a state is stably present in a cell before activating target genes. Therefore, the function of a GRN can be dictated from the types of subcircuits and their relative placement within the network. This idea, as will be expanded on below, has very important consequences for how GRNs might evolve.

Evolution of GRNs

Much of the great body of literature in the field of EvoDevo examines the evolutionary and developmental consequences of changes in gene regulation. Comparisons of GRNs provide a powerful extension to this approach as these analyses permit an understanding of how these evolutionary changes alter development. One especially important goal in this research is to use the framework of the GRN to understand whether all types of changes are equally likely, i.e., whether some changes modify GRNs in less disruptive ways that therefore are more likely to pass the filter of selection. The notion that there are constraints on developmental possibilities has been widely discussed and debated (e.g., Raff, 1996), but it remains unclear whether this occurs, and if so, what mechanisms might underlie constraint. Identifying the types of GRN topologies that are more or less likely to evolve and linking this to development provides a solution to this problem.

There are still relatively few comparison of detailed GRNs, but some of the best known comparison of GRNs have been performed between groups of echinoderms (Hinman and Cheatle Jarvela, 2014; Hinman *et al.*, 2009). This is because the best example of a detailed developmental GRN is for the sea urchin, a member of one of the classes of echinoderms (Davidson *et al.*, 2002; McClay, 2011; Peter and Davidson, 2011; Sethi *et al.*, 2012). The sea star, a representative of another class of echinoderm, was selected as an ideal comparative model as it has an overall fairly similar developmental plans but with several distinct differences. One of the most remarkable observations from these comparative analyses was the finding that certain types of GRN subcircuitry may have different capacity for evolutionary change (Davidson and Erwin, 2006). The best described example of this is with positive feedback circuitry. A comparison between early development in sea urchin and sea stars showed that among the earliest regulatory events is the activation of a feedback subcircuit (Hinman *et al.*, 2003; McCauley *et al.*, 2010). Remarkably this feedback circuitry is maintained in common, i.e., the same gene ortholog are engaged in positive autoregulation to form a feedback loop. Not all regulatory connections are identical, but the overall positive feedback function of the subcircuit is conserved. In both the sea urchin and sea star this subcircuitry occurs downstream of early signaling processes during early cleavage and thus the function of these circuits may be to simply provide a 'lock down' of specification state to one germ layer cell type. While these subcircuits are conserved, there are significant differences in the GRN acting earlier and later in development. (Hinman and Davidson, 2007; McCauley *et al.*, 2010). Most significantly in the mesoderm, the sea urchin GRN downstream of this conserved subcircuit leads to the development of biomineralization genes required for the formation of the larval skeleton. The orthologous GRN

in sea stars forms other types of mesoderm. This demonstrates that conserved circuitry in early development does not constrain the later evolutionary capacity for development.

The evolution of novel features that underlie the diversity of animal forms must arise from changes to GRNs (e.g., Erwin and Davidson, 2009). There are a variety of well-understood mechanisms through which gene regulation can evolve. The consequences of changes in gene regulation can be explored in the context of how such changes will generate changes in GRN topologies. The loss and gain of individual nodes are likely to be fairly frequent events, and as discussed above (e.g., Wray, 2007) are most likely to be due to a change to CRMs. As shown in the example above, for individual interaction changes within otherwise conserved feedback loops, this may not affect an obvious change in function (Hinman *et al.*, 2007). Another well studied evolutionary mechanism are duplications, either of coding regions or CRMs (Kimura and Ohta, 1974). Duplicated genes allow for rewiring of circuits. Initially, the duplicated gene will continue to regulate its original subcircuit but over time this additional gene will lose and gain targets genes and presumably this will change development (e.g., **Figure 4(a)**). This has been implicated in the developmental a several vertebrate specific novelties, for example, neural crest (Green and Bronner, 2013), and biomineralization (Fisher and Franz-Odendaal, 2012). The two duplicates may also simply split their ancestral function. When this happens the associated changes to development are expected to be relatively minor. Alternatively, a CRM may duplicate during evolution. This duplicated CRM can acquire or lose binding sites that will allow the regulated gene to be expressed in a new context (e.g., **Figure 4(b)**). Potentially the subcircuit driven by the new domain of gene expression may then 'bring along' or co-opt the downstream target genes. This circuitry is now therefore also expressed in a new developmental context. This is an attractive solution to making novel structures during development and this mechanism for

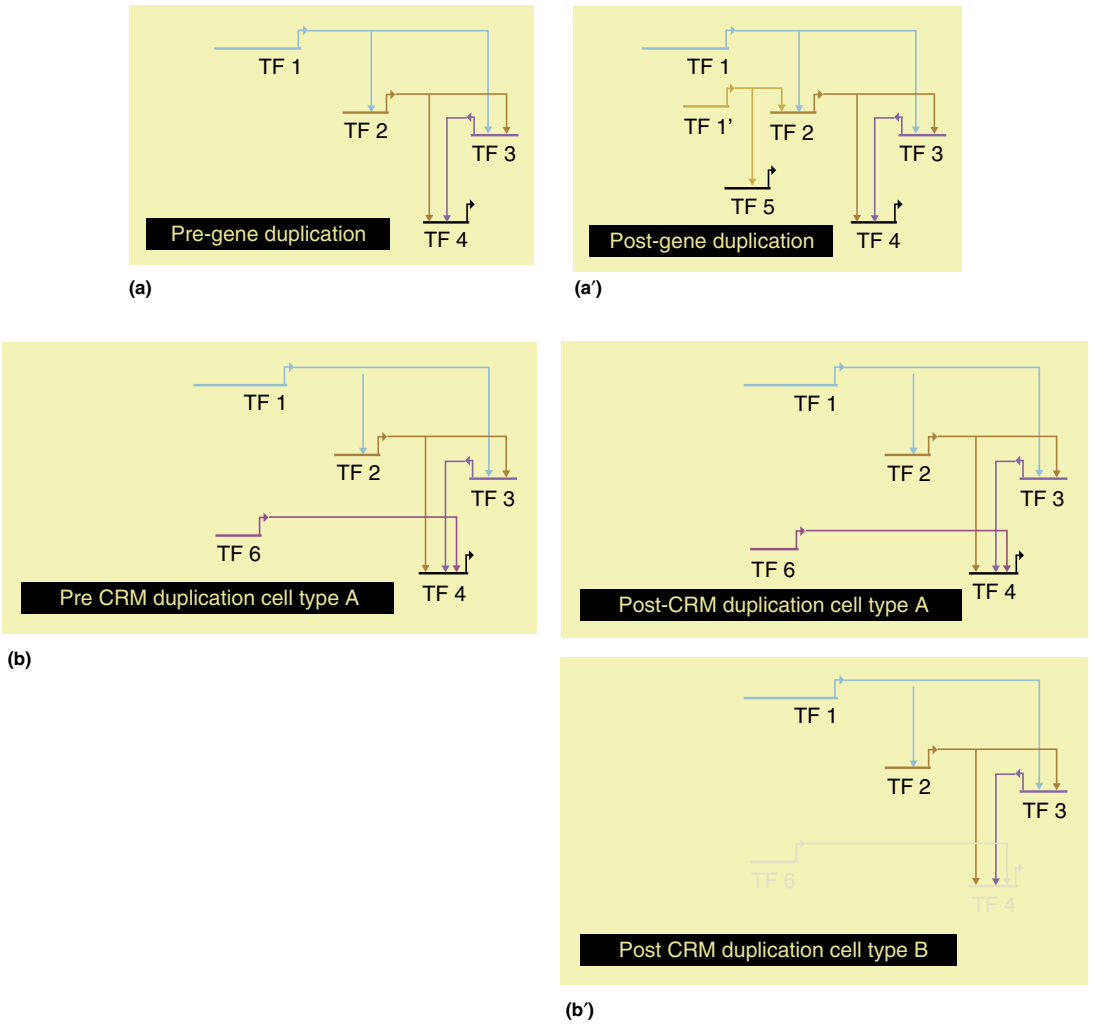

Figure 4 Evolution of GRNs. (a), (a′) Gene (coding) duplication. TF 1 is duplicated to TF1 and TF1′ in (a′). TF1′ continues to regulate some of its original target genes (TF2), losses its ability to regulate some target genes (TF3,4) and gains new target gene regulation (TF 5). (b), (b′). *Cis*-regulatory duplication. In this case TF1 duplicates its CRM (in (b′)) which leads to TF 1 being expressed in a different cell type "B." TF1 continues to regulate its targets in this new context if the other TFs needed for their expression are also present. Thus as TF 6 is not also expressed in Cell Type B, then TF4 is not co-opted.

quickly generating rapid changes may be pervasive in biology. Many examples have recently been suggested as indications of co-option. These almost entirely rely on finding many of the same (i.e., orthologous) genes expressed in two different structures. Thus for example, co-option has been suggested as a model for butterfly wing patterns from eye GRNs (Monteiro *et al.*, 2006); limb patterning (Bowsher and Nijhout, 2009; Lemons *et al.*, 2010) and beetle horns may have been co-opted from other axial patterning systems (Moczek and Rose, 2009); the turtle carapace may have been co-opted from limb patterning (Kuraku *et al.*, 2005); and sea urchin larval skeleton co-opted from the adult skeleton (Gao and Davidson, 2008). To be strictly defined as subcircuit co-option, however, requires that a cluster of pre-wired genes is brought along together (Monteiro and Podlaha, 2009). This means, therefore, that the downstream co-opted genes must be regulated by single CRMs that can function in both the original and new situation (e.g., TFs 2 and 3 are regulated using the identical CRM in both contexts rather than have also evolved new modules). As any CRM is regulated by many factors to ensure the fidelity and specificity of expression needed during development, this must influence the type of CRMs or GRN structure that can co-opt. Once co-opted these enhancers are pleiotropic and so in addition must be under selective pressure in both contexts (Monteiro and Podlaha, 2009). Any changes in the CRM would affect both the old and co-opted gene and associated circuit. These types of restrictions may have largely unexplored consequences on both how CRMs evolve and also on the structure of GRNs that permit this.

Conclusions

Comparisons of GRNs is a new approach for understanding how development can evolve. New technologies, which allow high throughput probing of genes and their interactions, will enable a rapid expansion of this approach as GRNs from more taxa become increasingly available. Such comparisons should reveal how subcircuits of genes evolve and how their placement within the GRN affects both their evolutionary potential for change and the consequence that this change has on the evolution of development.

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See also: Developmental-Genetic Toolkit for Evolutionary Developmental Biology. Regulatory and Coding Changes in Developmental Evolution, Roles of

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Gene Origin, Sex Chromosomes and

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Glossary

Dosage compensation It is a process by which gene expression between males and females is equalized.
Heteromorphic chromosomes Homologous chromosome pair that is not morphologically identical: differing in features such as size, shape, and staining properties, for example, sex chromosomes.
Meiotic sex chromosome inactivation (MSCI) It is a process in which sex chromosome expression is inactivated or down-regulated through a global process of chromatin structure change during meiotic phase of the heteromorphic sex gametogenesis.
Sexual antagonistic selection The case of opposing selection pressure on the two different sexes.

Introduction

Gene evolution is revealed by the study of genes that recently emerged in the evolutionary history of a taxon, so-called new genes. The interest in study gene evolution differs from conventional molecular evolution that has been focused on small-scale changes such as nucleotide substitutions, insertions, and deletions (Long *et al.*, 2003; Kaessmann, 2010; Chen *et al.*, 2013; Long *et al.*, 2013). Gene evolution is concerned with the changes at gene level, typically with gain of a new gene, loss of an old one, the change in gene structure, and their consequence on the function and evolution of genomes. Because a gene is a basic genetic unit that code for a specific function, gene evolution is often associated with evolution of gene functions and phenotypes (Long *et al.*, 2003; Kaessmann, 2010; Chen *et al.*, 2013). Sex chromosomes provide a unique environment in the genome for the evolution of new functions and phenotypes, as they are intrinsically related to the distinct evolution of males and females (Ellegren and Parsch, 2007; Parsch and Ellegren, 2013). Indeed, as the genomic era advance, evolutionary studies with independently originated sex chromosomes from several different taxa reveal that genes evolved as a major process (e.g., Ellegren and Parsch, 2007; Bachtrog, 2013). In this review, we not only describe sex chromosomes gene content with examples of well-studied organisms, but also discuss evolutionary processes and forces related to their roles on the origination of new genes and death of old ones.

Molecular Processes that Generate New Genes

New genes can form through no less than a dozen of mutation mechanisms (**Figure 1**), for example: recombination of exon, lateral gene transfer, *de novo* origination, and duplication of preexisting genes, which are more frequently investigated (more can be found in summary in Long *et al.*, 2013 and Chen *et al.*, 2013). The most common mechanism, duplication by retrotransposition or at the DNA level, generates genes with similar sequence and function to the parental genes and therefore are prone to accumulate detrimental mutations and become functionless, i.e., a pseudogene (Ohno, 1970; Kimura, 1983). Alternatively, these duplications over an evolutionary period can undergo neofunctionalization, a process of diversification where a gene acquires a new function that might be maintained in a population, spread and become fixed in the species population (Ohno, 1970).

Potential Impact of Sex Chromosomes on New Gene Evolution

Different from the autosomes, sex chromosomes are those that males and females do not share. In some sexual systems, one of the sex chromosomes carries a sex-determining gene, e.g., the human Y chromosome carries a male determining gene (Goodfellow and Lovell-Badge, 1994). In other cases, the sex is determined by the ratio of one chromosome over the others, e.g., *Drosophila* (Bull, 1983). Sex chromosomes are often different from each other in terms of size, shape, and gene content (Bachtrog, 2013). It has been found that sex chromosomes originated independently many times in nature, generally by a similar process in which one member of an autosomal pair acquire one or more sex-determining genes giving rise to a proto X-Y or Z-W chromosomes. Suppression of recombination between those chromosomes is favored by natural selection, which together with genetic drift leads to a progressive degeneration of the Y or the W chromosome, by accumulation of repetitive sequences and massive loss of genes (Charlesworth and Charlesworth, 2000). The chromosome that continues to recombine in one sex, including X in females and Z in males, does not degenerate and is hemizygous (haploid) in the opposite sex. Therefore, mechanisms that compensate their dosage such as somatic X inactivation in females may be favored by natural selection (Charlesworth, 1978).

Although not all sex-determining systems bear a heteromorphic-conserved sex chromosome like in human and other genetic model organisms, in most of those systems, sex chromosomes evolve under different selective and mutational environment than the autosomes (Viçoso and Charlesworth, 2006). Mutational processes and genetic drift have huge impact over the evolution of sex chromosomes, however, gene content differences between them is not solely a result of

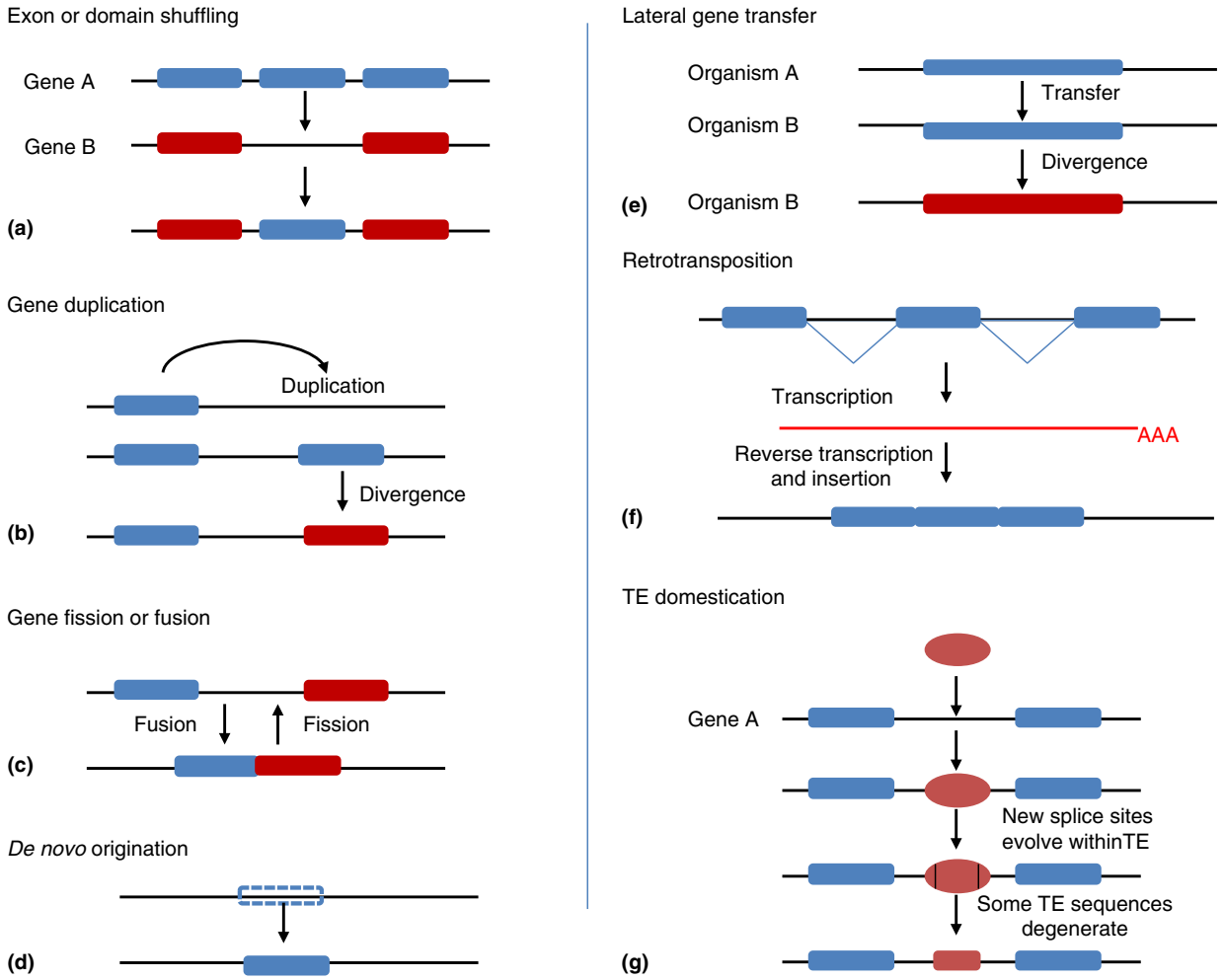

Figure 1 Molecular mechanisms for creating new genes. Major molecular processes that can generate new protein-coding genes. For more descriptions, discussion, and other mechanisms, see references Long *et al.* (2003), Chen *et al.* (2013), and Long *et al.* (2013). (a) Chimeric genes can be formed by recombination of exons or domains from different genes; (b) gene duplication; (c) gene fission divides one gene into different genes while gene fusion combines together neighboring genes; (d) *de novo* origination refers to the generation of a completely novel protein from the accumulation of mutations in noncoding genomic sequences; (e) genes can be transferred between organisms, specially found in bacteria; (f) genes can be generated by the transcription of a parental copy in mRNA and posterior reverse transcription and insertion of this sequence into a new position in the genome. The result will be an intronless new gene that can diverge acquiring new function; (g) transposable element (TE) can be domesticated forming new genes.

degeneration and gene loss of the Y or W chromosomes (Viçoso and Charlesworth, 2006). Even the X and the Z chromosome usually do not carry the same genes as the ancient proto-sex-chromosomes and gene content continues to evolve with both loss and gain of genes. Evolutionary forces applied differently in females and males over time have great differential consequences on the sex chromosomes.

A sexual antagonistic gene has beneficial fitness effect on males but detrimental effects on females or vice-versa (Arnqvist and Rowe, 2005). The detrimental effect in one sex can decrease if the expression in this sex is reduced. Therefore, sexually antagonistic forces can drive sex-biased genes, i.e., genes more expressed in one sex than the other. The sexual antagonistic hypothesis proposes that sexual antagonistic and possible sex-biased genes have different probability to be fixed in the X/Z chromosome compared to the autosomes (**Figure 2**) (Charlesworth *et al.*, 1987; Rice, 1984). The early theoretical prediction by Rice (1984) is that the polymorphic antagonistic recessive alleles that favor males and disfavor females would prefer the X-linkage, as shown in recent observations in *Drosophila* populations (Innocenti and Morrow, 2010). However, the fixation of sexual antagonistic genes often shows opposite distribution. For example, considering a long evolutionary process, the X chromosome would have less opportunity to accumulate male-biased genes, as they are present in a single copy in males and in two copies in females (Wu and Xu, 2003). For the same logic, the X chromosome is more prone to accumulate female-biased genes. This prediction depends on the dominant or recessive character of the sexual antagonistic mutation but expectation for differential accumulation of sex-biased genes between X/Z chromosomes and autosomes is general for the evolution of

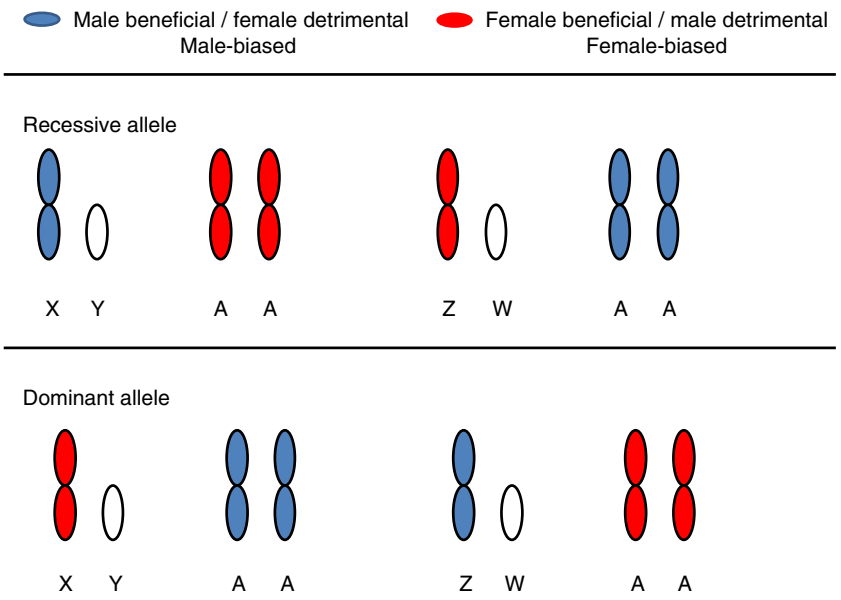

Figure 2 Chromosomal distribution of sexual antagonistic mutations/sex-biased genes. In a male heterogametic system (XY), it is unlikely that an X-linked recessive mutation beneficial to females and detrimental to males will be spread in the population. Those mutations will be exposed to negative selection in single copy in males and will not be visible to positive selection at first in heterozygous females (Rice, 1984). Alternatively, a dominant X-linked mutation will be present in females more often (two-thirds of the time) and will have more chance to be positive selective if beneficial to females. Alternatively, X-linked male-beneficial/female detrimental recessive mutation will be positively selected right away in males. However, if those mutations are dominant, negative selection will occur more frequently in females (Rice, 1984). Following the same principles, the reverse chromosomal patterns are expected for female heterogametic systems (ZW) (Ellegren and Parsch, 2007). These dynamics and the assumption that sexual antagonistic mutations will present ultimately sex-biased expression predict that sex chromosomes and autosomes will have distinct distribution of sex-biased genes.

sex chromosomes (see **Figure 2** for details) (Charlesworth *et al.*, 1987; Rice, 1984). In female heterogametic systems, similar rules but with sex roles reversed are assumed to be more likely to occur (Ellegren and Parsch, 2007).

Dosage compensation usually evolves with sex chromosomes as a balance of the expression levels between autosomal and X/Z-linked genes (Ohno, 1967; Charlesworth, 1978). Different mechanisms evolve in different organisms such as the inactivation of the X chromosome in female somatic cells of some mammals (Julien *et al.*, 2012). In *Drosophila*, however, there is a global change of chromatin structure of the X chromosome leading to the hypertranscription of its genes in males (Baket *et al.*, 1994). It has been proposed that the mechanism of dosage compensation in *Drosophila* would inhibit the presence of male-biased genes in the X chromosome, as further up-regulation of X-linked genes in males would be more difficult to be achieved (Bachtrog *et al.*, 2010; Viçoso and Charlesworth, 2009). Therefore, this hypothesis expected a paucity of male-biased genes in the X chromosome content (Bachtrog *et al.*, 2010; Viçoso and Charlesworth, 2009).

Meiotic sex chromosome inactivation (MSCI) may also have effects on the gene composition of the X/Z chromosomes (Lifschytz and Lindsley, 1972; Betran *et al.*, 2002). During male meiosis, the X chromosome is down-regulated through a global process of chromatin structure change (McKee and Handel, 1993; Turner, 2007). In mammals, where MSCI is well documented, inactivation in male germline would prevent the deleterious products from the recombination of nonhomologous sequences especially those from heteromorphic sex chromosomes (Lee 2005; Turner *et al.*, 2005). Nonetheless, there is experimental evidence for the existence of MSCI in other taxonomic groups such as *Drosophila*, nematodes, and grasshoppers (Vibranovski *et al.*, 2009a, 2009b; Cabrero *et al.*, 2007; Deng *et al.*, 2011; Hense *et al.*, 2007; Kelly *et al.*, 2002). If the X chromosome is inactive during male meiosis, X-linked testis-biased genes cannot be expressed (Betran *et al.*, 2002; Vibranovski *et al.*, 2009a, 2009b). Therefore, evolution of MSCI associated with sex chromosomes would broadly favor the origination of male-biased genes out of the X chromosome (**Figure 3**) (Lifschytz and Lindsley, 1972; Betran *et al.*, 2002). The opposite pattern should be expected for Z chromosome inactivation in female meiosis.

Meiotic drive is a phenomenon where a mutation in one allele distorts meiotic transmission in its own favor and result in the distortion of the normally 1:1 offspring sex-ratio (Gershenson, 1928; Hamilton, 1967). Usually meiotic drive alleles located on the X chromosome lead to the extinction of a population with excess of females due to the lack of males. Alternatively, suppressors in the Y chromosome and in the autosomes could be favored (Fisher, 1930; Thomsom and Feldman, 1975). Normally those autosomal suppressors are expected to function during male meiosis and therefore are male-biased expressed (Tao *et al.*, 2007). The evolution of such suppressors could lead to the accumulation of male-biased genes out of the X chromosome (Tao *et al.*, 2007).

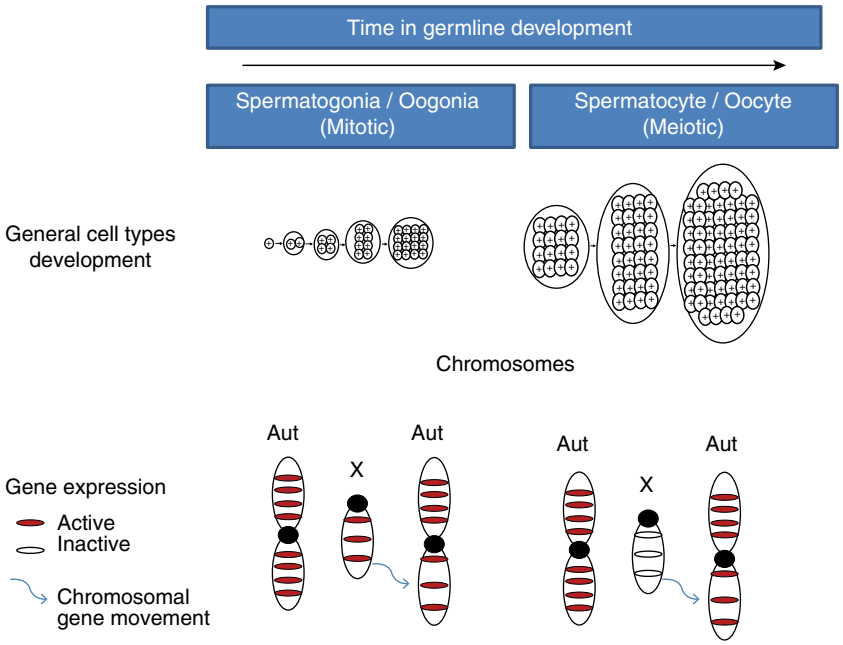

Figure 3 Meiotic sex chromosome inactivation (MSCI) and the movement out of the X chromosome. MSCI is the inactivation or down-regulation of the X- or Z-linked genes during male or female meiosis, respectively (Turner, 2007; Schoenmakers *et al.*, 2009). Duplicated genes from the X or Z to the autosomes will have more chance to be expressed in testis or ovaries, respectively (Lifschytz and Lindsley, 1972; Betran *et al.*, 2002).

Gene Evolution with the XY Systems

X Chromosomes

Several organisms with XY chromosome system of sex determination have been extensively studied: *Drosophila*, mammals, worms, mosquitos, and other insects (Betran *et al.*, 2002; Dorus *et al.*, 2006; Parisi *et al.*, 2003; Ranz *et al.*, 2003; Khil *et al.*, 2004; Reinke *et al.*, 2004; Wang *et al.*, 2005; Baker *et al.*, 2011; Baker and Wilkinson, 2010). In most of them, the general predictions regarding the demasculinization of the X chromosome in the overall evolutionary history have been confirmed, but each system shows peculiarities of specific evolutionary driving forces. In the *Drosophila* X chromosome, there is a paucity of male-biased protein coding and noncoding genes and an excess of female-biased ones revealed by several studies comparing transcriptomes of males and females (Parisi *et al.*, 2003; Ranz *et al.*, 2003, Sturgill *et al.*, 2007; Gao *et al.*, 2014). X chromosome demasculinization was found not only in testis and ovaries comparisons but also, though in lower levels, in studies of male and female carcasses (Parisi *et al.*, 2003). Those studies uncovered the role of sexual antagonistic dominant mutations as MSCI is expected to drive gene evolution only in male gonad tissue (Parisi *et al.*, 2003).

In addition, early genomic studies in *Drosophila* showed that retrogenes, new genes originated by retroposition from a parental gene, preferentially move out of the X chromosomes to the autosomes, so-called X→A gene movement (Betran *et al.*, 2002; Bai *et al.*, 2007; Dai *et al.*, 2006). Interestingly, this movement contributed to the X desmasculinization, as those new genes are frequently testis expressed as opposed to their broadly expressed parental genes (Betran *et al.*, 2002; Bai *et al.*, 2007; Dai *et al.*, 2006). Also, new genes generated by other mechanism involving DNA duplication also show X→A pattern indicating that natural selection instead of an intrinsic retrotransposition mutational bias drives the nonrandom movement (Vibranovski *et al.*, 2009b; Meisel *et al.*, 2009). The evidence in support of natural selection was detected also from population genomic analyses of copy number variation of retrogenes in *Drosophila* (Schrider *et al.*, 2011) and humans (Schrider *et al.*, 2013). MSCI was shown to be one of those selective forces driving the gene movement out of the X as autosomal retrogenes present specific higher male meiotic expression than their inactivated X-linked parental genes (Vibranovski *et al.*, 2009a). Dosage compensation also seems to play a role on the demasculinization of the *Drosophila* X chromosome by limiting further increase of male-biased gene expression close to the compensated regions (Bachtrog *et al.*, 2010).

In mammals, studies with mouse spermatogenic transcriptome have shown that the paucity of X-linked male-biased genes is more restricted to meiotic and some level of post-meiotic cells consistent with the action of MSCI (Khil *et al.*, 2004). Genes specifically expressed in spermatogonia and sertoli cells, MSCI-free tissues, are overrepresented on the X chromosome (Wang *et al.*, 2001; Soumillon *et al.*, 2013). Interestingly, brain-specific genes are also enriched in the X chromosomes, indicating that recessive sexual antagonistic mutations beneficial to males might be prevailing in the evolution of genes expressed in spermatogonia, sertoli cells, and brain (Zhang *et al.*, 2010b; Soumillon *et al.*, 2013). Recently, X chromosome enrichment of male-biased genes expressed in the brain was found in *Drosophila*, but the study also suggests association with dosage compensation (Huylmans Parsch, 2015).

Retrogene movement out of and into the X chromosome has been also found in excess in humans and mouse genomic

studies (Emerson *et al.*, 2004). However, only X-derived autosomal genes present association with testis expression (Emerson *et al.*, 2004). Actually, the movement is most likely to be driven by MSCI as, similar to in *Drosophila*, those retrogenes compensate the expressions of their inactive X-linked parental genes during meiosis (Potrzebowski *et al.*, 2008; Vibranovski *et al.*, 2009a, 2009b; Dai *et al.*, 2006).

Recently, the genome sequencing of *Drosophila* species and other vertebrates allowed the use of phylogenetic relationships to date the origin of *D. melanogaster*, human, and mouse new genes (Zhang *et al.*, 2010a,b). This approach revealed that paucity of male-biased genes in both *Drosophila* and mammals X chromosomes is only observed for genes older than a few million years. Recently originated male-biased genes are found to be overrepresented on the X chromosome consistent with fixation of recessive male-beneficial alleles by sexual antagonism. In both cases, the proportion of X-linked genes decreases gradually with time leading to an excess of male-biased genes on the autosomes, suggesting that X demasculinization is an evolutionary process (Wu and Xu, 2003; Zhang *et al.*, 2010a,b; Gao *et al.*, 2014). In *Drosophila*, the switch from X-linked to autosomal enrichment of male-biased genes occurs before the split of the *melanogaster* subgroup (<3–6 million years ago) (Russo *et al.*, 1995; Zhang *et al.*, 2010a), whereas in mammals there were two bursts of gene gain on the X chromosome (Zhang *et al.*, 2010b). It is evident in mammals that, however, the MSCI contributed to the switch dynamics in addition to sexual antagonistic forces (Zhang *et al.*, 2010b; Mueller *et al.*, 2008). Inactivation does not affect young genes in meiotic cells, only old ones suggesting that new genes are immune at first to the chromosomal-wide mechanism of silencing (Zhang *et al.*, 2010b). Also, feminization of the X chromosome is associated with the process of demasculinization in both mammals and *Drosophila* as most of the genes expressed in ovaries are relatively old (Zhang *et al.*, 2010a,b).

In *Anopheles gambiae*, the malaria vector mosquito, the X chromosome is also depleted with male-biased genes and enriched with female-biased ones (Baker and Russell, 2011; Baker *et al.*, 2011). Only testis and ovaries transcriptomes were able to detect the biased chromosomal distribution emphasizing the importance of using of gonad tissues to study sex-biased genes (Baker and Russell, 2011; Baker *et al.*, 2011; VanKuren and Vibranovski, 2014). The mosquito testis is several times smaller than its ovary and their differential rate of dilution in whole-body transcriptome turns the detection imprecise (Baker and Russell, 2011; Baker *et al.*, 2011). It is interesting that excess of retrogene moving off the X chromosome has been also detected in *A. gambiae* (Toups and Hahn, 2010). The movement associated with male-biased expression (Baker and Russell, 2011) is only detected after the split from this species and *Aedes aegypt* – a homomorphic sex chromosome species – from their common ancestor. This indicates that X→A pattern is probably a property of organisms with heteromorphic sex chromosome (Toups and Hahn, 2010).

In another model organism, the worm *Caenorhabditis elegans*, the X chromosome desmasculinization and feminization are also observed (Reinke *et al.*, 2004). The X chromosome is depleted with sperm-enriched genes (Reinke *et al.*, 2000, 2004). Transcriptome analyses together with histone modification studies provide evidence for X inactivation both in *C. elegans* spermatogenesis and in the hermaphrodite germline, suggesting the role of MSCI with other forces on the evolution of demasculinization (Kelly *et al.*, 2002; Reuben and Lin, 2002; Bean *et al.*, 2004; Bessler *et al.*, 2010; Reinke *et al.*, 2004).

Y Chromosomes

Sex chromosomes in placental mammals and marsupials have a common origin about 200 million years ago after the split from monotremes which carry independently originated five Y chromosomes (Lahn and Page, 1999; Potrzebowski *et al.*, 2008; Grützner *et al.*, 2004). The sex-determining gene SRY probably triggered the differentiation of all therians sex chromosomes, including humans (Goodfellow and Lovell-Badge, 1994; Lahn and Page, 1999). Suppression of crossing-over between proto X and Y should be advantageous as no proto-Y-linked new allele that confers a selective advantage to males, but neutral or detrimental to females would be able to recombine to the proto-X (Charlesworth and Charlesworth, 2000; Bachtrog, 2013). Although human Y chromosome still bears a cluster of X-Y paralogues with active recombination, discrete clusters of genes with different degree of divergence – called 'evolutionary strata' – provide evidence that progressive reduction in recombination have taken place along the therian sex chromosome evolution (Lahn and Page, 1999). The human Y chromosome, therefore, became enriched with repetitive sequences, very heterochromatic and containing genes with male-specific functions due to gene retention and amplification (Bellot *et al.*, 2010; Skaletsky *et al.*, 2003; Lahn and Page, 1999). Two recent studies (Bellott *et al.*, 2014; Cortez *et al.*, 2014), however, reconstructed the evolutionary function of the Y chromosome along several mammals revealing that gene content in the Y chromosome is not only a reflection of the male reproduction and spermatogenesis function. Gene retention and survival in the Y chromosome in those mammals are enriched with regulatory function and most probably are very sensitive to dosage constraints caused by deterioration on the proto-Y.

The *D. melanogaster* Y chromosome is completely heterochromatic and contains just a small number of protein coding genes in comparison to the X chromosome (Gepner and Hays, 1993; Carvalho *et al.*, 2000; Carvalho *et al.*, 2001; Vibranovski *et al.*, 2008; Carvalho *et al.*, 2003). The Y chromosome does not determine sex; therefore flies without it are viable but sterile due to the presence of fertility factors (Bridges, 1916; Brosseau, 1960; Kennison, 1981; Gatti and Pimpinelli, 1983; Hazelrigg *et al.*, 1982). *Drosophila* genomic and functional studies have shown that Y-linked genes are expressed and/or have a role in spermatogenesis such as *kl-2*, *kl-3*, and *kl-5* genes that encode for proteins involved in the sperm flagella motor (Hardy *et al.*, 1981; Gepner and Hays, 1993; Carvalho *et al.*, 2000; Carvalho *et al.*, 2001; Vibranovski *et al.*, 2008; Carvalho and Clark, 2013).

Interestingly, there is not a single-copy gene in the Y chromosome of *D. melanogaster* with a homologue in the X chromosome (Carvalho *et al.*, 2000; Carvalho *et al.*, 2001; Vibranovski *et al.*, 2008). The majority of the genes, however, are recent acquisitions of the autosomes (Carvalho *et al.*, 2000; Carvalho *et al.*, 2001; Vibranovski *et al.*, 2008). Comparative

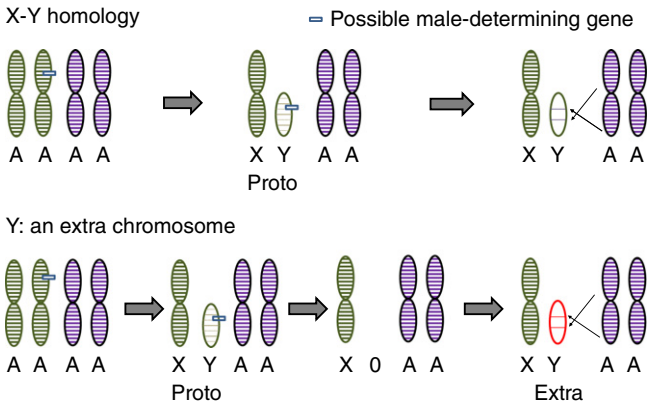

Figure 4 Scenarios for the Y chromosome origin in *Drosophila*. Two possible scenarios for the Y chromosome origin and evolution resulting in the current observation that single-copy Y-linked genes are homologous to autosomal ones (Bernardo Carvalho *et al.*, 2009). Only male karyotypes are shown. X–Y homology – one member of one autosome pair acquires a male determining gene; the process of degeneration and evolution of dosage compensation lead to the formation of a proto X-Y; the degeneration is complete while Y chromosome acquired new genes duplicated from the autosomes. Y: extra chromosome – the homologous Y chromosome has been completely degenerated and lost. An extra chromosome (shown in red) acquires the capacity to pair with the X and new genes duplicated from the autosomes. Note that for both scenarios, the male determining gene has been lost during evolution or never existed. Therefore, the Y chromosome does not determine sex. Rather, as observed in *Drosophila*, sex is determined by the relative dosage of X chromosome in comparison to the autosomes.

genomic studies with other *Drosophila* species have shown that the Y chromosome gene content is not very conserved due to gene gain being more frequent than gene loss (Koerich *et al.*, 2008). For example, the *Drosophila pseudoobscura* Y chromosome is not homolog to the Y of other *Drosophila* species and the genes present in the ancestral *Drosophila* Y chromosome are found in the *D. pseudoobscura* dot autosomal chromosome (Carvalho and Clark, 2005). The general lack of homology of Y-linked coding protein genes with the X chromosome and the high rate of gene turnover suggest that *Drosophila* Y is not a degenerated autosome like in mammals but might has been originated by the acquisition of an extra chromosome instead (**Figure 4**; Bernardo Carvalho *et al.*, 2009).

Regardless the origin, *Drosophila* Y-linked genes show some sign of natural selection efficacy reduction (Singh *et al.*, 2014). However, it seems that there is purifying selection on strong deleterious mutations despite its smaller effective size and lack of recombination (Singh *et al.*, 2014). Moreover, several of those genes already show sign of positive selection along the *Drosophila* genus evolution (Singh *et al.*, 2014). This, together with the recent evidence on the important role of the *Drosophila* Y chromosome in regulating the expression of other autosomal and X-linked genes emphasizes the significant phenotypic impact that the Y chromosome has evolved (Lemos *et al.*, 2008; Singh *et al.*, 2014).

Neo-Sex Chromosomes

New sex chromosomes can be also formed in nature by chromosomal fusion between autosomes and ancestral sex chromosomes. At the time of their formation, those new chromosomes – named neo-X and neo-Y – have the same gene content as the original autosome. However, as neo-sex chromosomes segregate in the same manner as the X and Y chromosomes, changes in many aspects occurs with time making them gradually resembling genuine sex chromosomes (Bachtrog, 2013). The studies of independently formed neo-sex chromosomes with different age of origins have revealed rich information about how heteromorphic pair of sex chromosomes usually differentiates (Bachtrog, 2013), for example, the general evolutionary forces and molecular mechanisms driving such differentiation and the order of evolutionary events (Bachtrog, 2013).

Neo-sex chromosomes were independently formed by the fusion of autosomes with the same ancestral sex chromosome in several *Drosophila* species and lineages, for instance, in *D. willistoni*, *D. pseudoobscura*, *D. miranda*, *D. albomicans* (Lucchesi, 1978). With the advance of comparative genomics, the studies of the last three species provide a powerful framework to compare evolutionary events in an old, an intermediate and a young sex chromosome, as their origin were respectively approximately 15, 1, and 0.1 million years ago (Lucchesi, 1978; Bachtrog, 2013). The analysis of *D. pseudoobscura* neo-sex chromosomes has shown that it took only15 million years for a *Drosophila* autosome to evolve completely into a sex chromosome (Bachtrog, 2013). Neo-X chromosome is fully dosage compensated and already shows significant paucity of male-biased genes and excess of female ones (Marin *et al.*, 1996; Sturgill *et al.*, 2007; Assis *et al.*, 2012). In addition, selective forces have already started to drive the relocation of genes out of the X chromosome to the autosomes (Vibranovski *et al.*, 2009b; Meisel *et al.*, 2009). An old neo-Y chromosome, due to degeneration, is mostly heterochromatic and bears only a few active genes (Carvalho and Clark, 2005). Alternatively, a young neo-Y chromosome like the one present in *D. albomicans* almost does not show signs of DNA degeneration and pseudogenization (Zhou *et al.*, 2012). However, several neo-Y linked genes are lower expressed in comparison to their neo-X-linked homologues, suggesting transcription down-regulation as the first step in sex chromosome differentiation (Zhou and Bachtrog, 2012b). The *D. miranda* neo-Y chromosome shows intermediate features: partially heterochromatic, accumulation of repetitive DNA such as

transposable elements, approximately half of the genes with homologues in the neo-X already show signs of loss of function (Zhou and Bachtrog, 2012a). Active genes in the neo-Y are not a random small subset of the ancestral autosome. Masculinization of the neo-Y chromosomes is noted by the retention of genes with male-beneficial effects and evolution of male-biased expression for genes involved in reproductive functions (Zhou and Bachtrog, 2012a,b). In addition, as in mammals, the retained genes tend to enrich with regulatory functions, suggesting that dosage sensitive genes have more chances to be maintained on the neo-Y (Zhou and Bachtrog, 2012a,b). On the other hand, the intermediate neo-X chromosome of *D. miranda* already shows signs of demasculinization as male-specific genes are more prone to be lost or down-regulated in a considerably short evolutionary time (Kaiser and Bachtrog, 2014).

Another example of neo-sex chromosomes that have been reported is the one found in stalked-eye flies (Baker and Wilkinson, 2010). Those species present a sexual dimorphism in eyespan that is linked to the success of male reproduction (see review in Baker *et al.*, 2012). Recently, studies with comparative genomic hybridization – an efficient approach for non-model organism – revealed a neo-X chromosome formed in several species of the *Teleopsis* genus (Baker and Wilkinson, 2010). Interestingly, chromosomal gene movement between sex chromosomes and autosomes was also observed confirming that gene evolution is a major process on the formation of sex chromosomes.

Gene Evolution with the ZW Systems

ZW systems, in which the female is heterogamety, have been found in several organisms, e.g., birds, reptiles, and some species in fishes and insects, but only recently more extensively studied. There are several reasons for the increase of interest in species with ZW chromosomes. First, the genomic era made the genetic studies with non-model organism more feasible. Second, as we expanded our taxonomic view about the sex chromosomes, it is realized that ZW are more commonly found than previously thought (Bachtrog *et al.*, 2014). Third, the comparison between XY and ZW systems have shown to be very useful to separate the sex chromosome effects from sex effects (Ellegren, 2011). Individuals harboring different chromosomes (heterogamety) present different sexes in ZW and XY: female and male, respectively. Therefore, patterns and processes associated with harboring the heterochromatic, differentiated W or Y chromosome can be disentangled from sexual selection and evolution of sex (Ellegren, 2011).

We focus our review on gene evolution and origination in ZW systems with differentiated chromosomes such as avian and some Lepidoptera species. ZW chromosomes in birds seem to share largely homology and have started to differentiate around 130 million years ago or even older (Shetty. *et al.*, 1999; Nam and Ellegren, 2008; Cortez *et al.*, 2014). The same Z chromosome found in Lepidoptera, including moths, butterflies, and silkworm seems to be already present in the common ancestor shared with Trichoptera more than 190 million years ago (Traut *et al.*, 2007).

W Chromosomes

In birds, there is still a debate regarding the sex determination mechanism. It is not known if sex determination depends on the Z chromosome dosage relative to autosomes or if there is one or several female-determining genes or factors on the W chromosome (e.g., Cortez *et al.*, 2014; Smith *et al.*, 2009). The knockdown of the gene *DMRT1*, located on the Z chromosome in all birds, directs the gonad feminization, suggesting this gene as the sex determining one in birds (Smith *et al.*, 2009). However, there is still the possibility that *DMRT1* is a downstream gene on the sex-determining pathway lead by other Z-linked and/or W-linked genes (Ellegren, 2011). Alternatively, sex in birds could be determined by Z chromosome dosage suggested by the fact that, in chicken, there is no full chromosome-wide dosage compensation of Z-linked genes (Ellegren *et al.*, 2007; Itoh *et al.*, 2007). Nonetheless, there is one feature of W chromosome in birds that is known for sure: the formation of different evolutionary strata consistent with the model that the loss of recombination may occur in steps caused by distinct successive inversion events (Nam and Ellegren, 2008; Cortez *et al.*, 2014).

Studies with chicken have shown that the W chromosome share similar features with the Y chromosome, such as large portion of repetitive heterochromatin and small size as well as decrease of genetic diversity (Berlin and Ellegren, 2004; International Chicken Genome Sequencing Consortium, 2004). Therefore, those features are probably not a result of selective sweeps due to sexual selection in sperm competition. Rather, the reduced diversity seems to be independent of the type of heterogamety (Ellegren, 2011). The W chromosome in chicken, however, shows a distinct pattern in comparison to therian Y chromosomes: expression levels and spatial patterns are preserved in comparison to the proto-sex chromosome (Cortez *et al.*, 2014) that could be related to the lack of global dosage compensation (Ellegren *et al.*, 2007; Itoh *et al.*, 2007).

In Lepidoptera, the majority of basal clades lack a W chromosome (Z0 females), suggesting that W chromosome arose later and it is not homologue to the Z chromosome as proposed for the Y chromosome in *Drosophila* (Traut *et al.*, 2007; Bernardo Carvalho *et al.*, 2009). However, there is still a possibility that the W chromosome has been lost in those basal clades. In *Bombyx mori*, the model organism for Lepidoptera, domesticated more than 5000 years ago and economically important for the production of silk, there is a genetic factor on the W chromosome that determines the female sex (Traut *et al.*, 2007; Goldsmith *et al.*, 2005). Although no precise localization has been defined, sex is not determined by the ratio of Z chromosomes relative to autosomes (Traut *et al.*, 2007; Goldsmith and Marec, 2010).

Z Chromosomes

Both chicken and silkworm present a deficit of female-biased genes and an excess of male-biased genes on the Z chromosome (Wang *et al.*, 2012; Xia *et al.*, 2007; Arunkumar *et al.* 2009; Kaiser and Ellegren, 2006; Storchová and Divina, 2006; Mořkovský *et al.*, 2009; Mank and Ellegren, 2009). These observations are in agreement with the model of partly dominant mutations with sexual antagonistic effects (**Figure 2**; Rice, 1984). However, the genes that are biased expressed in

somatic cells of chicken female germline are overrepresented on the Z chromosome, raising the possibility that recessive mutations beneficial to females but harmful to males have their role in the avian evolution (Mořkovský *et al.*, 2009). Nonetheless, oocytes do not show such pattern (Mořkovský *et al.*, 2009) and therefore the MSCI, even if brief (Schoenmakers *et al.*, 2009), could also contribute to the overall picture of the chromosomal distribution of sex-biased genes in birds.

Another possible explanation for the excess of male-biased genes in the chicken Z chromosome is the lack of dosage compensation (Ellegren *et al.*, 2007; Itoh *et al.*, 2007). Two copies of the Z-linked genes in males as opposed to just one in females would result in a general male-biased expression of the Z chromosome. However, recent works analyzing different strata showed that the Z chromosome gene expression might be more influenced by masculinizing selection, rather than by selection for dosage compensation (Wright *et al.*, 2012).

Gene trafficking between sex chromosomes and autosomes also revealed the dynamics of gene origination in ZW systems. In chicken, little movement of retroposition events, including the formation of retrogenes (International Chicken Genome Sequencing Consortium, 2004; Toups *et al.*, 2011), were detected, probably due to some mechanisms including inefficiency of the reverse transcriptase in recognizing polyadenylated mRNAs (Haas *et al.*, 2001). One study failed to find excess of gene movement in or off the avian Z chromosomes analyzing duplications mediated at DNA level (Toups *et al.*, 2011). However, other study observed that genes that have been repeatedly relocated to the Z chromosome during avian evolution show testis-specific expression (Bellott *et al.*, 2010). In silkworm, the excess of female-biased gene movement out of the Z was detected (Wang *et al.*, 2012).

Final Remarks

The multiple independent originations of sex chromosomes had profound impact on the evolution of organisms. The impact can be on the processes of sex chromosome formation including chromosome degeneration and dosage compensation mechanism or on the underlying mechanisms such as sexual antagonistic conflicts, sexual selection, meiotic sex chromosome inactivation, meiotic drive etc. Independently of the types of heterogametic system (XY or ZW), the achieved scientific advances have shown that sex chromosomes are an assured source in nature to trigger the evolution of new genes to novel functions and phenotypes.

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See also: Mutation and Genome Evolution. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth

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Genetic Architecture

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Nomenclature

E Vector of genetic effects
G=(G$_{ij}$) Vector of genotypic values; $G_{ij}=\mu+\alpha_{ij}+\delta_{ij}$
R Reference point of an individual-referenced model of genetic effects
S Genetic-effects design matrix
a Individual-referenced additive genetic effect
d Individual-referenced dominance genetic effect
p$_i$ Allele frequencies; $p_i=p_{ii}+\frac{1}{2}\Sigma p_{ij}$
p$_{ij}$ Genotype frequencies; Indicator variables at individual-referenced formulations
α Population-referenced additive genetic effect
α$_i$ Average additive effect; $\alpha=\alpha_2-\alpha_1$ (with multiple alleles, $\alpha^{ij}=\alpha_j-\alpha_i$)
α$_{ij}$ Breeding value; $\alpha_{ij}=\alpha_i+\alpha_j$
δ Population-referenced dominance genetic effect
δ$_{ij}$ Dominance deviation
μ Population phenotype mean

Glossary

Additive genetic effect Genetic effect that retains properties of the alleles that add up, whether in a population context or not and regardless of whether there are also interaction effects present or not. In the population context, this is the genetic effect directly related with phenotype resemblance due to sharing alleles – rather than genotypes – and thus with selection response (conditioned by resemblance between parents and offspring).
Average additive effect of allele A_i Expectation over a population of the effect on phenotype of a substitution of one allele at locus A by allele A_i.
Breeding value Expectation of the phenotype of the offspring of an individual in a random mating population, in relation to the population mean.
Dominance genetic effect Genetic effect that accounts for evolutionary properties of a one-locus genotype, particularly due to interaction effects (departures from additive expectations) of alleles within that locus.
Epistatic genetic effect Genetic effect that accounts for evolutionary properties of multilocus genotypes, particularly due to interaction effects (departures from additive expectations) of alleles across loci.
Genetic architecture Set of loci underpinning phenotypic variation of a trait in a population, including the sets of alleles present at each locus and the genetic (additive and interaction) effects involved.
Genetic effect Parameter that accounts for effects of allele substitutions and thus has a direct genetic and evolutionary meaning. It comes from the reparameterization of a genotype-to-phenotype (GP) map using a specific model of genetic effects.
Genotype-to-phenotype map Function assigning genotypes to phenotypes, often provided as a vector of genotypic values.
Genotypic value Expected phenotype of a particular genotype.
Imprinting genetic effect Genetic effect that reflects properties of one-locus genotypes in which allele properties depend upon the sex of their parent-of-origin, particularly accounting for interaction effects of alleles due to that dependence.
Individual-referenced genetic effects Genetic effects coming from the reparameterization of a GP map in terms of allele substitutions performed from the reference of an individual genotype.
Model of genetic effects Mathematical expressions reparameterizing a GP map into (additive and interaction) effects of allele substitutions with reference to a defined (individual or population) context.
Population-referenced genetic effects Genetic effects coming from the reparameterization of a GP map in terms of allele substitutions averaged across a population with particular genotype frequencies.
Reaction norm Expected phenotypes displayed by a genotype under a range of values of an environmental variable.

Genetic Source of Phenotypic Variation

Gregor Mendel inferred the existence and key properties of inheritance factors related to several binary phenotypes of pea plants (*Pisum sativum* L.). The foundations of genetics as a scientific field were established on his finding at the beginning of the twentieth century. Toward one and a half centuries after Mendel, we keep on trying to disclose loci with genetic variants underpinning phenotypic variation of traits in populations. The desired information is the relation between genotypes and phenotypes, the genotype-to-phenotype (GP) map, which can be expressed as a vector of genotypic values, **G** – gathering the expected phenotypes of each of the genotypes considered.

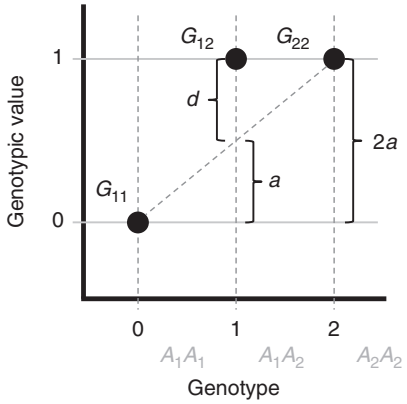

Figure 1 Graphical representation of the individual-referenced genetic effects in **Table 1**. The genotypes are in the horizontal axis represented as content of allele A_2, and the genotypic values (G_{ij}) are in the vertical axis. The difference between the genotypic values of the two homozygotes determines the additive effect (of substituting allele A_1 by allele A_2) and the dominance effect is the departure of the heterozygote from its additive expectation (due to the presence of interaction between the two alleles).

Table 1 Individual-referenced genetic effects derived from a phenotype studied by Mendel, pea color

Phenotype	*Genotype*	G_{ij}	*Genetic effects*
Green	A_1A_1	0	$R=0$
Yellow	A_2A_2	1	$R+2a=1=>a=½$
Yellow	A_1A_2	1	$R+a+d=1=>d=½$

Notes: Mendel inferred that genetic factors (genotypes) determined the phenotypes. A GP map consists in just assigning numerical (genotypic) values (G_{ij}) are to the phenotypes – in this case 'yellowness' is measured so that green peas are assigned to 0 and yellow ones to 1. Choosing the green pea as reference, the additive effects of allele substitutions come directly from comparison against A_2A_2 (involving two-allele substitutions). Finally, the dominance effect can be derived by accounting for the genotypic value of the heterozygote, where the two different alleles coincide and may interact. A graphical interpretation is given in Figure 1.

Genetic Effects

However, the GP map is seldom directly informative about evolutionary properties. In the simplest case – a genetic architecture of one locus with two alleles, A_1 and A_2 (and assuming also for simplicity that phenotype is fitness) – the outcome of selection depends on the genotypic value of the heterozygote (G_{12}) relative to the midpoint of those of the homozygotes (G_{11}, G_{22}). Such dependence is easier to express in terms of additive, a, and dominant, d, effects (as defined in Figure 1): fixation of the allele in the highest homozygote occurs whenever $-|a|<d<|a|$, *becoming* asymptotic as d approaches $|a|$, a polymorphic equilibrium is attained when $d>|a|$, and fixation of any of the two alleles is possible (depending on the initial frequencies) when $d<-|a|$. Thus, it is in general compulsory to reparameterize the genotypic values of a genetic architecture into genetic effects. This can be done in two different ways, using two different kinds of mathematical formulations that fit to two different genetic meanings.

Individual-referenced formulations

Genetic architectures of traits vary across populations since, on the one hand, one locus may bear different (from a single one to many) variants in different populations and, on the other hand, the effects on phenotype of variation at that locus may also differ depending upon the (genetic and/or environmental) background. Nevertheless, it is possible and actually sensible to initially express a genetic architecture regardless of the frequencies of the considered alleles at any population, through individual-referenced formulations. A toy example of such approach using Mendel's experiments is given in Table 1.

In order to reflect how genetic variation underpins phenotypes, genetic effects account for the effects of allele substitutions, which implies the choice of a particular starting point, or reference. Individual-referenced formulations use an individual genotype as reference point. As shown in Figure 1 and Table 1, comparing homozygotes allows us to extract effects that add up whilst interactions appear in the parameterization due to deviations from the additive expectations. Interactions between alleles within a locus are called dominance, whereas those affecting alleles at different loci shall be called epistasis.

Although not absolutely necessary for understanding that the models of genetic effects reparameterize the GP map into additive and interaction effects, it is very illustrative to have a look at how such reparameterizations can be conveniently condensed with matrix notation as $\mathbf{G}=\mathbf{SE}$:

$$\begin{pmatrix} G_{11} \\ G_{12} \\ G_{22} \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 1 & 1 \\ 1 & 2 & 0 \end{pmatrix} \begin{pmatrix} R \\ a \\ d \end{pmatrix} \qquad [1]$$

In the previous expression it is easy to track that the genotypic values are decomposed into additive and interaction (dominance) effects of allele substitutions from the reference of A_1A_1. In detail, the first row of the genetic-effects design matrix, $\mathbf{S}$, indicates that the genotypic value of A_1A_1, G_{11}, is the reference point ($G_{11}=R$). The second row indicates that the genotypic value of A_1A_2, G_{12}, differs from the reference in one additive effect and one dominance effect ($G_{12}=R+a+d$), and the third row indicates that the genotypic value of A_2A_2, G_{22}, differs from the reference in two additive effects ($G_{22}=R+2a$). Thus, expression [1] exactly fits Figure 1 and Table 1.

The matrix notation enormously facilitates computations. For instance, we can check how the genetic effects are defined in terms of the genotypic values by just inverting the genetic-effects design matrix, $\mathbf{S}$, leading to:

$$\begin{pmatrix} R \\ a \\ d \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ -1/2 & 0 & 1/2 \\ -1/2 & 1 & -1/2 \end{pmatrix} \begin{pmatrix} G_{11} \\ G_{12} \\ G_{22} \end{pmatrix} \qquad [2]$$

The first row of $\mathbf{S}^{-1}$ designates which is the reference point, whilst the other two rows provide the definitions of the genetic effects in terms of the genotypic values, $a=(G_{22}-G_{11})/2$ and $d=G_{12}-((G_{22}+G_{11})/2)$. A general expression for any reference point can be obtained by just replacing that row by p_{11}, p_{12}, p_{22} (which would here work just as indicator variables, taking values 0 or 1, instead of as frequencies). Inverting again, after that replacement, we obtain a general expression of the

genotypic values from any reference point:

$$\begin{pmatrix} G_{11} \\ G_{12} \\ G_{22} \end{pmatrix} = \begin{pmatrix} 1 & -(p_{12}+2p_{22}) & -p_{12} \\ 1 & 2(p_{11}-p_{22}) & 1-p_{12} \\ 1 & p_{12}+2p_{11} & -p_{12} \end{pmatrix} \begin{pmatrix} R \\ a \\ d \end{pmatrix} \quad [3]$$

Population-referenced formulations

We also need formulations appropriate to reflect the properties of populations with particular genotype frequencies. Such formulations stem from the seminal work of Fisher (1918), and can be obtained through a linear regression of the genotypic values on the allele content (**Figure 2**). These formulations keep on decomposing the genotypic values into additive and interaction effects, although with a different meaning – fitting the population context. The reference point becomes the population mean phenotype. The additive component of each genotypic value, G_{ij}, is its breeding value, α_{ij}, reflecting whether the offspring of individuals with that genotype are expected to score below or above the population mean phenotype. Its dominance component, δ_{ij}, is the deviation of the breeding value from the genotypic value.

The breeding value of genotype A_iA_j is the sum of the average effects of the alleles it bears, $\alpha_{ij}=\alpha_i+\alpha_j$. The average effect of allele A_i is the expected phenotype change that would be caused by substituting an allele of an individual of the population by that allele (see, e.g., Falconer and MacKay, 1996, p. 113). Therefore, population-referenced formulations are still parameterized by effects of allele substitutions, which, in contrast with the individual-referenced formulations, are averaged over the whole population considered.

It is also possible to express population-referenced formulations in matrix notation, as (Zeng *et al.*, 2005; Álvarez-Castro and Carlborg, 2007):

$$\begin{pmatrix} G_{11} \\ G_{12} \\ G_{22} \end{pmatrix} = \begin{pmatrix} 1 & -2p_2 & -2p_2^2 \\ 1 & p_1-p_2 & 2p_1p_2 \\ 1 & 2p_1 & -2p_1^2 \end{pmatrix} \begin{pmatrix} \mu \\ \alpha \\ \delta \end{pmatrix} \quad [4]$$

which is a general formulation for any allele frequencies, with p_1, p_2 as frequencies of A_1, A_2, respectively, and Greek letters (μ, α, δ) instead of Latin ones for stressing the different meaning of the genetic effects relative to the individual-referenced formulations – expressions [1–3]. Expression [4] decomposes the genotypic values into additive and interaction components from the reference of a population, as $G_{ij}=\mu+\alpha_{ij}+\delta_{ij}$. Additionally, it holds $\alpha(=\alpha^{12})=\alpha_2-\alpha_1$ and in general (with multiple alleles) $\alpha^{ij}=\alpha_j-\alpha_i$, $i<j$; hence $\alpha^{ik}=\alpha^{ij}+\alpha^{jk}$, $i<j<k$ (i.e., they actually are additive parameters).

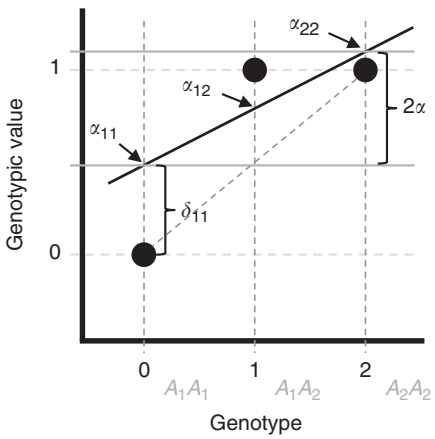

Figure 2 Population-referenced genetic effects for the same simple genetic architecture of **Table 1** and **Figure 1**. The frequency of A_2 is 0.7 and Hardy–Weinberg proportions hold. The departures (δ_{ij}, only δ_{11} marked) of the genotypic values from the values predicted by the weighted (by the genotypic frequencies) linear approximation (which are the breeding values, α_{ij}) are due to dominance (i.e., to the three genotypic values not laying in the same line). The slope of the approximation, α, is given by the difference between the breeding values of the homozygotes and reflects the average effects of allele substitutions.

Genetic variance decomposition

Population-referenced formulations of genetic effects are directly related to genetic variance decomposition – the variances of the (additive and interaction) components of the genotypic values. The most important component is the additive variance, V_A, which is the variance of the aforementioned breeding values, α_{ij}. With **Figure 2** in mind, it is easy to appreciate that the values population-referenced genetic effects take may vary remarkably for different population frequencies. Hence, so will the genetic variance components, which makes complete sense since they are built to keep a consistent biological meaning in different population contexts. The additive variance, for instance, is an index of the potential of a population to change the mean phenotype under directional selection in a one-generation step. Indeed, populations with different genotype frequencies may respond differently to selection and therefore have different additive variances (coming from different population-referenced genetic effects) for the trait under study.

What is true for different populations becomes even more remarkable when comparing different (individual- and population-referenced) formulations. Formalizing the decomposition of the genetic variance enabled Fisher (1918) to found the theoretical basis of quantitative genetics on Mendelian inheritance, but he would not at that juncture (when genes were not even known to lay in the DNA) bother to develop in detail additional formulations representing specific effects of substitutions of particular alleles from the reference of a given genotype. Such a task makes much more sense in the gene-mapping era, when it consequently becomes sensible also to beware the differences in meaning between the new individual- and the classical population-referenced genetic effects.

Indeed, comparing **Figures 1** and **2** it becomes clear that individual- and population-referenced genetic effects of the same genetic system are expected to be different. More to the point, it is noteworthy that, on the one hand, large additive variances are consistent with absence of individual-referenced additive genetic effects, since all individual-referenced genetic effects may contribute to the additive genetic variance (see, e.g., **Figure 1** in Álvarez-Castro, 2014; Álvarez-Castro and Le Rouzic, 2015), whereas on the other hand, nil additive variance may occur in the face of significant individual-referenced additive effects – particularly at polymorphic equilibria (see, e.g., Álvarez-Castro and Yang, 2011).

Genetic Architecture

The previous examples illustrate that sustaining a concept of genetic architecture on population-referenced genetic effects would be tricky to handle. Indeed, genetic architecture would that way become a property of a population with specific genotypic frequencies. Although little or no population-referenced interaction effects and variances do not warrant little or no individual-referenced interaction effects, the opposite is in point of fact true. Individual-referenced genetic effects are thus better candidates for grounding a definition of genetic architecture, as a number of loci underpinning phenotypic variation on a trait and their evolutionary properties, expressed as additive and interaction effects.

It is noteworthy, though, that the concept of genetic architecture is in any case not completely unaware of the population context. Evolutionary genetics is all about variation and different populations bear different genetic variation. In the extreme, one locus may bear a number of alleles underlying phenotypic variation at one population whilst being monomorphic – and therefore inconsequential to genetic architecture – at another one. It may also be useful to recall at this point that population-referenced genetic effects are necessary for properly making use of a genetic architecture, since they enable us to analyze its evolutionary implications at the particular context of a population with specific genotype frequencies. Transforming any kind of (e.g., individual-referenced) genetic effects, $\mathbf{E}_1$, into the ones fitting a particular population, $\mathbf{E}_2$, can easily be done using simple matrix algebra (Álvarez-Castro and Carlborg, 2007):

$$\mathbf{E}_2 = \mathbf{S}_2^{-1}\mathbf{S}_1\mathbf{E}_1 \qquad [5]$$

For being able to perform this transformation it is necessary to know, on top of the initial vector of genetic effects, $\mathbf{E}_1$, the genetic-effect design matrices of the two cases considered, $\mathbf{S}_1$ and $\mathbf{S}_2$. It is therefore necessary to develop such matrices for any putative genetic architecture to be considered, both concerning individual- and population-referenced formulations and from any reference point (Álvarez-Castro, 2012; Álvarez-Castro *et al.*, 2012a). Albeit we have initially considered, for simplicity, the population-referenced case of Hardy–Weinberg proportions (expression 4), it is also possible to provide a more general expression also accounting for departures from those proportions (Álvarez-Castro and Carlborg, 2007):

$$\begin{pmatrix} G_{11} \\ G_{12} \\ G_{22} \end{pmatrix} = \begin{pmatrix} 1 & -2p_2 & -\dfrac{p_{12}p_{22}}{2p_1p_2 - 1/2p_{12}} \\ 1 & p_1 - p_2 & \dfrac{p_{11}p_{22}}{p_1p_2 - 1/4p_{12}} \\ 1 & 2p_1 & -\dfrac{p_{11}p_{12}}{2p_1p_2 - 1/2p_{12}} \end{pmatrix} \begin{pmatrix} \mu \\ \alpha \\ \delta \end{pmatrix} \qquad [6]$$

Here, p_{ij} stands for the frequency of A_iA_j in the population considered. The only interaction effect we have addressed so far is dominance, the one already discovered by Mendel. Other important facts of genetic architecture follow.

Multiple alleles

As a first step beyond the one-locus two-allele case, we address in this section the case of a multiallelic locus. The multiallelic individual-referenced formulation can easily be obtained as a natural extension of expression [1] or, even more easily, of expression [2] (Yang and Álvarez-Castro, 2008). Kempthorne (1954) pioneered multiallelic population-referenced developments, providing expressions that determined the multiallelic genetic effects implicitly under Hardy–Weinberg proportions. In the same year, a PhD student of his published two-allele formulations accounting for departures from the Hardy–Weinberg proportions (Cockerham, 1954). Both approaches were more recently generalized with developments by Álvarez-Castro and Yang (2011) leading to expressions of the type $\mathbf{G} = \mathbf{SE}$, providing explicit population-referenced formulations with multiple alleles and accounting for departures from the Hardy–Weinberg proportions.

Epistasis

The classical works by Kempthorne (1954) and Cockerham (1954) had in common a motivation for implementing epistasis on Fisher's (1918, 1930) foundational developments. Toward the end of the twentieth century, a group of scientists felt encouraged enough to address the complexity of these interactions, which already justified a first compendium at the turn of the century (Wolf *et al.*, 2000) and a second installment 15 years later (Moore and Williams, 2015). Epistasis is a kind of interaction between or among alleles that, in contrast with dominance, occurs across loci. A simple instance of epistasis is shown in **Figure 3**.

Concerning models of genetic effects, the matrix notation used above enormously facilitates implementations of epistasis, by computing multilocus genetic-effects design matrices as Kronecker products of single-locus ones (Tiwari and Elston, 1997), which holds for multiallelic loci and for both (individual- and population-referenced) formulations. This way, epistasis genetic effects naturally emerge as interactions between the marginal ones (for the two-locus case, additive-by-additive, additive-by-dominance, dominance-by-additive, and

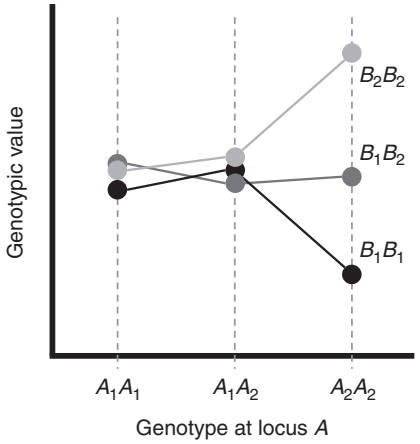

Figure 3 Representation of a two-locus two-allele GP map with epistasis. In this case, locus B is canalized (allele substitutions within it have little effect) when allele A_1 is present. In general, epistasis occurs as long as the solid lines in the figure are not parallel.

dominance-by-dominance) or among them (for higher order interactions that may occur when more than two loci are involved). Despite some work done in this direction, developing epistatic population-referenced formulations fully accounting for linkage disequilibrium remains a challenge (Yang, 2004; Mao *et al.*, 2006).

On the other hand, directionality of the genetic architecture of a trait has proven to be a relevant index since it is informative about the mid- to long-term evolutionary properties of a trait (Hansen, 2006, 2015). Very briefly, directionality has been designed to measure whether, and how much (on average), allele substitutions increasing trait value in the direction of selection have synergistic effects. When the directional epistasis index is significantly positive (positive epistasis), the response will eventually become significantly higher than expected under additivity, whereas if it is negative, canalization (low population-referenced additive genetic effects and response to selection) is prone to occur. When the index is virtually zero (nondirectional epistasis), the response to selection does not depart significantly from its additive expectation.

Imprinting and epigenetics

Also, attention on non-Mendelian inheritance has increased since the late twentieth century (see, e.g., Halgrímsson and Hall, 2011). Particularly, imprinting – a mechanism by which the inherited alleles are different depending on their parent-of-origin – has deserved special focus. In the simplest case (one two-allele locus), the GP map just has to include an additional term, G_{21}, since two different heterozygotes are possible – depending on which of the two alleles has maternal origin. This in its turn implies accounting for an extra genetic effect, which can be done either with two dominance effects (e.g., Santure and Spencer, 2011; Álvarez-Castro, 2014) or with an extra imprinting effect (e.g., Wolf *et al.*, 2008; Xiao *et al.*, 2013; Álvarez-Castro, 2014).

Gene-by-environment interactions

Since an individual may experience different environments during its life history, classical evolutionary genetics acknowledged the reaction norm as the target unit of selection (see, e.g., Sarkar, 2004). We may be more interested in considering that different individuals may experience significantly different environments. In both cases, gene-by-environment interactions may be a key factor to consider in the analysis of the genetic implications of phenotype variation (see **Figure 4**). Since these interactions appear when the effects of allele substitutions do not just sum up with the effects of changing environmental backgrounds, the way they can be modeled is similar to that of epistasis (see, e.g., Ma *et al.*, 2012). However, as opposed to genotypes, environment may be a continuous parameter and, to the increase of complexity, the reaction norms of the genotypes may not be linear functions (Yang, 2014).

Mapping Genetic Architectures

Gene-mapping experiments became widespread at the end of the latest century as molecular techniques enabled the inspection of a sufficient number of markers at a reasonable cost.

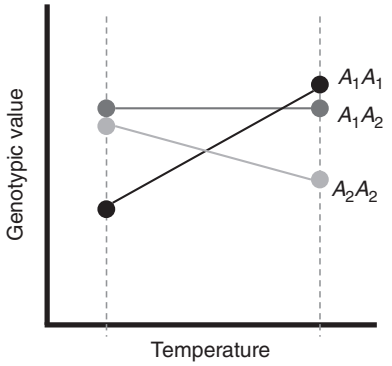

Figure 4 Representation of gene-by-environment interactions. Substitutions at the two-allele locus, *A*, have different effects depending on the environment as long as the solid lines (reaction norms) in the figure are not parallel. In this case, temperature does not affect the phenotype of the heterozygote, while each homozygote has higher values at opposite temperatures.

The initial quantitative trait loci (QTL) experiments (see **Figure 5**) consisted in performing selection for a trait in opposed directions to obtain a large trait mean difference in the two resulting populations, which would then be inbred. Molecular markers with different alleles at the two populations would then be identified and the populations themselves would be intercrossed – and allowed to recombine. Genotyping (at the chosen markers) and phenotyping the resulting individuals, would make it possible to map regions of the genome with genetic variability responsible for the trait mean difference between the two selected lines (for a more detailed description, see, e.g., Lynch and Walsh, 1998).

The key point of this experimental design is to generate linkage between each region of the genome of each of the lines and one allele of at least one molecular marker. As molecular techniques enabled sufficiently denser marker maps, the chance of a marker falling close to (and thus to retain linkage with) a responsible mutation increases. This is the principle behind genome-wide association (GWA) studies, which can be applied without the need of any previous design of selection and intercross – and thus enable mapping experiments at non-model species, particularly including humans.

In any case, gene-mapping methodologies aim for the location (as precise as possible) of loci with genetic variation responsible for trait variation at the population under study, and the genotype (or at least genotype probability) of each individual of the study at each of those loci. That is the information required for building up a GP map and to obtain estimates of genetic effects.

Obtaining the Genotype-to-Phenotype Map

Let us start by considering how we could estimate a GP map from the aforementioned information – the phenotypes of the individuals of our sample, P_i, and their genotypes for a set of mapped responsible loci. The parameters we would like to obtain are the expected phenotypic value of each genotype, G_i. The classical expression $P = G + E$ (e.g., Falconer and MacKay, 1996) accounts for the genetic and environmental influence

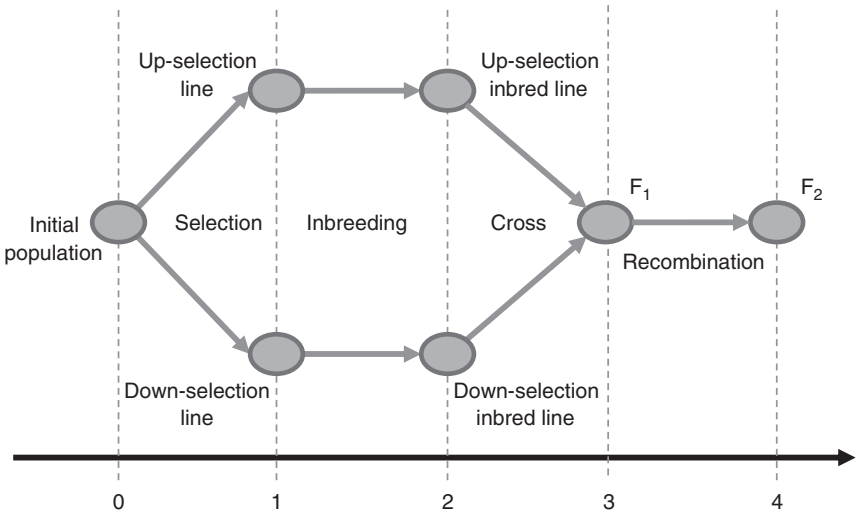

Figure 5 Schematic view of a QTL experimental design for a model species. The starting point (0) is a population with variation for the trait of interest, which is selected upwards and downwards to obtain two populations with sufficiently different mean trait values (1). Those are inbred, for example, through sib-mating for a number of generations. At the resulting inbred populations (2), molecular markers are obtained having different alleles for each of the lines – the individuals at the up-selection inbred line would have genotypes $M_1M_1/M_2M_2/M_3M_3/...$, whereas the ones at the down selection inbred line are $m_1m_1/m_2m_2/m_3m_3/...$ The two lines are then intercrossed to obtain an F_1 population (3), whose individuals are all $M_1m_1/M_2m_2/M_3m_3/...$ At this point there are several options to allow recombination. An F_2 population is represented in the last step (4), with recombining individuals of the kind $M_1M_1/M_2m_2/m_3m_3/...$ Other options are backcrosses and F_n populations.

on phenotype at the individual level (here E stands for environment, not to be confused with the vector of genetic effects, **E**, in the expressions above). For each of the individuals of our study, it can be rewritten as $P_i = G_i + E_i$. If individuals i and j in our sample have the same genotype, then $G_i = G_j$, and hence in general $P_i = G_k + E_i$, where k stands for the genotype of individual i.

In other words, the column vector gathering the phenotypes of the individuals in our sample, $\mathbf{P} = (P_1, P_2, P_3, \ldots)^T$, is larger than the column vector of the genotypic values, **G** – which for the one-locus (A) two-allele (A_1 and A_2) case is just $\mathbf{G} = (G_{11}, G_{12}, G_{22})^T$ as in the expressions above. Each individual can be assigned to its genotypic value as long as its genotype is known. In practice, this is done using a matrix called incidence matrix, **Z**. If the first three individuals of our sample have genotypes A_1A_1, A_1A_2, and A_2A_2, respectively, then the first three rows of the incidence matrix shall be (1, 0, 0), (0, 1, 0), and (0, 0, 1), respectively. The vector of genotypic values can now be estimated by solving the regression (see, e.g., Nettelblad *et al.*, 2012):

$$\mathbf{P} = \mathbf{ZG} + \varepsilon \qquad [7]$$

In this expression, the environmental values of each of the individuals are the residuals of the regression. Indeed, for each individual this expression reads $P_i = G_k + \varepsilon_i$ – note the analogy of expression [7] with the aforementioned P = G + E. For considering gene-by-environment interactions, an extra term needs to be added.

Estimating Genetic Effects

As stated above, it is preferable to estimate genetic effects directly from the data, rather than the raw GP map. The split of the genetic influence into additive and interaction components is traditionally represented as G = A + D, where A stands for additive and D for dominance (an extra term may be included to account for epistatic interactions). That split has been performed above using genetic-effects design matrices, **S**, which makes it straightforward to substitute **G** in expression [7] and turn it into a regression to directly estimate genetic effects from data as:

$$\mathbf{P} = \mathbf{XE} + \varepsilon \qquad [8]$$

In this expression, $\mathbf{X} = \mathbf{ZS}$ and for each individual it reads $P_i = \mu + \alpha_{kl} + \delta_{kl} + \varepsilon_i$, where $\alpha_{kl} = \alpha_k + \alpha_l$. Greek letters are used here instead of Latin ones (R, a, d) because the best choice of genetic-effects design matrix for performing the regression in expression [8] is the population-referenced one fitting the frequencies of the genotypes of the individuals represented in the vector **P**, which conveniently enables an orthogonal estimation of genetic effects.

Hitherto, it has been assumed that the loci underlying variation on the trait under study are known, but that is precisely the information often pursued, which increases complexity in two important senses. First, it shall often be the case of feeding the incidence matrix, **Z**, with genotype probabilities rather than certainties, since we want to test the hypothesis that loci underlying trait variation (QTL) lay at locations whose genotype probabilities may be inferred from flanking markers. Opportunely, it is possible to preserve orthogonality in expression [8] (and therefore also the genetic meaning of the estimates obtained) under these circumstances (Nettelblad *et al.*, 2012).

Second, even when putative QTLs have been located, it will be necessary to choose among several possible genetic architectures, by comparing several submodels resulted from discarding either certain genetic (or gene-by-environment) effects

within a locus or even one to several complete loci. Orthogonality is highly desirable at this point, since it implies that subtracting effects form a model (from a genetic architecture under evaluation) does not alter the estimates of the ones remaining. There exist quite interesting literature on the specificities of model selection (e.g., McKinney *et al.*, 2006; Zou *et al.*, 2010; Steen, 2012) and in general on methodologies related to QTL analysis (see Rifkin, 2012 for a compendium).

Genetic Architectures Discovered

Beyond single QTLs, very many interesting facts of genetic architectures have already been discovered. Epistasis has been found widespread especially by studying model species (e.g., Mackay, 2014). More specifically, it has been shown that mapped interactions (particularly, epistasis) improve prediction of evolutionary properties as compared with information of marginal loci alone (Álvarez-Castro *et al.*, 2012b) and that directional epistasis is consistent with real data (Le Rouzic, 2014). It is crucial to consider the implications of epistasis also for the study modularity of genetic networks (Wagner *et al.*, 2007), loci affecting expression (eQTL; Chen, 2012; Li *et al.*, 2012), loci affecting the pleiotropic relationships of different traits (rQTL; Pavlicev *et al.*, 2013), and variance-controlling QTL (Rönnegård and Valdar, 2011; Shen *et al.*, 2012).

The increase of molecular data opened the possibility of mapping genetic architectures using GWA studies, also with particular progress made in the direction of detecting epistatic interactions (see, e.g., Wei *et al.*, 2014; Wang and Biernacka, 2015). In data for which dense marker maps are available, blocks of alleles of molecular markers (haplotypes) occur, so that even when there are only two versions of each marker, the haplotypes are multiallelic. Multiallelic systems may also be detected through QTL mapping experiments (Rönnegård *et al.*, 2008) – although the mapping populations come from the cross of two lines, those may not be fully inbred as the original experimental design advises (see, e.g., Lynch and Walsh, 1998). **Figure 6** shows a three-allele genetic architecture found for human *ACP1* in European populations. There already exist sound evidences for imprinting effects as well (e.g., Wolf *et al.*, 2008; Xiao *et al.*, 2013) and a promising method for systematically searching for signals of general epigenetic inheritance has been developed (Varona *et al.*, 2015).

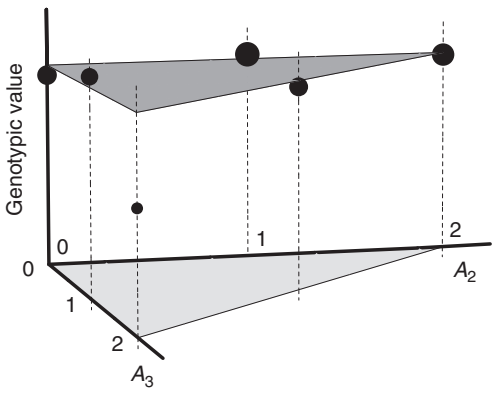

Figure 6 One-locus three-allele GP (in particular, genotype-to-fitness) map deduced using a population-referenced formulation. The model of genetic effects was fed with the frequencies of the genotypes at a population (represented by the size of the dots of the genotypic values) and minimized for the variance of population-referenced additive effects. The minimum was zero, indicating that the population may be at equilibrium, with the fitnesses found at the minimum. Note that indeed the plane of the linear approximation weighted by the genotype frequencies is parallel to the horizontal plane of the coordinates of the genotypes (for details see Álvarez-Castro and Yang, 2011; Álvarez-Castro and Le Rouzic, 2015).

Evolutionary Quantitative Genetics

The basic conceptual ground of genetics gained soundness as compatibility between Mendel's laws and inheritance of continuous traits was shown (Provine, 1971; Kim, 1994). More to the point, Fisher's (1918) work enabled Mendelian reinterpretation of the results of biometry – particularly, the regression toward mediocrity (Galton, 1886) – and kicked off new paradigms for the study of (gradual) evolution. Albeit quantitative genetics cannot elude the population dimension, and a population genetics of strictly discrete traits would not bring the brightest light, these instantly became two distinct lines of work. On the one hand, quantitative geneticists pursued (and achieved) to prompt the development of practical tools to aid plant and animal breeding, in which context, a model "does not have to be true to be useful" (Hill, 2014). On the other hand, population geneticists started a slower-paced hike toward a comprehensive understanding of mechanisms of natural selection and evolution, an endeavor in which (paraphrasing Hedrick and Murray, 1983) "we do not want to be right for the wrong reason".

Thus, the divide between population and quantitative genetics is of epistemological nature. Albeit this conditions differences in methodology, ultimately the two disciplines address mostly the same phenomena and thus it means no surprise that there has occurred profuse feedback between them, seeding a common ground for a merge into evolutionary quantitative genetics (see, e.g., Roff, 1997). To close this article, new opportunities blossoming in this common ground, watered by the advent of mapping experiments, are briefly commented (see **Figure 7**).

The traditional strategy of quantitative genetics used the data available (basically, phenotypes and relatedness) for improvement on production traits with a statistical machinery that enables handling phenotype (and genetic) variance decomposition whilst being parsimonious about the genetic architecture of the underlying Mendelian factors. This can be labeled as a top–down approach, in contrast with a bottom–up one consisting in predicting the outcome of selection based on the knowledge of the loci involved in trait variation and their genetic properties (additive genetic effects, interactions, etc.) at the population under study (see, e.g., Zuk *et al.*, 2012). Models of genetic effects were initially developed, at the beginning of the twentieth century, under the top–down paradigm, the only way possible at that time. Only at the turn of the century – as mapping experiments started to become

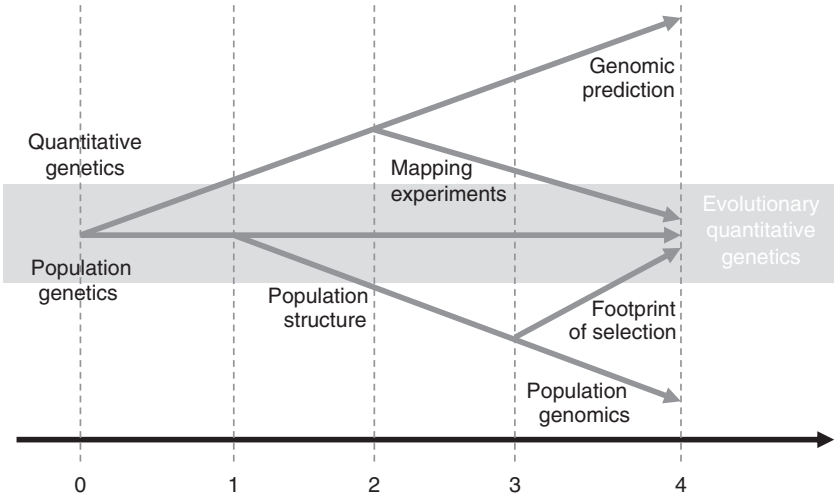

Figure 7 Diagram representing directions taken along history within population and quantitative genetics relative to their focus on the GP map (shadowed area). Initially (0), only population genetics addressed the GP map explicitly. The availability of the first molecular markers enabled population genetics to branch out with the analysis of neutral variation (applied to, e.g., population structure and phylogeny studies), thus also diverging from the GP map (1). The exponential development of molecular techniques eventually made gene-mapping experiments possible, which entailed a convergence with population genetics through the study of GP maps (2). Finally, advanced studies of neutral variation tracked the footprint of selection and, together with candidate-gene approaches, triggered a re-convergence of this line of work toward the study of the GP map (3). Currently, there keep on existing population and quantitative genetics directions both within and outside the GP map (4).

feasible and eventually widespread – it became apparent that models of genetic effects had to be updated for bottom–up applications (see Álvarez-Castro and Yang, 2015 for a compendium).

One of the initial worries consisted in being able to express genetic architectures in ways independent from the (experimental) population under study. Cheverud and Routman (1995) brought this issue to the table with their physiological model, which prompted Hansen and Wagner (2001) to develop their multilinear model with individual-referenced (which they called functional) genetic effects and to introduce the concept of change of reference. A second worry was about being able to express complex models (particularly, with epistasis) within an operational mathematical framework, which was attained by means of a practical matrix notation (Tiwari and Elston, 1997). Third, it was necessary to make an effort in generalizing approaches made in different directions, which required addressing several levels of action.

Álvarez-Castro and Carlborg (2007) used the matrix notation to unify individual- and population-referenced (statistical) formulations of genetic effects, with the expressions shown above (1–6). Le Rouzic and Álvarez-Castro (2008) provided a first software package for the use of that framework – the NOIA (natural and orthogonal interactions) model. Yang and Álvarez-Castro (2008) and Álvarez-Castro and Yang (2011) extended NOIA to multiple alleles, thus generalizing the aforementioned classical implementations of epistasis by Kempthorne (1954) and Cockerham (1954), with multiple alleles and departures from the Hardy–Weinberg proportions, respectively. Further facts of genetic architectures keep on being implemented in NOIA (e.g., Ma *et al.*, 2012; Xiao *et al.*, 2013; Álvarez-Castro, 2014).

Overall, the inspection of GP maps with the advent of mapping experiments opened new thrilling opportunities not only to integrate population and quantitative genetics, but also both into a systems biology perspective (Álvarez-Castro and Yang, 2014). Such a shift has already proven to be productive and would rather be accompanied by a certain dose of patience, for major achievements within a new and complex scientific paradigm are expected to come with work and time rather than instantly (Álvarez-Castro, 2012). Incidentally, this shall not preclude rewarding work to keep on being done also outside the domains of the GP map, both in population and quantitative genetics (see **Figure 7**).

Acknowledgment

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See also: Epigenetic Inheritance. Gene Interactions in Evolution. Genotype-by-Environment Interaction. Quantitative Genetic Variation

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Genetic Drift, Models of Random

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Glossary

Allele vs. allele copy In this article, an 'allele copy' refers to one (of $2N$) physical instance of an allele at a locus; it does not imply a copying process such as gene duplication or replication from parent to offspring generation.

Binomial distribution A probability distribution describing the following process: an activity with two possible outcomes (e.g., a coin flip) is done repeatedly. The number of times that an outcome having constant probability p of occurring in one attempt happens in n attempts is said to be binomially distributed with key parameters n and p.

Coalescent theory A backwards-time method of analyzing population genetic data, focusing on the mathematical properties of the genealogy of a sample of allele copies.

Diffusion theory An approximate method of analyzing complex stochastic processes.

Effective population size (N_e) For a population that may violate one or more assumptions of an idealized Wright–Fisher population, the size of an equivalent idealized Wright–Fisher population. 'Equivalent' here means matching in some aspects of their population genetic characteristics (e.g., variance in allele frequencies or degree of inbreeding).

Fixation An allele is 'fixed' or 'goes to fixation' when its frequency in the population equals 1.

Geometric distribution A probability distribution describing the following process: an activity with two possible outcomes (e.g., did it rain today or not?) are repeated until the first time one of the outcomes occurs (a day with no rain). The number of unsuccessful trials (number of rainy days) before the first success is said to have a geometric distribution.

Markov chain A type of stochastic (random) process. The key feature of this type of process is that it is 'memoryless' – none of the history of the process, other than the current state, matters to the future prospects.

Multinomial distribution A probability distribution, very similar to the binomial distribution, but with more than two possible outcomes.

Poisson distribution A probability distribution describing the following process: an event has a constant (but low) probability of happening (e.g., getting a flat tire on a well-maintained car under normal driving conditions). Given some set time interval (10 years worth of driving), the total number of events (flat tires) is said to have a Poisson distribution.

Time reversible A property of some stochastic processes. The process looks the same (probabilistically) whether looking forward in time or backwards.

Overview

From the earliest days of population genetics, evolutionary biologists have used a variety of mathematical models to describe and understand the dynamics of random genetic drift. By far the most common model analyzed is the Wright–Fisher model, although in recent years an alternative, the Moran model, has proved to be a useful complementary tool. Although these two main models were first proposed well before widespread DNA sequencing and the development of the field of molecular evolution, it is in the service of molecular evolutionary studies that they have seen their utility expand. This article outlines the basic mathematical structures, results, and applications of the two main models of random genetic drift used by evolutionary biologists: the Wright–Fisher model and the Moran model. A third category of random genetic drift models, the Cannings models, is referenced briefly.

Core Assumptions of Genetic Drift Models

All of the models considered here assume a simple population structure (a single population of constant size) and an idealized view of how individuals in the population interact to form a new generation (individuals are monoecious – no separate sexes – and mate at random). Because the core descriptions of genetic drift models focus on random sampling as the only factor affecting allele frequencies, they often assert many of the Hardy–Weinberg assumptions in order to remove other factors from consideration: random mating of parents, no mutation, migration, or natural selection. Once the basic structure of the stochastic model is established, these other evolutionary forces can be reintroduced and incorporated into more complex models of evolution in a finite population (see Ewens (2004) for several examples of these more complex models, and Gale (1990) for an exploration of some of the approximation methods used for their analyses).

Two Different Genetic Drift Models

The random genetic drift models considered here have a critical structural difference in how they view the transmission of alleles and genetic information from parent to offspring. The Wright–Fisher model is a particular type of Cannings model (Cannings, 1974). This category of models focuses on the population-scale transmission of information from the parental generation to the offspring generation; all parents reproduce (and then die) simultaneously in such models, so that generations are non-overlapping. The Cannings-type models

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(including the Wright–Fisher model) have an appealingly direct intuitive connection to the behavior of some species (such as annual plants) with non-overlapping generations in nature.

The Moran model is quite different in structure. Here, the focus is on the birth and death of individual allele copies, and only one or two members of the population are involved in reproduction and/or death at a particular time. Thus, generations are overlapping. This makes the Moran model initially appear to be more appropriate for the majority of species (including humans) that have continuous reproduction and overlapping generations. Historically, however, it has received much less attention than the Wright–Fisher model, perhaps because the specific structure is harder to connect directly to a familiar natural system. For example, the Moran model, with its focus on birth and death of single allele copies, is strictly speaking a haploid model, and, although in practice it can be applied approximately to diploids, this apparent limitation may hinder making intuitive connections to the natural world. The barriers to adoption for the Moran model may be receding in recent years, as its advantages in mathematical tractability have become more widely recognized and the model itself has become more familiar. Additionally, its inherent time-reversibility makes it an attractive starting point for modern 'retrospective' population genetics analyses such as coalescent theory. Nevertheless, the main mathematical framework for evolution in a finite population remains the Wright–Fisher model.

Wright–Fisher Model

Over several decades in the middle of the twentieth century, Sewall Wright and R.A. Fisher (along with J. B. S. Haldane) set the theoretical foundations for much of what became the field of population genetics (Provine, 1971). Both Wright (1931) and Fisher (1922, 1958) recognized that it was necessary to have a mathematical description of the random fluctuations of allele frequency due to sampling in a finite population. We refer to the model that they developed as the Wright–Fisher model.

Mathematical Framework of the Wright–Fisher Model

The Wright–Fisher model is structured in the following way (Figure 1):

- generations are non-overlapping: all individuals reproduce and die simultaneously;
- the population is characterized by the current numbers of each allelic type;

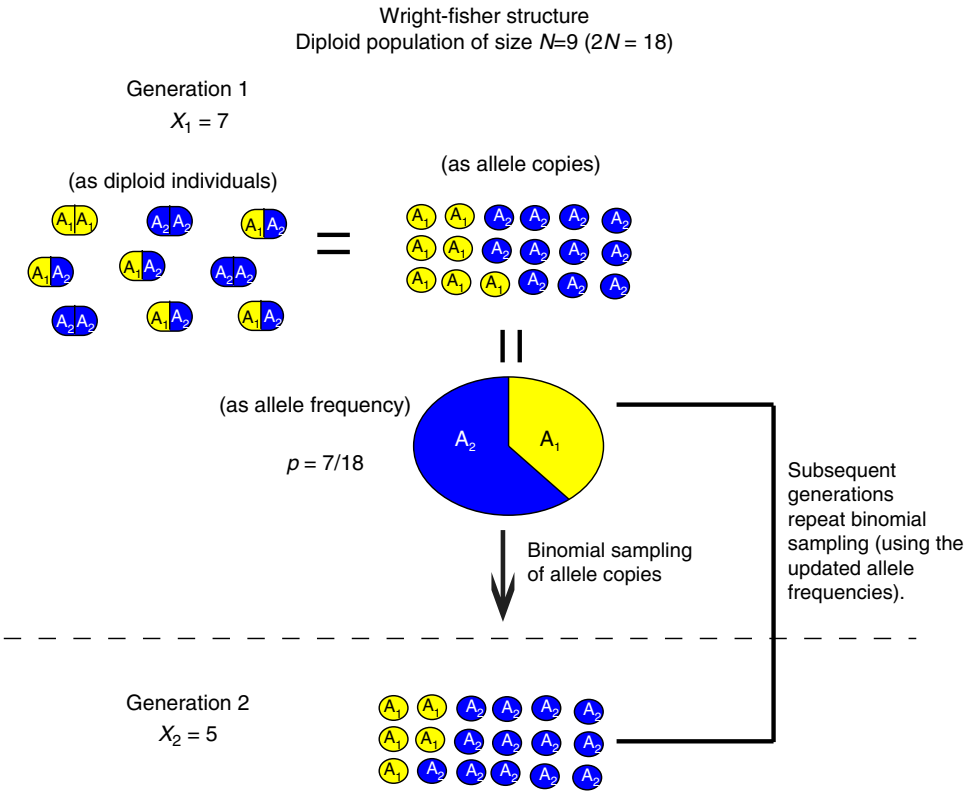

Figure 1 Structure of the Wright–Fisher model. The population is characterized by the number (X_t) of a focal type of allele, which are converted to fractions ($p = X_t/2N$) to represent an infinite pool of gametes. An entire new generation of $2N$ allele copies (shown: one of several possibilities) is produced through binomial sampling of the old generation's gamete pool. At this point, the sampled offspring alleles may be assembled into a new set of diploids, or else simply used to calculate a new p for a new generation of binomial sampling.

- new generations are formed by binomial sampling of allelic types from the parental generation when there are two allelic types, and multinomial sampling when there are more than two alleles.

The Wright–Fisher model is a specific type of Cannings (1974) model. All Cannings models share the features of non-overlapping generations and stochastic sampling of alleles (from an infinite parental 'gamete pool') from one generation to the next; particular Cannings models are distinguished by their method of generational sampling and their characteristic amount of variation in offspring number. For the Wright–Fisher model, a new generation of alleles is generated by binomial (or, in the case of more than two alleles, multinomial) sampling of the current generation's gamete pool. Much of the analytical power of the Wright–Fisher model comes from our knowledge of binomial and multinomial sampling.

Mathematically, this model is straightforward to describe: given a single locus with two alleles, A_1 and A_2, let X_t be a random variable describing the number of A_1 alleles in a diploid population of size N. If, in the present generation t, $X_t = i$, then the probability that the next generation contains $X_{t+1} = j$ copies of A_1 is given by the binomial sampling formula:

$$P_{ij} = \binom{2N}{j} \left(\frac{i}{2N}\right)^j \left(\frac{2N-i}{2N}\right)^{2N-j}$$

where $\binom{2N}{j} = \frac{2N!}{j!(2N-j)!}$ gives the binomial coefficient.

As might be expected with a model of random genetic drift, the trajectory or outcome for any particular population cannot be predicted absolutely – the next generation described by P_{ij} is described only in terms of the probabilities of many possible outcomes (**Figures 2** and **3**). Many interesting results can, however, be derived about the long-term or expected behavior of the process by using mathematical methods designed to study stochastic processes.

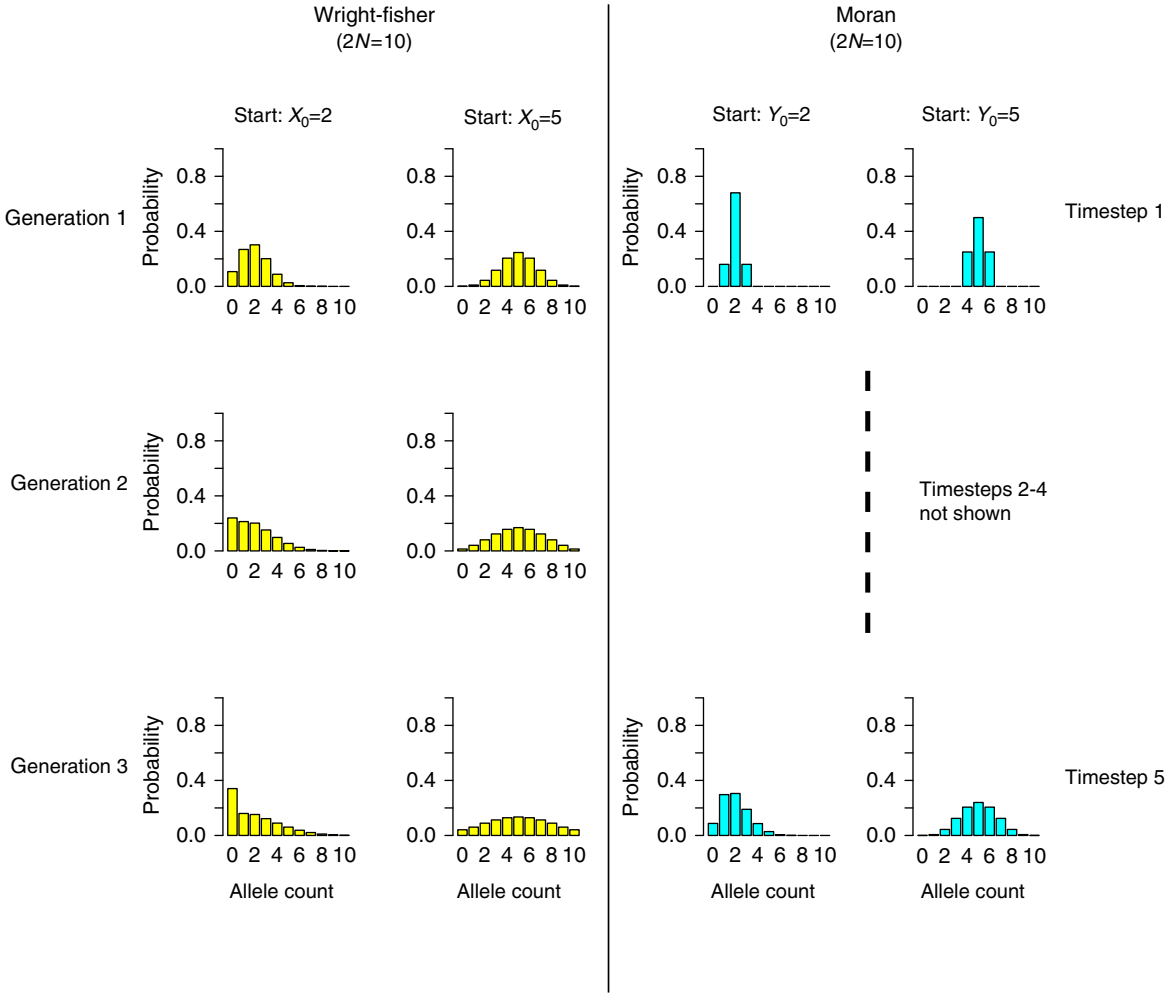

Figure 2 Probability distributions over time for both the Wright–Fisher and Moran models. Each graph shows the probability that a population which began with either 2 or 5 copies of the focal allele (out of a total $2N=10$ copies) has, in subsequent generations, 0, 1, 2, etc. of that allele. The Wright–Fisher results on the left show the predictions under three consecutive generations of random genetic drift, while the Moran model on the left shows 1 and 5 timesteps. In this case, the 5-timestep Moran result approximates one generation of Wright–Fisher evolution.

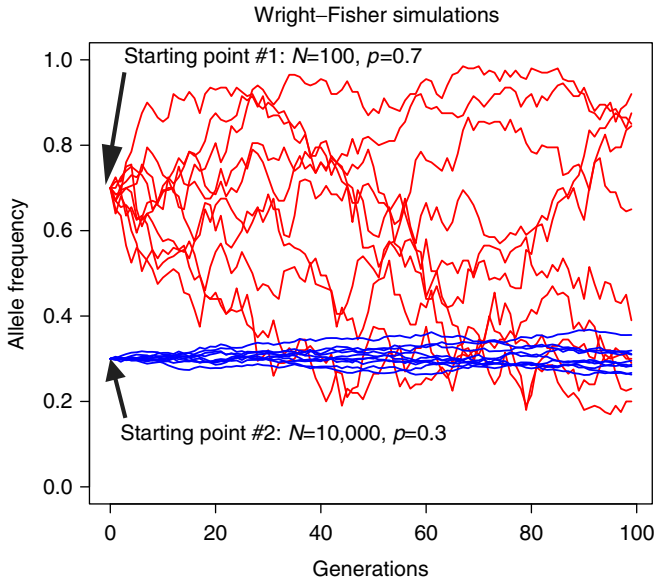

Figure 3 Simulations of random genetic drift under a Wright–Fisher model. Two different population sizes and starting conditions are shown. Red lines: $N=100$, $p_0=0.7$. Blue lines: $N=10\,000$, $p_0=0.3$. Each line is a separate simulation, and uses the binomial sampling scheme of the Wright–Fisher model to follow the frequency of the focal alleles remaining in the population over time.

Over multiple generations, the repeated application of the Wright–Fisher sampling strategy forms a particular type of stochastic process, a Markov chain, with transition matrix $\boldsymbol{P}$ composed of elements P_{ij}. The Markov chain can be written

$$x(t+1) = P \cdot x(t)$$

where $\boldsymbol{P}$ has been transposed and the (column) vector

$$x(t) = [x_0(t), x_1(t), \cdots, x_{2N}(t)]$$

is composed of probabilities, with each $x_i(t)$ representing the probability that the population has i copies of the allele at time t, $x_i(t) = \text{Prob}[X_t = i]$. Figure 2 (left side) shows these probabilities for a diploid population of size $N=5$ over three generations of the Wright–Fisher Markov chain, with the two columns giving two different starting conditions.

Direct Results from the Wright–Fisher Model

Unfortunately, while we can readily write down the form of the transition matrix of the Wright–Fisher Markov chain and can iterate it by hand for any particular population (Figure 2), a full exact analysis of this Markov chain appears to be largely intractable. Nevertheless, substantial insights into the long-term consequences of random genetic drift under the Wright–Fisher model can be gained using methods that draw only upon the basic structure of the model and a thorough understanding of the single generation transitions and binomial sampling.

Probability of fixation

Under one generation of binomial sampling, the expected value of the allele frequency in generation $t+1$ is just its frequency in generation t. In fact, this will remain the case in all subsequent generations. Thus, if the current allele frequency is p, p must also be the long-term expected value for the allele frequency. In the very distant future, the allele must either be fixed ($X_\infty = 1$) or lost ($X_\infty = 0$), and so, allowing $\text{E}[X]$ to indicate the expectation of X,

$$\begin{aligned} p &= E[X_\infty] \\ &= 0 \cdot \text{Prob}[X_\infty = 0] + 1 \cdot \text{Prob}[X_\infty = 1] \\ &= \text{Prob}[X_\infty = 1] \\ &= \text{fixation probability} \end{aligned}$$

which gives the familiar result that the eventual fixation probability of a neutral allele is equal to its current frequency.

Variance in allele frequencies over time

In order to understand a stochastic process such as random genetic drift it is essential to characterize the variability in the process as well as the average behavior. It is clear in Figure 2 that many possible fates are possible for a population undergoing random genetic drift, and Figure 3 shows several possible trajectories for allele frequency in simulated populations. Using the Wright–Fisher model, it is possible to quantify the variation in allele frequencies expected over time, and thus obtain a rough measure of the magnitude of the effects of random genetic drift.

If x is the current count of the focal allele and thus $p = x/2N$ the current allele frequency, then from the properties of the binomial distribution, after one generation the allele count will have variance $2Np(1-p)$. The variance in allele frequencies becomes

$$\begin{aligned} \text{Var}[\text{frequency}] &= \text{Var}\left[\frac{\text{count}}{2N}\right] \\ &= \frac{\text{Var}[\text{count}]}{(2N)^2} \\ &= \frac{p(1-p)}{2N} \end{aligned}$$

Over time, allele frequency trajectories in replicate populations diverge from each other, and the variance in allele frequency between them increases (**Figure 3**). The above equation quantifies the variance produced in a single generation, given the population size and the current frequency. Quantifying the variance provides a measure of not only the expected impact of genetic drift on individual trajectories, but also, importantly, the timescale upon which drift operates and the speed at which alleles are fixed or lost from the population. Smaller population sizes lead to higher generation-to-generation variances (**Figure 3**; red lines, $N=100$; blue lines, $N=10\,000$), corresponding to our intuition that smaller population sizes should experience more pronounced effects of random genetic drift than do larger populations.

Reproductive output of individual alleles

With binomial sampling, as the $2N$ allele copies in the offspring population are generated, at every 'pick' of an allele copy for the new generation an allele copy in the parent population has a $1/2N$ chance of being chosen to leave a descendant. If N is very large, $1/2N$ is very small and the number of 'picks' is very large. The total number of descendants left by an allele copy will then be approximately Poisson distributed with mean 1, or

$$D_{WF} \sim \mathrm{Po}(1)$$

Approximate Results from the Wright–Fisher Model

Many other interesting results from this model, notably those involving time, are only obtainable through approximate methods, many of which were first developed by Fisher and Wright in their initial analyses of the model and then elaborated on by later workers, chiefly Motoo Kimura (1955).

Time to fixation or loss of a neutral allele

Given initial allele frequency p, the expected time before a neutral allele is either fixed or lost from the population is:

$$\text{Expected time to fixation or loss} \approx -4N\{p\ \log p + (1-p)\log(1-p)\}$$

Time to fixation (conditional on fixation)

If only fixation events are considered, the expected time until fixation occurs, given current allele frequency p is:

$$\text{Expected fixation time; given fixation} \approx -4N\left\{\frac{1-p}{p}\log(1-p)\right\}$$

This time is of particular interest when the allele is a new mutant, $p=1/2N$:

$$\text{Expected fixation time for a new mutant given fixation} \approx (4N-2)\ \text{generations}$$

Approximation Methods

The above quantities can be derived using the common approximation method of Taylor expansions (Ewens, 2004). Many additional important results, however, are available through diffusion methods, which have been widely applied in population genetics (Feller (1951) and Hartl and Clark (2007) gives a good overview of the basic method as used in population genetics; see also Crow and Kimura (1970) and Ewens (2004) for more mathematically involved discussions and several applications). These mathematical tools were developed essentially in parallel in the fields of physics and biology, with the biological development driven by Wright and Fisher in the service of their genetic drift model. The techniques involve approximating discrete-time stochastic processes such as the Wright–Fisher Markov chain by similar continuous-time processes that are more amenable to analysis.

One useful consequence of a successful diffusion analyses is that it converts the basic Wright–Fisher model into a 'time-reversible' process, so that the process looks identical probabilistically whether one is looking forward or backward in time. This is not only convenient mathematically, it gives the familiar forward-time Wright–Fisher model added authority in the context of modern analysis methods which rely heavily on backward-time coalescent models. Meanwhile, the main alternative to the Wright–Fisher model of random genetic drift, the Moran model, uses an inherently time-reversible stochastic process.

Moran Model

P.A.P. Moran (1958, 1962) developed an alternative stochastic model of allele frequency evolution over time. Moran's model both differs strikingly from and complements the Wright–Fisher model: where the Wright–Fisher model uses discrete non-overlapping generations, in the Moran model generations overlap. Strictly speaking, the Moran model is a haploid model (although it can, with some cost in complexity, be modified to accommodate diploids), while the Wright–Fisher model has more inherent flexibility regarding ploidy. Lastly, while the Wright–Fisher model has an intuitively straightforward structure that is mathematically difficult to analyze, the Moran model has a much more mathematically tractable framework that comes perhaps at some cost to intuition.

Mathematical Framework of the Moran Model

The Moran model does not specify discrete generations for the population as a whole; instead, it follows the fate of individual allele copies in 'timesteps.' Each timestep involves the death of an allele copy and the reproduction (exact duplication) of an allele copy to replace the one that died (**Figure 4**). This simple structure ensures that the population size remains constant. The mathematical description of the process is further simplified by assuming that the two events (death and reproduction) are simultaneous, so that an allele copy's probability of being chosen for reproduction does not depend upon whether or not it was chosen for death.

We again begin with a single locus with two alleles, B_1 and B_2. Let Y_t be a random variable describing the number of B_1 alleles in a haploid population of size M. If, in the present generation t, $Y_t=i$, by the rules of the Moran model the only possible fates for Y_t in a single timestep (one death, one birth) are to increase by 1, to decrease by 1, or to stay the same.

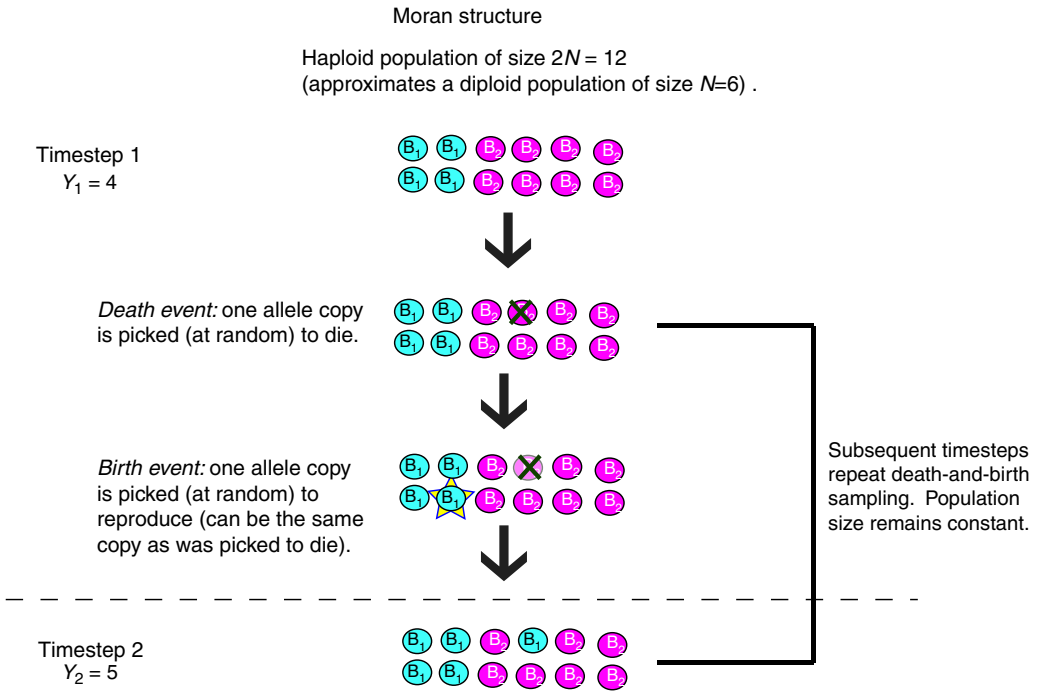

Figure 4 Structure of the Moran model. The population begins in a haploid representation, and is characterized by the number (Y_t) of a focal type of allele. Only (at most) two allele copies are affected in each timestep, and Y can only increase or decrease by (at most) one in any timestep. Each timestep consists of one birth and one death (possibly the same individual) of an allele copy. 'Birth' results in a replicate of the chosen allele copy replacing the allele copy chosen for death.

The probabilities of each type of outcome are:

$$P_{i,i-1} = \left(\frac{i}{M}\right)\left(\frac{M-i}{M}\right) \quad \text{for } i>0$$

$$P_{i,i+1} = \left(\frac{M-i}{M}\right)\left(\frac{i}{M}\right) \quad \text{for } i<M$$

$$P_{i,i} = \left(\frac{i}{M}\right)\left(\frac{i}{M}\right) + \left(\frac{M-i}{M}\right)\left(\frac{M-i}{M}\right)$$

In each case, the overall probability is obtained by multiplying probabilities of two independent events: an allele is picked to die, and an allele (possibly the same) is picked to reproduce. No other transitions are possible ($P_{i,j}=0$ if $|i-j|>1$), and so the Markov chain describing the timestep-by-timestep changes in the population has a relatively simple transition matrix compared to that of the Wright–Fisher model, with only the diagonal and immediately adjacent elements non-zero. This simple structure yields a model that is considerably more mathematically tractable than the Wright–Fisher model, particularly when extensions incorporating additional evolutionary forces (e.g., mutation, natural selection) are added. In addition, continuous-time diffusion approximations can still be applied to the Moran model, allowing for direct comparisons between the two models.

Direct Results from the Moran Model

Many of the basic results outlined for the Wright–Fisher model above have analogs in the Moran model. In particular:

Fixation probability of an allele

We can use the same intuitive argument given for the Wright–Fisher model and find again that the fixation probability of a neutral allele is equal to its initial frequency:

Probability of fixation = p0

Variance in allele frequencies over time

The variance in allele count from timestep to timestep is no longer the binomial variance of the Wright–Fisher model. It can be calculated from the general variance formula:

$$\mathrm{Var}[Y] = E\left[Y^2\right] - (E[Y])^2$$

where the capital E again indicates an expected value. Beginning with i B_1 alleles and letting $M=2N$ (so that the frequency of B_1 is $p=i/2N$, facilitating comparison with the Wright–Fisher results), after one timestep the expectation for Y is:

$$E[Y] = i$$

and

$$\begin{aligned} E\left[Y^2\right] &= (i-1)^2 P_{i,i-1} + (i+1)^2 P_{i,i+1} + (i)^2 P_{i,i} \\ &= (i^2-2i+1)P_{i,i-1} + (i^2+2i+1)P_{i,i+1} \\ &\quad + (i)^2 P_{i,i} \\ &= (i^2-2i+1)p(1-p) + (i^2+2i+1)p(1-p) \\ &\quad + i^2\left\{p^2 + (1-p)^2\right\} \\ &= 2p(1-p) + i^2 \end{aligned}$$

After one timestep of the Moran model, the variances in allele count and frequencies are:

$$\text{Var}[\text{count}] = 2p(1-p)$$

and

$$\text{Var}[\text{frequency}] = \frac{2p(1-p)}{(2N)^2}$$

There are two important mathematical differences between these results and the Wright–Fisher results. First, there is an extra $2N$ in the denominator of the variance in allele frequency. This is related to the difference between a Wright–Fisher generation (in which all $2N$ allele copies turn over at once) and a Moran timestep (which turns over allele copies one at a time). It might be expected a priori that $2N$ timesteps of the Moran model would be equivalent to 1 generation of the Wright–Fisher model. However, there is also an additional factor of two in the numerator of the Moran variance, meaning that drift in the Moran model proceeds roughly twice as fast as might be expected using this simple correspondence. Thus, in Figure 2 the Moran model prediction looks very similar to the 1-generation Wright–Fisher prediction after only five (rather than $2N=10$) timesteps have elapsed. In this case, the intensification of the drift process is due to an increased variance in offspring number, or reproductive output.

Reproductive output of individual alleles

In the Wright–Fisher model, the number of offspring of an allele copy was distributed as a Poisson random variable. The Moran model, by contrast, leads to a geometric distribution of offspring number. In every timestep in which a particular allele copy experiences an event, that event can either be reproduction (adding to the count of offspring) or death (the waited-for event that ends the series), with equal probability. The total number of offspring (reproductive events before the series-ending death) thus follows a geometric distribution with $p=1/2$:

$$D_{Mor} \sim \text{Geo}\left(\frac{1}{2}\right)$$

which has a mean of 1 and variance of 2 (contrast with the Wright–Fisher model's Poisson distribution of reproductive output, which has mean and variance of 1). The increased variance in offspring number increases the variability of outcomes and thus makes the population behave, with respect to random genetic drift, as if it is a much smaller population – the 'effective population size' (N_e) is half the census size.

Time-Based Results for the Moran Model

In the analysis of the Wright–Fisher model, approximate methods were required to obtain the expected time to fixation and other similar results. By contrast, all of the results below can be obtained in exact form from the Moran model Markov chain (see Ewens (2004) for an overview).

Given initial allele count i, we have:
Expected time to fixation or loss

$$= 2N(2N-i)\sum_{j=1}^{i}\frac{1}{2N-j} + 2Ni\sum_{j=i+1}^{2N-1}\frac{1}{j}$$

Expected fixation time, given fixation

$$= 2N\left(\frac{2N-i}{i}\right)\sum_{j=1}^{i}\frac{j}{2N-j} + 2N(2N-i-1)$$

and, for a new mutant:

$$\text{Expected fixation time for a new mutant, given fixation} = 2N(2N-1) \text{ time steps}$$

As predicted from our examination of the variance in allele frequencies from generation to generation, the timescale of random genetic drift (in the form of fixation of new mutants) in the Moran model differs from the timescale of the Wright–Fisher model by a factor of $2N/2$. The similar behavior of each model (once population size and time have been rescaled appropriately), despite very different structures (Figures 1 and 4), is encouraging. No population in nature has ever followed either model exactly in its mode of reproduction, but the convergence in results seen here suggests that it will very often be entirely appropriate to approximate a wide variety of naturally complex systems by one of these simple models.

See also: Coalescent and Models of Identity by Descent. Effective Population Size

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Genetic Variation in Populations

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Glossary

Allele A variant form of a gene.
Amplified (or anonymous) fragment length polymorphism (AFLP) Polymerase chain reaction-based molecular tool that uses restriction enzymes to cut genomic DNA into fragments and adaptors to select a subset of the fragments for visualization on an electrophoretic gel.
Cytogenetics Microscopic analysis of chromosomes in individual cells.
Haplotype Alternative forms for a multisite genotype in a defined DNA region.
Identity by descent Identical alleles in two or more individuals in a population due to shared ancestry.
Identity by state Identical alleles or phenotypes in two or more individuals in a population derived from separate mutations, not shared ancestry.
Insertions and deletions (indels) Class of DNA changes that involves the addition (insertion) and/or the removal (deletion) of small numbers of base pairs in the sequence.
Karyotype The number and appearance of chromosomes in the nucleus of a eukaryotic cell.
Microsatellite sequences Tract of repetitive DNA comprised of two to four base pairs repeats occurring at a high number of different locations throughout the genome
Minisatellite sequences Tract of repetitive DNA comprised of 10–60 base pair repeats occurring at a high number of different locations throughout the genome.
Mitochondrial DNA (mtDNA) DNA located in the mitochondria, which are cellular organelles responsible for energy production within eukaryotic cells.
Phylogenomics Analysis that involves genome data and the reconstruction of evolutionary relationships between taxa.
Polymerase chain reaction (PCR) Molecular technology that is used to amplify single copies of DNA regions several orders of magnitude for analyses.
Polymorphism The condition of occurring in many different forms.
Population genetics The field of study of the distributions and changes in allele frequencies within and between populations over time.
Population genomics Population genetics extended to include analyses involving whole genome data.
Restriction fragment length polymorphism (RFLP) analysis Molecular tool that uses restriction enzymes to cut genomic DNA into fragments for visualization of genetic variation on an electrophoretic gel.
Single-copy nuclear DNA (scnDNA) DNA regions that occur only once in the nuclear genome.
Single nucleotide polymorphism (SNP) Class of DNA variation that involves changes in nucleotide identity of individual base pairs in the sequence.
Y DNA Molecular markers mapping to the Y sex chromosome of an organism.

Introduction

Nothing is as fundamental to evolutionary biology than genetic variation. That is because genetic variation is the engine of all evolutionary change. Genetic variation can be defined as hereditary differences within or between populations and species. Without these hereditary differences, there is nothing on which natural selection can act. Going beyond natural selection alone, without genetic variation, there can be no change of any sort over time. In short, without genetic variation, there can be no evolution.

Population genetics is the study of the distributions and changes in the genetic compositions of populations and species over time. Population geneticists have developed numerous abstract mathematical models that allow them to predict how genetic variation will change in the future under the influence of different evolutionary processes, such as mutation, natural selection, nonrandom mating and sexual selection, random genetic drift, and gene flow between populations. In practice, population geneticists examine patterns of extant genetic variation and use abstract theory to infer how each of these evolutionary processes may have played a role in shaping this variation in the recent and more distant past. With this approach, population geneticists are able to identify specific loci under the influence of natural selection, deduce the relationships of different populations and species across both space and time, and infer historical changes in population sizes and dispersal events that are important for facilitating or constraining divergence and the emergence of new species.

Types of Genetic Variation

Population geneticists are interested in all types of genetic variation and have been throughout history. The types of variation that have been utilized by evolutionary biologists have ranged widely from morphological variation to chromosomal variation to assessing variation at individual loci to whole genome scans today. As molecular genetic techniques have advanced, so has the ability of population geneticists to detect patterns of genetic variation at higher and higher levels of resolution.

Morphological Variation

Biodiversity in the world is manifested most obviously by morphological variation. Morphological differences between

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species are the most apparent. However, almost all natural populations also show some form of variation in morphological traits between individuals. Before an actual mechanism of inheritance was established, it was still understood that a good portion of this morphological variation was heritable, and therefore, represented what we now know to be genetic differences between individuals. The desire to explain the generation and maintenance of morphological variation at all levels (from individuals through species) has been a driving force throughout the history of the development of evolutionary thought (Young, 2007).

Morphological traits are often classified as being either Mendelian or quantitative. Variation in both types of morphological traits represents genetic variation, albeit with different underlying genetic bases. For Mendelian traits, generally only a few loci with relatively large effects on phenotype are involved (referred to as Mendelian Genetics; see Griffiths *et al.*, 2015). For quantitative traits, many more loci are involved each with smaller individual effects on phenotype in addition to environmental influences on their expression (referred to as Quantitative Genetics; see Falconer and Mckay, 1996). The foundation of our understanding of genetics was formulated through the analyses of the transmission of phenotypic traits across generations, whether looking at Mendelian or quantitative traits. Although different mathematical formulations are used to analyze these different classes of traits, both follow the same basic underlying principles of inheritance.

With respect to evolutionary genetics, perhaps there is no model organism that has been as influential on advancing our understanding of genetic variation as *Drosophila* has been. Developed as a model organism by Thomas Hunt Morgan and his colleagues in the early 1900s, *Drosophila* has continued to have profound impacts on population, developmental and molecular genetics for 100 years (Kohler, 1994; Powell, 1997). One particular colleague of Morgan's who changed the face of evolutionary genetic studies was Theodosius Dobzhansky, who took the rigors of genetics being developed in the laboratory and applied them to natural populations. While most variation associated with fitness in natural populations does not have a simple genetic basis, Dobzhansky was able to use technological tools developed in *Drosophila* (such as Balancer chromosomes, which can be used to isolate whole chromosomes without recombination) to assess the rate and accumulation of lethal mutations in natural populations. This research proved very important for empirically testing theoretical models describing the action of natural selection operating on lethal mutations and the evolutionary dynamics involved in maintaining genetic variation underlying fitness (Dobzhansky and Wright, 1941).

Chromosomal Variation

A major breakthrough in assessing genetic variation in natural populations came about with the advent of cytogenetics and the ability to visualize the karyotype of individuals. Here population geneticists were able to look directly at genetic traits rather than only assessing genetic variation based on the expression of different phenotypes. A peculiar property of *Drosophila* is the polytene chromosomes in their salivary glands where hundreds of rounds of DNA replication occur without cell division. The result is visible chromosomes with distinctive banding patterns that can be used as unique identifying genetic markers. The banding patterns could be compared between individuals and stretches where the banding patterns were inverted relative to each other (termed inversions), were very useful for looking at genetic variation in populations. A major advantage was that one could observe genetic variation in the microscope directly. Examining inversion polymorphisms became the cornerstone of early population genetics and allowed researchers to look at natural selection operating on large blocks of linked loci in wild populations of *Drosophila* (Dobzhansky, 1937). Inversion polymorphisms were also used to construct some of the first phylogenetic relationships based on genetic information and were informative both for looking at relationships within species (*Drosophila pseudoobscura*: Dobzhansky, 1938) as well as between species (Hawaiian Picture-winged *Drosophila*: Carson, 1983) for over half a century.

Although polytene chromosomes are unique to a subset of organisms, karyotype analyses can be performed on any eukaryotic organism. The variation revealed in chromosome shape and number opened up the door for cytogenetics to be useful for evolutionary studies beyond *Drosophila*. Many organisms were found to have supernumerary chromosomes (also sometimes referred to as B chromosomes), which would vary in number and provide material for assessing genetic variation in species for which crosses are not possible (Palestis *et al.*, 2004; Jones *et al.*, 2008).

Protein Variation

The application of protein electrophoresis in the 1960s to the study of genetic variation in natural populations marks a critical turning point in the development of population genetics. Protein electrophoresis allowed for the separation of soluble proteins based on size and charge. Differences in amino acid composition could shift a protein's electrophoretic properties allowing for the detection of genetic differences between individuals. A substantial advantage to utilizing protein electrophoresis is that no breeding of the study organism was necessary. This opened the door for the population genetic analysis of any natural population of organisms and allowed for generalizations to be made beyond what could be gleaned from the handful of model organisms available at the time.

Population geneticists could look at how variable proteins were across multiple loci simultaneously (i.e., average polymorphism) and how often individuals carried different alleles at a particular locus (i.e., average heterozygosity) (Hartl and Clark, 2007). These measures gave a better metric for determining how variable populations were across a vast spectrum of organisms and revealed that although generally speaking increases in heterozygosity went hand in hand with increases in polymorphism, there was quite a bit of variability across populations as governed by differences in population size, mating, natural selection, and gene flow.

More variation than was previously imaginable was revealed (Hubby and Lewontin, 1966; Harris, 1966), leading

researchers to propose alternative explanations for the maintenance of variation beyond directional natural selection (e.g., balancing selection with heterozygotes having highest fitness and background purifying selection acting on recessive deleterious mutations). Another explanation proposed at that time that gained traction was that not all the genetic variation we see is subject to natural selection, because not all of the genetic variation revealed leads to differences in fitness, supporting the neutral theory of evolution proposed by Kimura and Crow (1964).

DNA Variation

If protein electrophoresis represented a renaissance in population genetics, the advent of direct deoxyribonucleic acid (DNA) analyses represented a revolution and led to a new branch of population genetics referred to as molecular evolution (Graur and Li, 2000). DNA analysis remains the principle approach used to describe genetic variation to this day. An advantage is that genetic variation can be assessed in any stretch of DNA, and is no longer confined to soluble proteins or even coding regions of the genome. Additionally, DNA studies no longer rely on gross mobility shifts as in protein electrophoresis, but can obtain a much higher level of resolution of the genetic changes. Most importantly, DNA analysis allows for the determination of *identity by descent*, the ability to trace multiple copies of a DNA segment in a population back to a single common ancestor in the past. Theoretical population genetics relies on identity by descent as a central organizing principle for describing evolutionary change. This is in contrast with *identity by state*, where two proteins may appear identical but actually represent proteins with different evolutionary histories because of changes that cannot be revealed by protein electrophoresis (i.e., showing convergence in phenotypic state). This is not to say that convergence cannot occur with DNA markers as well, but the resolution is often high enough to be able to distinguish between identity by state and identity by descent. Previously identified protein electrophoretic types were revealed to harbor an abundance of genetic variability in the noncoding and flanking regions of the locus not detectable from protein electrophoresis alone. This increased molecular resolution was very important for determining the evolutionary relationships between different alleles and inferring the mode of selection operating on the protein level that was most important for maintaining the genetic variation (Aquadro *et al.*, 1986; McDonald and Kreitman, 1991).

To assess genetic variation, Restriction Fragment Length Polymorphism (RFLP) analysis was commonly used. RFLP analysis involves cutting a target segment of DNA with restriction enzymes and looking at variation in lengths of the fragments that result. RFLPs reveal differences in the sequences underlying the restriction enzyme cut sites. A more direct assessment of changes occurring on the DNA level is to sequence the DNA segment of interest to reveal single nucleotide polymorphisms (SNPs) or small insertions and deletions (indels). In this manner, a direct read of the bases along a stretch of DNA can be made. Direct sequencing was technically more demanding than RFLP analyses, but as technological advances were made using polymerase chain reactions (PCR) to amplify specific DNA regions of interest that required very little amounts of starting material, direct sequencing became the popular standard for assessing genetic variation that remains to this day. The generation of sequence data has led to new measures of genetic diversity based on polymorphic nucleotides segregating in a population (θ, theta) and average proportion of nucleotide differences between all possible pairwise comparisons of sequences in a population sample (π, pi) (Graur and Li, 2000; Hartl and Clark, 2007). RFLPs also use similar measures, albeit with lower resolution, if it is assumed that there is only one nucleotide site change per restriction site difference. These are now the standard measures of genetic variation that are used today, similarly to how polymorphism and heterozygosity measures were used for protein electrophoresis in the past.

Highly Polymorphic Molecular Markers

While DNA sequencing was still relatively expensive for large population surveys, other methods for detecting DNA variation were developed in parallel, taking advantage of repeated genetic elements that are highly variable and very abundant across the genome in most eukaryotes. Higher levels of variability often translate into greater power in detecting population level processes operating during evolution, which makes these genetic markers valuable tools still used to this day (Avise, 2004; DeYoung and Honeycutt, 2005). These techniques also allow for the assessment of multiple loci in comparison to sequencing which normally relied on single-locus evaluations.

Repeated genetic elements fall into different categories based on the length of the repeat and the number of times it is repeated in the genome. Minisatellite DNA refers to sequences of approximately 10–60 base pairs in length that show a variable number of tandem repeats throughout the genome. Microsatellite DNA is comprised of even shorter repeated units (di-, tri-, or tetra-base pair repeats) that occur in higher numbers of tandem arrays throughout the genome. Both of these molecular markers have been very useful for DNA fingerprinting of individuals in populations and for assessing variability on short time scales. Multi-locus analyses of these markers can be very complex, because of the number of fragments being analyzed and overlap in size classes that cannot be easily attributed to a specific locus. However, composite single-locus approaches have helped.

A combination of PCR-based and RFLP-based approaches is Amplified (or Anonymous) Fragment Length Polymorphism (AFLP) analysis. In this case, total genomic DNA is digested with restriction enzymes. The cut sites serve as anchors for adding adaptor sequences which are then used as primer sites for PCR amplification, resulting in a range of amplified fragments that vary in size. Although the specific loci being surveyed in this case are unknown, these approaches are very good for surveying large numbers of loci simultaneously which has proven to be very useful in mapping studies. There are some problems with amplification in AFLP studies, however, where amplification of one allele in a heterozygote maybe favored over the other allele as well as other issues with

reproducibility, indicating that the results would need to be interpreted with caution.

All of these molecular markers have the advantage of revealing high variability, which is very important for certain applications in population genetics; however, these approaches also lose the ability to infer identity by descent directly. Additional assumptions are needed for the evolution of these markers in order to place them in a population genetics framework. Without accurate estimates of actual identity by descent, direct use in population genetic models of these highly variable markers becomes less powerful. Combining microsatellite analyses with sequence information from the flanking regions has been useful for understanding the evolutionary dynamics of these markers and recovering more direct identity by descent information in population studies (Schlotterer, 2000; Chatrou *et al.*, 2009). In any event, by using highly variable markers over very short generational time scales, researchers can be pretty confident about assuming identity by descent. The much higher levels of variability and relatively low costs, outweigh the loss of direct descent in certain applications.

Applications of Different Tools

Different measures of genetic variation capture different temporal scales of relatedness between individuals, populations, and species. Therefore, different classes of molecular data (e.g., RFLP, DNA sequence, microsatellites, etc.) will be more effective at answering different types of evolutionary questions than others. There are advantages to using the highly variable markers (such as minisatellite or microsatellite markers) across several loci as compared to using sequencing approaches with fewer loci. Even with the same type of molecular tool being employed, different parts of the genome experience different evolutionary histories because of their mode of inheritance and mutational constraints. As a result, the genome is actually a mosaic of evolutionary information with different parts of the genome representing different depths of evolutionary time (Graur and Li, 2000; Avise, 2004; Templeton, 2006).

For example, animal mitochondrial DNA (mtDNA) is haploid and maternally inherited in most species. These characteristics leave mtDNA more subject to drift than other parts of the genome and can reveal population dynamics that have occurred over relatively recent time scales. Similarly, animal Y DNA is also haploid (there is very little homologous DNA in comparisons of the X and Y chromosomes) and is paternally inherited making this molecular marker also more subject to genetic drift effects. In addition, high variance in male reproductive success further reduces the effective population size for Y DNA causing it to evolve even more rapidly than mtDNA. As a result, Y DNA is a very good marker for differentiating populations and looking for episodes of recent gene flow. Single-copy nuclear DNA (scnDNA) is associated with autosomes, which are diploid and biparentally inherited. These markers evolve more slowly than mtDNA and Y DNA markers because they are less subject to drift and experience lower rates of mutation due to more efficient DNA repair mechanisms. As a result, scnDNA markers typically exhibit more standing genetic variation in populations than mtDNA and *Y* DNA and can reveal population histories dating further back than the haploid, unisexually inherited markers. Molecular markers on sex chromosome (X or Z, depending on species) show temporal patterns of evolution on average that are intermediate between mtDNA and Y (or W) DNA markers and autosomal markers because of sex chromosomes being diploid in one sex, but hemizygous in the other (i.e., being paired with a nonhomologous sex chromosome with which it does not freely recombine).

Plants also have mtDNA, yet this haploid genome exhibits unusual patterns of variation and tends to be highly variable with respect to gene order, but very conserved with respect to sequence changes, making it more difficult to use as a molecular marker for evolutionary analyses. Plant chloroplast DNA (cpDNA), on the other hand, follows more standard models of evolutionary change similar to mtDNA in animals, albeit with rates of mutation that are much slower, making it a better molecular marker for questions involving more distantly related taxa (Avise, 2004).

The types of molecular markers used in a given study depends on the questions being addressed, the availability of molecular markers in the study system, the amount of variation present for those markers, and costs (DeYoung and Honeycutt, 2005). The best approach is to use multiple markers from more than one genome region. Different regions of the genome are expected to respond differently to the same underlying population processes, allowing for independent estimates of events that have occurred at different temporal scales (Templeton, 2005). Researchers can also tailor their molecular markers to suit different rates of evolutionary change by choosing particular loci experiencing different selection constraints or even confine their analyses to different parts of the DNA region (e.g., DNA changes that do not alter the protein amino acid sequence, such as synonymous change, versus DNA changes that do alter the resulting amino acid sequence, such as nonsynonymous changes, which are expected to evolve at different rates with synonymous changes following more neutral expectations of change).

Which molecular marker is chosen depends on the evolutionary time scale that the question being addressed encompasses. For questions that rely on identification of unique individuals in the population, such as parentage analyses, the markers that evolve more quickly and provide higher levels of variation across multiple loci will be more informative than more slowly evolving markers. Conversely, for questions that aim to relate evolutionary taxa across deeper time scales, markers that evolve more slowly would necessarily need to be employed. **Table 1** provides an approximation for which markers would be most useful for which applications.

Future Directions: Whole Genome Approaches

With technological advances allowing for increased automation of sequencing, more studies of natural populations are aiming to assess genome-wide patterns of genetic variation, opening up new areas of inquiry in population genomics (Ellegren, 2014) and phylogenomics (Lemmon and Lemmon,

Table 1 Applicability of Different Molecular Markers to Different Questions in Evolutionary Biology

Hierarchical Level	*Protein electro*	*RFLP mtDNA*	*RFLP scnDNA*	*AFLP/minisats/ microsats*	*SEQ mtDNA*	*SEQ Y DNA*	*SEQ scnDNA*	*SEQ cpDNA*
DNA fingerprinting	*	–	–	***	*	–	–	–
Parentage/relatedness	**	*	*	***	*	*	*	–
Conspecific populations	***	***	*	**	**	**	**	*
Closely related species	*	***	**	*	***	***	***	**
Intermediate taxa levels	–	*	***	–	***	**	***	***
Deep (>50 mya) separations	–	–	*	–	**	*	**	***

*** highly informative; ** informative; * somewhat informative; – inappropriate. Not all categorizations are absolute and will depend on the evolutionary constraints of the particular molecular marker chosen within each category and the evolutionary history of the specific taxa in question.

Abbreviations: minisats and microsats, ministaellite and microsatellite sequences; protein electro, protein electrophoresis; SEQ, DNA sequence information.

Source: Adapted from Avise, J.C., 2004. Molecular Markers, Natural History, and Evolution, second ed., Sinauer Associates, Inc., Sunderland, MA, including additional information reproduced from Templeton, A.R., 2005. Haplotype trees and modern human origins. Yearbook of Physical Anthropology 48, 33–59.

2013). Although these whole genome approaches do not necessarily provide greater resolution of variability on the molecular level, they do provide greater statistical power resulting from the shear magnitude of the number of genetic elements that can be analyzed, which has led to new insights and approaches to studying evolutionary processes.

Up to this point, traits that had been identified as being important in adaptation were first identified phenotypically and then used to map the underlying loci to analyze the evolutionary processes involved. Through the application of whole genome approaches, adaptive traits no longer need to be defined a priori and whole genome scans looking for patterns of variation relative to neutral expectations allow for the direct identification of DNA regions that have been under different types of selection (e.g., positive selection versus purifying selection). Different types of selection will leave specific genetic footprints of increased or decreased genetic variation in the genome that can then be studied further.

The same type of whole genome scan approach can be used to look at which parts of the genome may be more subject to gene flow between populations or newly evolving species. Identifying regions that are more or less permeable to gene flow between diverging populations can reveal DNA regions that may be important for providing reproductive barriers (either because of reproductive incompatibilities in the genome or strong selective difference of traits in different ecological contexts) in the early stages of species divergence (Feder *et al.*, 2012; Sousa and Hey, 2013). These types of considerations have also been very important for using whole genome information for phylogenetic reconstruction, where careful data selection of DNA regions to include in the analyses is critical for obtaining accurate taxa relationships (Lemmon and Lemmon, 2013). Once relationships have been established, they can be used to test hypotheses about the origins of adaptive genetic variation leading to phenotypic divergence (Feder, *et al.*, 2012; Ellegren, 2014).

Other Considerations and Controversies for Future Research

Just as the historical trend has been to move closer and closer to the genetic elements over time, there is a more recent reversing trend to try to move back to analyzing the adaptive traits themselves. This has taken different forms, but one large-scale approach has been evolutionary proteomics, which looks at the whole genome dynamics of protein expression, regulation, and interactions within an evolutionary context (Diz *et al.*, 2012). Although these types of studies are moving back to identity by state rather than identity by descent, the argument is that we are ultimately getting closer to studying the actual traits that matter (i.e., protein phenotypes involved in adaptation, as opposed to only the DNA sequences that underlie them). Another emerging large-scale approach has been the analyses of whole genome gene expression (termed the transcriptome). Researchers are combining whole genome sequencing with gene expression analyses to directly connect changes in DNA sequences to changes in expression and the ultimate development of morphological differences within and between species (e.g., humans and chimpanzees: Prescott *et al.*, 2015), coming back full circle to the detailed analysis of the type of variation that was originally of interest to population and evolutionary geneticists.

Although these studies investigating changes in gene regulation and the role of development in evolution have been exciting, they have also reignited controversies centered around determining whether structural changes in proteins or changes in gene regulation have been more important in adaptation (Hoekstra and Coyne, 2007; Wray, 2007). Part of this controversy stems from lumping morphological change with all types of adaptive change, which may not always be morphological in nature. In any event, the relative importance of regulatory versus non-regulatory genetic variation underlying adaptation is yet to be determined.

The controversy surrounding the nature of genetic variation underlying adaptive traits may also lie in differences in scale that have often been used to provide evidence of the relative importance of regulatory and non-regulatory change in evolution. A relevant, yet still controversial, insight stemming from studies of evolution and development centers on the nature of genetic variation at different scales of divergence (Stern, 2011; Hollocher *et al.*, 2013). The assumption has been that within-species genetic variability and genetic differences between species are similar and follow the same basic assumptions of evolutionary change. In that respect, the assumption has been that the study of evolutionary dynamics occurring within species can be used to extrapolate

to the processes underlying speciation. However, patterns are beginning to emerge that suggest that changes underlying long-term evolution may be regulatory in nature, while the vast majority of mutations underlying short-term evolution are not regulatory. Therefore, short-term, within-species variation is quite different from the long-term changes that move to fixation in entire species. If this generalization proves to be true, then this insight could fundamentally change our understanding of the evolution of morphology and our concepts of the genetic variation underlying species divergence.

Additional complexity is presented by changes that involve epigenetics. Up to this point, all discussion of genetic variation have been concerned with changes in DNA sequence, which then can be inherited across generations to serve as the engine of evolutionary change. However, with the discussion of gene expression and development, comes an enhanced appreciation of the role epigenetic changes may play in evolutionary processes. Epigenetic changes are those genetic modifications that control gene expression that give rise to variable phenotypes even when the underlying genetic sequences are identical (West-Eberhard, 2003; Jablonka, 2013). The relevance and evolutionary significance of epigenetics is not completely understood and highly controversial with several researchers claiming that the evolutionary consequences of epigenetic variation have not been adequately accommodated in standard models of evolution (Laland, *et al.*, 2014), while others say that they have (Wray *et al.*, 2014). These ideas have been extended to include other processes beyond the interplay of epigenetics and development, looking at the role of behavior and learning on the subsequent evolution of populations (termed niche construction; Bateson and Gluckman, 2011; Jablonka and Lamb, 2014), revealing the potential for these processes to shape evolutionary trajectories and influence rates of change. Whether one sides with one camp or the other (and there is some evidence that both camps are talking about similar phenomena, but with different emphases; Scott-Phillips, *et al.*, 2014), the impact of epigenetics and other nongenetic modes of inheritance is an area of investigation that complements ongoing studies of changes in DNA sequence to help advance our collective understanding of the roles all different types of variation play in evolution.

See also: Genetic Variation, Maintenance of. Hardy–Weinberg Equilibrium and Random Mating. Quantitative Genetic Variation

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Genetic Variation, Maintenance of

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Glossary

Balancing selection Any form of natural selection that balances or actively maintains genetic variation in a population.

Effective population size (N_e) An idealized population size in which the effects of genetic drift are the same as in the population under study. This concept allows us to compare populations with geographical or age structures, different number of males and females, etc. In most cases, the effective size is less (sometimes considerably less) than the actual size.

Equilibrium (in the context of allele frequencies) Values that do not change from one generation to the next. An equilibrium can be stable, unstable, or neutral. A stable equilibrium is one to which the population returns if, for some reason (e.g., random changes due to genetic drift), the values are disturbed by some small amount. Heterozygote advantage in a two-allele model gives a stable polymorphic equilibrium. An unstable equilibrium is one from which a population moves further if the allele frequencies are perturbed (e.g., the polymorphic equilibrium for heterozygote disadvantage with two alleles). Allele frequencies neither return to nor move further away from a neutral equilibrium.

Genetic load The reduction in the mean fitness of a population compared to the fitness of the optimal individual.

Heterozygote advantage A model of selection (especially one with just two alleles) in which heterozygotes have higher fitnesses than homozygotes.

Heterozygote disadvantage A model of selection (especially one with just two alleles) in which heterozygotes have lower fitnesses than homozygotes.

Polymorphic Consisting of two or more variants (usually alleles).

Segregational load A form of genetic load arising from segregating variation maintained by balancing selection.

Introduction

Natural populations of most diploid organisms are characterized by genetic differences among individuals. This variation is vital to the adaptation and long-term survival of each species: genetic variation is often described as the 'fuel' for natural selection. Just why this genetic variation is present, however, is unclear. The two most prominent population-genetic theories (selectionism and neutralism) appear to predict patterns of variation inconsistent with those observed in nature. This mismatch between theory and observation has been dubbed 'the paradox of variation' and held to be the central problem in population genetics. On the one hand, selectionist explanations emphasize the current adaptive value of genetic variation: different genotypes have different viability or fertility and their frequencies are actively maintained by selection. On the other hand, neutralists argue that variation is selectively equivalent and that genetic drift is the primary process governing allele frequencies, which change randomly over time. Of course, different explanations may be correct for different genes and some combination of the two theories may apply. In this article we outline these theories, discussing their various strengths and weaknesses.

The Evidence

Many debates in population genetics in the 1950s centered around the levels of genetic variation that were present in natural populations. The technology of the time, however, was not able to provide a definitive estimate; it was not until the advent of gel electrophoresis in the 1960s that the question was apparently settled. In short, electrophoretic data were interpreted as showing that genetic variation was at high levels in nearly all populations, a conclusion that was reinforced when the results of direct DNA sequencing became available. Today it is clear that genetic variation is ubiquitous in populations of nearly all diploid species (and many haploid taxa, too) for almost all genes. Just why this variation is present, however, is not immediately clear.

Hypothesis 1: Natural Selection

One possible explanation is that this variation is actively maintained – balanced – by natural selection, a view sometimes described as the 'selectionist paradigm' or the 'balance school.' This view derives from the argument that individuals harboring genetic variation have a selective advantage: variation is intrinsically beneficial to its bearers (Lerner, 1953). Certainly, it is clear that highly inbred (and hence largely homozygous) individuals have generally lower fitnesses. Heterozygotes may also have an advantage in being more phenotypically flexible, being able to present two versions of the gene product being coded at the locus in question. For example, the allelic differences may result in proteins that have their optimal catalytic functions at slightly different temperatures and so heterozygotes can function better over a wider range of physiological or environmental conditions than either homozygote.

Hypothesis 2: Genetic Drift and Mutation

A second possible explanation is that the observed variation, rather than being actively maintained, is in constant flux, with

the overall level of variation being at some steady state even though its constituents are continually being turned over. The 'neutral hypothesis' or 'neutralist school' argues that standing variation is equivalent with respect to selection, a consequence of its generation by (neutral) mutation and its elimination by genetic drift (Kimura, 1968). The role of selection is confined to the purging of deleterious mutations (which constitute the vast majority of novel mutations). This view derived, in part, from considerations of the molecular biology of genes and proteins, where it was difficult to envisage a genuine selective difference for much of the variation observed (e.g., differences in codons' 3rd positions that made no difference to the protein being encoded).

Problems with the Selectionist Hypothesis

Both hypotheses have theoretical and empirical weaknesses. For example, the intuition underlying the balance school suggests that selection in the form of heterozygote advantage underlies the observed levels of genetic variation. Interestingly, this model is unique among the classical, one-locus, two-allele models of constant viability selection in maintaining both alleles at a stable equilibrium: heterozygote advantage is both necessary and sufficient to ensure that any population with allele frequencies close to those at this equilibrium will evolve toward that equilibrium. All other classical models with constant viabilities (e.g., deleterious recessive) predict that genetic variation should eventually be depleted. (Compare Figures 1 and 2.)

Unfortunately, when this basic model is extended to more than two alleles, the situation is less clear. For a start, the very meaning of 'heterozygote advantage' becomes ambiguous. We could mean simply that each heterozygote is fitter than its two corresponding homozygotes, or we could require the stronger condition that all heterozygotes are fitter than the fittest homozygote. Ironically, whatever our definition, it turns out that heterozygote advantage is neither necessary nor sufficient to maintain all the alleles at a stable equilibrium. In other words, it is possible to find fitness parameters that do not fit our definition that afford a stable fully polymorphic equilibrium, as well as other fitnesses that do meet our definition that nevertheless lead to the elimination of one or more alleles. Lewontin *et al.* (1978) exhibit counterexamples to both the necessity and sufficiency of 'heterozygote advantage' in maintaining even 3 alleles at equilibrium.

A related issue is that the sorts of fitnesses that are able to maintain larger number of alleles at an equilibrium are highly unusual. In a simulation study, Lewontin *et al.* (1978) generated random sets of $n(n+1)/2$ viabilities for a population with n alleles (and hence $n(n+1)/2$ different genotypes) for $n=2$, 3, 4, 5, and 6 in different simulations, and asked what proportion of these sets allowed a stable equilibrium with all n alleles present. For $n=2$, heterozygote advantage is both necessary and sufficient and thus we know the answer is 1/3, since there are 3 viabilities needed (for the two homozygotes and one heterozygote) and the chance that the heterozygote fitness in the largest of the 3 viabilities is 1/3. For $n>2$, however, there is no intuitive explanation. Simulations showed that the proportion rapidly becomes minute: for $n=3$, the proportion was ~ 0.042, for $n=5$, it was 6×10^{-5}, and for $n=6$, not one in 100 000 sets yielded a stable 6-allele polymorphism. Note that the viability sets that did maintain all n alleles did not necessarily display heterozygote advantage, however defined (except for $n=2$). Clearly, being able to maintain n alleles for larger values of n is a very unusual property of fitness sets.

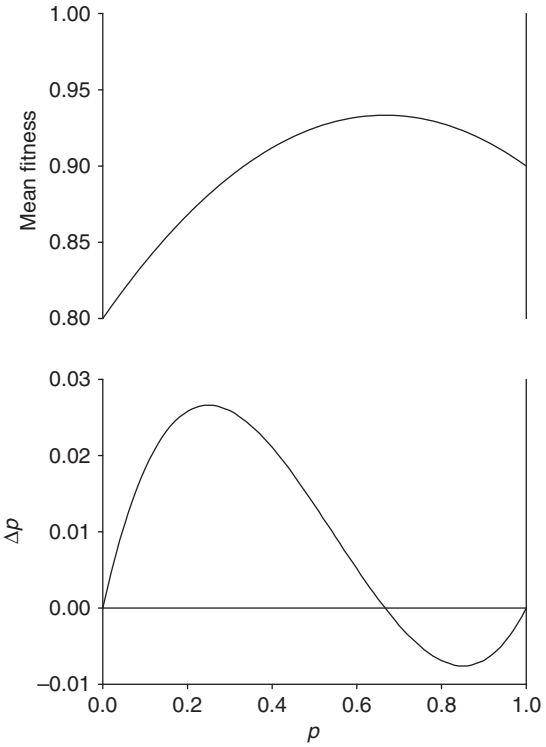

Figure 1 Mean fitness of the population (above) and change in frequency of allele A_1 (below) under an example of heterozygote advantage with 2 alleles. The fitness of A_1A_1 homozygotes is 0.9, that of A_2A_2 homozygotes 0.8, compared to A_1A_2 heterozygotes with a fitness of 1.0. Note that for frequencies of A_1 below $^2/_3$, the change in frequency of A_1 is positive (i.e., it increases); above this value the change is negative (i.e., A_1 becomes rarer). Hence, A_1 moves closer to the stable equilibrium frequency of $^2/_3$ every generation. At the same time A_2 moves toward a stable equilibrium frequency of $^1/_3$. Since both alleles are present at this equilibrium, it is polymorphic. Its stability means that this form of selection maintains variation in the population. This equilibrium frequency also maximizes the population's mean fitness.

These findings were interpreted as showing that the likelihood of such sets occurring in nature was minute. But such a conclusion is a logical fallacy: the size of parameter space is not a measure of likelihood, especially in a system that evolves over time. In another simulation study, Spencer and Marks (1988) showed that recurrent mutation at a locus could lead to the occasional successful invasion of a population by a new allele and the building up of polymorphism over time. Indeed, after 10 000 generations, with just one mutation per generation, the simulated populations possessed an average of more than 5 alleles (Marks and Spencer, 1991). These ideas have been applied to various other forms of selection (e.g., frequency-dependent selection, spatially variable selection,

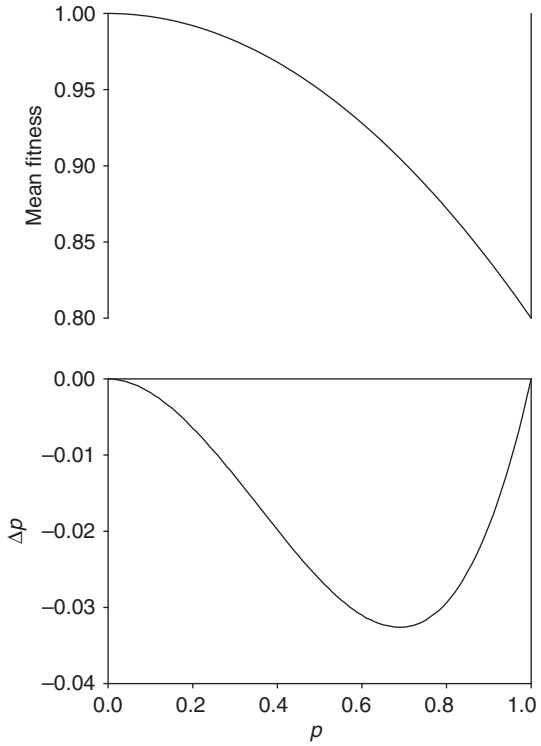

Figure 2 Mean fitness of the population (above) and change in frequency of allele A_1 (below) under an example of deleterious recessive with 2 alleles. The fitness of A_1A_1 homozygotes is 0.8, compared to A_1A_2 heterozygotes and A_2A_2 homozyotes with fitnesses of 1.0. Note that for all non-trivial frequencies of A_1, the change in frequency of A_1 is negative (i.e., A_1 becomes rarer). Hence, A_1 moves closer to the stable equilibrium frequency of 0 every generation. At the same time A_2 moves toward a stable equilibrium frequency of 1. Since only A_2 is present, this equilibrium is monomorphic, not polymorphic. Its stability means that this form of selection does not maintain variation in the population. Nevertheless, this equilibrium frequency also maximizes the population's mean fitness.

differential selection on males and females) and the overall conclusion is similar: variation is constructed over time under simple combinations of mutation and selection. Nevertheless, it is not clear if these models are sufficiently realistic. For example, one possible disconnect is that many of the simulations generated some form of heterozygote advantage and yet convincing cases of this form of selection acting in natural populations are extremely rare.

A different theoretical problem arises when variation at many loci is considered. Under simple models of selection such as heterozygote advantage at a single locus, the mean fitness of the population is lower than that of the ideal (heterozygous) genotype. This reduction in fitness is called the 'segregational load,' a form of 'genetic load.' When multiple loci are considered, it seems reasonable to assume that effects of each locus are independent and so fitnesses combine multiplicatively. Doing so, however, leads to an absurdity if large numbers of loci have variation maintained by these forms of selection: the mean fitness becomes ridiculously small compared to that of the ideal, multiply-heterozygous types (see Lewontin (1974) and Kimura (1983) for the details of this criticism). Different forms of selection, such as frequency-dependent selection, are less subject to this problem, but it has not been satisfactorily answered.

Finally, as mentioned above, convincing cases of genetic variation in natural populations that are actively maintained by selection are rare. In part, this absence of evidence reflects the difficulty of measuring selection in the wild, but if it is due to a real dearth of examples, then the selectionist hypothesis needs significant modification.

Problems with the Neutralist Hypothesis

One of the attractive features of neutralism is that it leads to clear, mathematically derived predictions (Kimura, 1983). For example, the rate of molecular evolution (i.e., the rate at which neutral alleles are replaced by others) should be equal to the neutral mutation rate, independent of population size. Evidence from numerous studies, however, suggests that population size may have an effect and that over short time scales, this prediction is not borne out.

The neutral theory also makes predictions about the levels of variation to be expected in populations. For instance, the expected level of heterozygosity, H, in a population of effective size N_e subject to neutral mutation at a rate μ is given by

$$H = \frac{4N_e\mu}{1 + 4N_e\mu}$$

Thus, larger populations (everything else being equal) should harbor more variation (whether measured using H or other measures), but this prediction is only weakly supported, with most species' levels of variation falling in a rather narrow range. Similarly, the amount of variation within a species should be positively correlated, as one moves across the genome, with the amount of divergence from a closely related species, and yet in a number of cases, this correlation turns out to be negative. Finally, again as one moves across the genome, the neutral theory predicts no correlation between recombination rates and polymorphism, but numerous observations show a positive relationship. Hahn (2008) and Wagner (2008) expand on these and other problems with neutralist predictions. Unfortunately, many of these predictions require the study system to be in a 'steady state,' which in practice requires a very long period of constant conditions (e.g., mutation rate, population size, absence of selection at nearby loci).

Possible Solutions

More complex forms of selection, such as frequency-dependent selection, density-dependent selection, spatially and temporally variable selection, and differential selection on males and females can also maintain variation. For example, 'negative frequency-dependent selection' in which rare types are favored can often preserve allelic variation. Such selection pressures naturally arise when the alleles concerned confer resistance to parasites (or disease). Hosts carrying common alleles are likely to have been encountered by the parasite, which may then have developed resistance to the effects of

these common alleles. Hosts bearing rare alleles, however, are less vulnerable to the parasite and so have higher fitness. The evidence for frequency-dependent selection acting in natural populations is much stronger, and it does not lead to the same segregational-load issues as heterozygote advantage. Similarly, density-dependent selection, in which selection coefficients depend on the population size can also maintain genetic variation without the same load issues. Spatially variable selection, reflecting the different selection pressures arising from environmental heterogeneity, and variation in selection pressures over time (at different stages of an organism's life cycle or from one generation to the next) would also seem to be more realistic than uniform selection. Nevertheless, depending on the form of selection in the different habitats or time periods, segregational load may still occur. Differential selection on males and females has been relatively neglected by theorists, but it too appears to occur reasonably often in natural populations. The assumption about independence of fitnesses at different loci could also be relaxed to allow epistasis (fitness interactions between loci), although how to do so for many loci is not immediately obvious. The algebraic complexity of many of these models has meant they are relatively poorly understood, especially when more than 2 alleles at more than one or two loci are involved.

A modified version of the neutral theory, the 'nearly-neutral theory' avoids several of the problems of the original proposal. In the original modification (Ohta, 1973) much standing variation is mildly deleterious, but a more expanded view also included mildly advantageous variation (Ohta and Gillespie, 1996). As in the neutral theory, genetic drift is the predominant process affecting allele frequencies, but the nearly-neutral theory predicts that in larger populations, molecular evolution is slower because drift has less effect in larger populations. Interestingly, nearly-neutral alleles also appear to constitute the standing variation in some models purely of selection. Marks and Spencer (1991) found that in their simulated populations, in which there was no genetic drift, many of the resulting polymorphisms were not detectable as different from those expected under neutrality, according to a standard statistical test. Effectively, many of the alleles surviving the selective filter had fairly small selective differences, and could be described as nearly neutral.

This convergence between explanations is, perhaps, no accident. One longstanding suggestion is that both the selectionist and neutral hypotheses apply, but to different aspects of the genome. For example, alleles at a highly polymorphic locus that differ in DNA sequence may fall into a small number of classes within which there are no selective differences; such differences only apply among classes. So the neutral hypothesis applies within classes and the selectionist hypothesis among them.

More fundamentally, perhaps, the two explanations may apply at different loci. Moreover, theoretical predictions may be confounded, especially if those loci are linked: genetic linkage between loci has fundamental consequences for levels of variation. For instance, if, at one locus, selection has driven a favorable newly arisen mutation to fixation, levels of variation at nearby linked loci will be reduced because only a few of the variants will have been present on haplotypes carrying the favored mutation. Such 'selective sweeps' (as these fixations are dubbed) can explain the correlations often observed between recombination rates and levels of genetic variation. Thus, even if the assumptions of the neutral hypothesis are satisfied, its predictions might be awry because a steady state at the locus of interest has yet to be reached: more time might be needed for neutral mutation to restore the predicted levels of variation at loci to which it applies. In an intriguing recent study, Corbett-Detig *et al.* (2015) showed that selective sweeps had a greater effect on levels of linked neutral variation in species with larger populations. Thus, the neutralist expectation of a strong correlation between population size and genetic variation is severely constrained by natural selection acting at linked loci, and the observed correlation is unexpectedly weak.

In summary, some combination of genetic drift, mutation (both neutral and non-neutral), and selection is responsible for maintaining the variation observed in natural populations; the arguments revolve around the relative importance of these population-genetic processes.

See also: Genetic Drift, Models of Random. Natural Selection, Introduction to

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Genome Evolution's Role in Developmental Evolution

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Glossary

Cis-regulatory mutations Mutations in the DNA affecting the regulation of a nearby gene.
Coding mutations Mutations in the DNA affecting the amino acid sequence of a protein.
Comparative genomics New field of biological research in which the genome sequences of different species are compared.
Copy number variant (CNV) A gene which varies in the number of copies from one individual to the next.
Horizontal gene transfer Transfer of genetic material between organisms without mating.
Phyla Taxonomic rank below kingdom and above class.
Pleiotropy One gene that influences multiple traits.
Pseudogene Nonfunctional relative of gene is called pseudogene.
Transposons DNA sequence that can change its position within the genome.

"Curiously the improved understanding of the nature of gene and mutation has not added, so far, to the understanding of evolutionary phenomena"
Ernst Mayr, 1963

Half a decade after Ernst Mayr expressed his frustrations in this famous statement, its significance has not entirely faded. Despite ongoing interest and a steady rise in knowledge about the genetic basis of evolution, we are still far from its comprehensive understanding. Recent technical developments, especially in increasingly cheaper and high-volume sequencing technologies, have improved the feasibility of whole genome comparisons and have raised our hope that we will be closer to a new level of understanding in the near future. The emerging field of comparative genomics has indeed provided us with a wealth of sequenced, annotated, and published genomes of species from almost all extant phyla over the last decade. Such a heavy focus on the genome is turning the field of evolutionary genetics more and more into the field of evolutionary genomics. But this wealth of genomic data does not immediately translate into insight. Genomes are big and diverse, and genome evolution is driven by various forces, hence the crucial question remains: what exactly in these genomes is responsible for the vast phenotypic diversity in the tree of life? Fortunately, previous work in classic evolutionary genetics can give important clues into where in the genome to look and what types of changes are likely to be important in evolution. This article will provide a glimpse into some of the ongoing areas of study in genome evolution and discuss some examples that have shaped our understanding of developmental evolution. The end of the article will address some recent trends in genome evolution and make a case of how important it is to continue these sequencing efforts, as even some of the simplest questions have yet to be resolved.

Transposons

Transposons are pieces of DNA that can change their position in the genome. Most transposons, however, have become inactive and do not 'jump' anymore. They were first discovered by Barbara McClintock as the cause of the irregular color pattern in some strains of maize, *Zea mays* (**Figure 1**). Her discovery at first earned her a lot of skepticism but was eventually accepted and led to a Nobel prize (McClintock, 1950). Transposons come in many flavors (for a review see Muñoz-López and García-Pérez (2010)) and make up a surprisingly large percentage of most genomes that have been studied so far (Chalopin *et al.*, 2015; Lisch, 2013). In our species, for example, almost half of our genome is comprised of transposable elements, although only less than one-tenth of a percent are still potentially active (Mills *et al.*, 2007). Active

Figure 1 Ears of corn, which formed the subject of Barbara McClintock's research on transposons as seen on display at the Nobel Museum in Stockholm. One can almost see the transposons hopping.

transposons can have far-reaching consequences. When transposons jump, they can mutate their host genomes by interfering with a gene or changing a regulatory region. Due to their repetitive nature, even when they remain inactive they can facilitate chromosomal rearrangements (such as deletions or duplications) through homologous recombination (Burns and Boeke, 2012). When transposons disrupt the function of genes, they can induce phenotypic changes. One such example comes from plant domestication and has been studied by generations of students of genetics. The famous wrinkled peas, which inspired Mendel to formulate his second law of inheritance, owe their shape to a transposon interruption of a starch-branching enzyme (Bhattacharyya *et al.*, 1990). Another example, one the wine drinker amongst us might appreciate, is a transposon integration in the Vvmby1A gene (not present in the red wine Cabernet grape) which caused the loss of color in the white wine producing Chardonnay variety (Kobayashi *et al.*, 2004). In addition to disrupting function, transposons can also modulate where or how much a gene is expressed. One study, Barbara McClintock surely would have appreciated, showed that one of the main loci responsible for the gross morphological differences between maize and its wild relative teosinte is a retrotransposon roughly 60 kb upstream of the tb1 gene, a gene that represses branching. The retrotransposon enhanced expression of this gene resulted in a dramatic reduction in the number of branches in modern maize, which in turn contributed to the success of corn as one of the most popular crops in the world (Studer *et al.*, 2011). Transposons are not only important in plants. Their role in shaping genome evolution extends to animals as well, including most likely our own. It is intriguing to note, that since the split between humans and chimpanzees, roughly 6 Mio years ago, over 10 000 transposons have changed their location (Mills *et al.*, 2006). It would be hard to believe that none of them had an effect on the evolution of either of the two species. If not adaptive, at the very least they have had a clear role in many human diseases (Ayarpadikannan and Kim, 2014).

Given their importance in genome evolution, it is clear that research on transposons will continue to provide important new findings in the future. Indeed in almost every new genome project one of the first things people focus on are the number and structure of transposable elements.

Coding Mutations

Coding mutations have been at the forefront of evolutionary genetics for a long time, simply because the genetic code makes it easy to identify mutations that alter proteins by solely looking at the DNA sequence. There are generally three types of mutations that change protein sequences: nonsynonymous substitutions, premature stop codons, and frameshifts, which ultimately lead to the former. Although their importance in evolution has come under attack recently (Carroll, 2008), their contribution to genome and developmental evolution remains undeniable. Case studies of coding mutations in developmental evolution come mainly from work on coloration. Examples range from changes in pigmentation in cavefish, to the coat pigmentation in beach mice (*Peromyscus polionotus*), and the black fur in some cat species, all due to single amino acid substitutions in pigmentation genes (Protas *et al.*, 2006; Hoekstra *et al.*, 2006; Schneider *et al.*, 2015). In human populations coding mutations have also changed the way we look. The paler skin of Europeans is at least partly due to such structural mutations (Lamason *et al.*, 2005). The occurrence of coding changes in the evolution of pigmentation is most likely attributable to the fact that the affected genes seem to have very specific functions and hence the mutations do not have pleiotropic effects. Nevertheless there are many examples for coding mutations driving evolution besides the ones affecting coloration. For instance, the reason that Andean geese can tolerate such high altitudes is, in part, due to a single amino acid change in their hemoglobin protein, which leads to increased O2 binding (Perutz, 1983). Visual acuity can come from coding changes as well. The opsins of the canary, for example, have shifted their sensitivity due to a single amino acid change, which transformed their violet pigment into a UV pigment (Yokoyama and Shi, 2000). Last but not least, for most known cases, where the genetic basis of hybrid sterility is known, there seems to be evidence for selection on mutations in coding regions (Orr *et al.*, 2004; Brideau *et al.*, 2006) suggesting an important role for structural mutations in speciation.

Regulatory Mutations

Coding mutations can also affect regulation of genes, especially if they occur in transcription factors (Lynch *et al.*, 2011), or if they affect mRNA stability (Schiavi *et al.*, 1994). However, most of the time when regulatory mutations are invoked, it is implied that they affect either the promoters of genes or cis-regulatory elements such as enhancers or repressors. Regulatory elements were first discovered by Jacob and Monod in their ground-breaking paper describing the lac-operon in 1961 (Jacob and Monod, 1961). Their work was so paradigm shifting that it earned them the Nobel prize in Medicine four years later. From this seminal finding it took only a few years until some influential papers speculated about the role of cis-regulatory elements in evolution (Britten and Davidson, 1971; King and Wilson, 1975; Bryson and Vogel, 1965; Jacob, 1977). Such arguments have not ceased since then, though there has been a recent surge of advocacy for the role of cis-regulatory evolution over the last decade (e.g., Stern, 2000; Stern and Orgogozo, 2008; Carroll, 2000, 2008; Wray, 2007). Arguments for why evolution would favor regulatory mutations over coding mutations usually converge around the assumption that cis-regulatory mutations are less pleiotropic than coding mutations. Due to the modularity of cis-regulatory elements it is expected that mutation of individual enhancers would allow for time or tissue-specific effects, rather than systemic effects associated with coding mutation (Carroll, 2008; Stern and Orgogozo, 2008). Also, mutations in enhancers are believed to frequently be dominant as each allele in a diploid organism is transcribed largely independently. A dominant effect of a mutation would in principle allow for selection in heterozygotes (Wray, 2007). While there is evidence for the role of promoter mutations in genomic evolution (Haygood *et al.*, 2007), promoter sequences often bind to a core set of widely used transcriptional regulators and are

therefore not believed to be the primary driver of regulatory variation. Since enhancers tend to be more variable, most work is focused on enhancer elements as a source of evolutionary divergence. Examples for cases of developmental evolution caused by changing regulatory elements include the bristle pattern on *Drosophila* larvae (Sucena and Stern, 2000), armor plate reduction in stickleback (O'Brown *et al.*, 2015), and the ability of digesting lactose in adult humans (at least in some of us) (Tishkoff *et al.*, 2007) and many more. For a more comprehensive list see Stern and Orgogozo (2008). The types of mutations that underlie cis-regulatory divergence can vary from a single nucleotide substitution (Shirangi *et al.*, 2009) to deletions of the entire regulatory element (Chan *et al.*, 2010). Recent studies using multiple stickleback populations allowed for some estimates about the contributions of coding and regulatory changes to evolution. Using a genome-wide set of loci, used repeatedly in parallel marine to freshwater stickleback adaptation, the authors estimated the contributions of coding changes to be roughly 20%, while regulatory changes could contribute up to 80% (Jones *et al.*, 2012). Another study focusing on cichlid genomes found that coding changes are more likely to be involved in the divergence of physiological or terminally differentiated traits like color, while regulatory changes seem to be more important in morphological changes especially when genes with pleiotropic effects are involved (Brawand *et al.*, 2014). There has been a long debate about whether there are differences in the types of mutations affecting morphology, physiology, or behavior (Hoekstra and Coyne, 2007). Current studies have been biased toward mutations affecting anatomical traits, in part because the latter two are much harder to study in the fossil record. Future work will help in addressing this question. The development and affordability of high-throughput RNA sequencing protocols and the growing use of eQTL studies have already started to open the door to unprecedented cross-species transcriptome analyses and comparisons (Necsulea and Kaessmann, 2014; Albert and Kruglyak, 2015). Such cross-species analyses will shed further light into the genetic basis of regulatory variation in genome evolution and ultimately link it to phenotypic, developmental, and adaptive evolution.

Gene/Genome Duplications

In principle, there are alternative ways to avoid the negative consequences of pleiotropy, besides changing the regulation of genes. One possibility is gene duplication followed by divergence of one of the duplicated copies. The idea that gene or genome duplication can be important in evolution and speciation can be found throughout the scientific literature of the last century (e.g., Tischler, 1915; Haldane, 1932; Müntzing, 1936; Bridges, 1936; Goldschmit, 1940) but gained the most popularity after Ohno's well formulated theory of duplication in evolution (Ohno, 1967, 1970). His argument circled around the notion that when a gene is duplicated, its duplicated copy is free to act as a 'play' copy potentially acquiring new functions while the ancestral role is preserved by the original gene-copy. However, the role of gene and genome duplication in developmental evolution has been somewhat neglected since Ohno. This is probably due to the technical challenge of assembling overly repetitive sequences. Therefore recently duplicated genes remain often undetected. However, despite the limited number of examples, there is no doubt that gene duplication is a fundamentally important mechanism in genomic evolution. For instance, most crop plants are polyploid, which contribute to an increase in robustness and crop yield (Renny-Byfield and Wendel, 2014). There are also plenty of other examples in domestication, one of them being the loss of scales in the common carp, a trait that makes it such an easy-to-prepare fish in the kitchen, which is a result of a duplicated gene selected for by breeders (Rohner *et al.*, 2009) (**Figure 2**). In addition to domestication, gene duplications have generated entire gene families such as olfactory receptors (Kratz *et al.*, 2002), opsins enabling color vision (Dulai *et al.*, 1999), or Hox genes (Brooke *et al.*, 1998) as only some prominent examples. After all, vertebrates have undergone two, and in the case of most bony fishes even three, whole genome duplications with far-reaching consequences (Taylor *et al.*, 2003). It is still a theory, but one which is gaining popularity, that the diversity in fishes, which are the most diverse vertebrate group, is linked to the fish-specific whole genome duplication (Glasauer and Neuhauss, 2014). In at least Cichlids there is evidence that the species richness of this family is partly due to duplicated genes (Brawand *et al.*, 2014). Another argument for duplications being important in evolution comes from studies that found frequent positive selection for amino acid changes within duplicated genes (Heger and Ponting, 2007; Birtle *et al.*, 2005). In human populations many genes are observed to be present in varying number of copies in different individuals, the so-called CNV (copy number variants). A recent study found that up to 15% of human genes vary in their copy number (Redon *et al.*, 2006). Over a broader time frame, nearly every gene can be considered to be a duplicate of earlier genes or some parts of the genome, so it is fair to say that the emergence of new genes plays an integral role in genome and developmental evolution.

Figure 2 *Cyprinus carpio*, the common carp has been bred as a food fish for millennia in China and Europe, mainly due to its robustness and relatively fast growth. One domesticated trait which makes it particularly popular in modern times is its reduction in scalation. The almost complete absence of body scales allows for an easy preparation in the kitchen as depicted in this franconian specialty version, the so-called mirror carp.

Origins of New Genes, Pseudogenes

Duplication and divergence is a major source of new genes. However, gene duplication is not the only way of generating new genes. They can emerge through a variety of different mechanisms from gene fusion over retrotransposition to originating *de novo* from noncoding regions of the genome (for a more exhaustive overview see Chen *et al.*, 2013; Kaessmann, 2010; Long *et al.*, 2003). There is no doubt that new genes have to be important in evolution. However, the extent to which this is the case may not be fully appreciated; for example, 21% of human protein coding genes have no known homologs in mice (Hoekstra and Coyne, 2007). Even compared to our closest living relative, the chimpanzee, 6% of the genes in one species have no known homologue in the other (Demuth *et al.*, 2006). One problem is that the functions of lineage-specific genes will often remain mysterious because no orthologues are available for study in experimentally tractable organisms. Fortunately there are some notable examples where novel genes were studied and were shown to play an important role in helping animals adapt to new environments. In Antarctic fish, antifreeze proteins have emerged *de novo* from ancestral noncoding DNA (Chen, 1997a) enabling survival in frozen environments. Remarkably, almost identical proteins have evolved independently in Artic fish as well, interestingly from entirely different genomic regions (Chen, 1997b) providing a striking case of convergent evolution. Another example, not completely *de novo*, but due to a transposition of an existing gene (fgf4) generated a variant of the gene in the dog genome. This variant, which was selected for by breeders, is the underlying genetic cause of the cute stubby legs of the dachshund and probably other short legged dog breeds as well (Parker *et al.*, 2009). These are just some of many other examples where recent evidence suggests that new genes have contributed to phenotypic diversity (for a more comprehensive list see Chen *et al.* (2013), Kaessmann (2010), and Long *et al.* (2003)). Even relics of genes, originally thought to be nonfunctional and therefore named pseudogenes, can still have effects on the regulation of other genes (Sasidharan and Gerstein, 2008). For example, in mouse oocytes several small RNAs controlling gene expression (so-called siRNAs) are derived from pseudogenes (Tam *et al.*, 2008).

Genome Reduction, Gene Loss (Compact Genomes), Deletions

However, not only is the gain of genes or genomic regions important, sometimes their loss can be as well. One famous example of gene loss in mammalian evolution is the loss of ancient egg-yolk genes in non-egg laying mammals (Brawand *et al.*, 2008). In terrestrial animals many odorant or pheromone genes are particularly transient (Ponting, 2008). Immunity genes are another case of frequently gained or lost genes (Goodstadt *et al.*, 2007). Overall, sequence deletion rates in mammals are high; in fact a quarter of the human genome has been lost since its split from the common ancestor with rodents (Mouse Genome Sequencing Consortium, 2002). But even within shorter evolutionary distances, deletions seem to have had an important impact on human evolution; a recent study found the genetic basis for the human-specific loss of penile spines (keratinized structures on the male sexual organ) compared to great apes and other mammals to be the consequence of a genomic deletion near the gene for the androgen receptor (McLean *et al.*, 2011). Evidently, genomic deletions can even make us more gentle lovers.

Horizontal Gene Transfer

Horizontal gene transfer also known as lateral gene transfer is the process by which an organism incorporates genetic material from another organism without mating. Horizontal gene transfer is traditionally believed to be restricted to bacteria and unicellular eukaryotes; however, recent work challenges this dogma and argues for a potentially broader significance of lateral gene transfer in animal evolution and development, including humans (Ivancevic *et al.*, 2013; Schönknecht *et al.*, 2014; Crisp *et al.*, 2015). For example, it has been argued that our ABO blood group gene has been horizontally transferred from bacteria (Crisp *et al.*, 2015).

Evolution of Sex Chromosomes

Sex chromosomes underlie strong selective pressures in evolution and as a consequence exhibit many unusual patterns in sequence and gene expression relative to autosomes (Vicoso and Charlesworth, 2006). Sex determining mechanisms have evolved independently several times and differ drastically between different clades, families, and even species (for a review on sex determination see Bachtrog *et al.*, 2014). In species with heterogametic sex chromosomes, such as mammals or birds, the lack of recombination between the two nonidentical chromosomes can lead to substantial genomic and structural changes. This is particular bad news for all men on this planet as it has been argued that the male determining human Y chromosome will vanish from the earth surface in the future (Wilson and Makova, 2009).

Open Questions

Despite substantial interest in genome evolution and a rising number of available genome sequences many substantial questions remain. This last paragraph will briefly mention some open questions in genome evolution and shortly reference the areas addressing them. Such brevity does not make the immense investigation justified and each of these topics could have easily filled a chapter of their own.

A still controversial question in the field is the extent in which genome size is contributing to organismal complexity. As the numbers of genes do not correlate well with organismal complexity in animals and lie only within a relatively narrow range from 13 100 genes in the mosquito to 23 300 genes in the sea urchin (Ponting, 2008), other factors must be more important. Evidently related to this observation is the question about the potential role of so-called 'junk' DNA, areas in the genome where we have not been able to attribute any obvious meaning. Another important area of inquiry deals with the

question of the role of population sizes and associated random genetic drift (Lynch and Conery, 2003) on genomic evolution. Further topics of rising interest are the role of micro RNAs (Berezikov, 2011) and long-noncoding RNAs in developmental evolution. Equally interesting are questions probing the importance of epigenetic effects, epistasis, and gene networks. Last but perhaps most fundamental is the question of how genomes evolved from RNA precursors in the first place (Goldman and Landweber, 2012). These are a few of many open questions and plethora of opportunities for future generations of genome and evolutionary studies.

Finally it is important to put forth the claim that genome-wide studies are fundamentally important and it is hoped the interest in whole genome sequencing will not cease. Fortunately, support for sequencing genomes remains high. As evidence, a particularly ambitious and valuable approach currently underway is the genome 10K project with the aim of sequencing 10 000 vertebrate genomes (Koepfli and Paten, 2015). However, one caveat of many genome-wide studies, which is hoped to be resolved soon, is that most genome-wide studies are conducted in the absence of phenotypic information. This deficiency limits their informative value about mutations and alterations in the genome which truly affect developmental evolution.

See also: Genome Organization, Evolution of. Regulatory and Coding Changes in Developmental Evolution, Roles of

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Genome Organization, Evolution of

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Glossary

Conserved linkage Genes that are on the same chromosome and in the same order in two or more species.
Deletion The loss of <10 kb of DNA from a chromosomal location.
Duplication The gain of <10 kb DNA at a chromosomal location either from DNA duplication or through reverse transcription of mRNA sequences.
Inversion A chromosomal rearrangement that reverses the order of genes through the introduction of two double-stranded breaks.
Karyotype Organization of the genome into chromosomes.
Neofunctionalization Result of a gene duplication where one copy retains the original function and the second copy has gained a new function.
Nonfunctionalization Result of a gene duplication where one copy retains the original function and the second copy becomes nonfunctional.
Ploidy The haploid number of chromosomes in the genome.
Polyploid A species with more than the haploid number of chromosomes.
Pseudogene A copy of a duplicate gene that has lost function.
Segmental duplication The gain of >10 kb of DNA at a chromosomal location that is 95% similar in sequence to other regions in the genome.
Specialization Result of a gene duplication where both copies acquire new functions that are distinct from the original gene.
Subfunctionalization Two genes that resulted from a gene duplication where each duplicate has acquired a unique part of the original single gene's function such that both genes are indispensable.
Synteny Refers to genes found on the same chromosome in two or more species.
Translocation Reorganization of genes that moves one or more genes from one location to another location on the same chromosome or different chromosomes.

Introduction

The genomics era has opened the door to detailed studies of how genes are organized in the genome and will allow tests of hypotheses about the mechanisms that generate and maintain karyotypic variation within and between species. A naïve view might be that genes are clustered according to shared function or how they are regulated (Hurst *et al.*, 2004). The HOX clusters are a well-documented example of a nonrandomly arrayed set of genes. Without detailed knowledge of what genes are in a species' genome and what the functions of the genes are, it is difficult to evaluate whether nonrandom clusters of functionally related genes are the rule or the exception. Adjacent genes that are divergently transcribed can have similar expression profiles given shared *cis* regulatory sequences unless insulator elements are recruited into the intergene region to prevent coordinate regulation (Yang *et al.*, 2012). Rearrangements may provide a second mechanism to prevent maladaptive expression of adjacent genes.

Genomic rearrangements can restructure the genome through deletions, duplications, inversions, or translocations (Coghlan *et al.*, 2005). A species karyotype can be modified by the fission and fusion of chromosome arms. Species can also duplicate all of the genetic information in their genomes either through hybridization with different species or spontaneous duplications of their own genomes. Not only can these changes be observed in natural populations of many organisms, but in humans, they are often associated with genetic diseases (Pettenati *et al.*, 1995; Shaffer and Lupski, 2000). A study of a million human conceptions found that 7% of spontaneous abortions result from some form of genome rearrangement or ploidy change (Sankaranarayanan, 1979). Until recent advances in genomics, it has been unclear how often rearrangements occur in human populations, but do not cause disease (Stefansson *et al.*, 2005).

Until recently, the study of genome structure was largely descriptive, but with the availability of complete genomes we are now beginning to be able to test evolutionary hypotheses about how these rearrangements originate and how they are established in populations. The effect that rearrangements have on chromosomes is that they act as modifiers of recombination either in a positive or negative way. It has long been known that genome rearrangements can have profound effects on the transmission of chromosomal variants in heterozygotes (Sturtevant and Beadle, 1936). For instance, crossing over between homologous chromosomes heterozygotes for chromosomal inversions or translocations leads to the formation of recombinant gametes with deletions and duplications that will produce inviable offspring. This reduction in the transmission of recombinant gametes can lock particular combinations of genes together. Processes that lead to increases in chromosome numbers may lead to a species better able to adapt to a heterogeneous environment because the population is better able shuffle the contents of their genomes via independent assortment of chromosomes (Otto and Barton, 2001). This article will discuss the structure of genomes, the types of chromosomal rearrangements, and how chromosomal rearrangements may influence the evolutionary process.

Structural Organization of Genomes

Genomes of viruses to eukaryotes are organized or packaged to facilitate transmission of genomes from one generation to the next. The length of most organismal genomes is larger than the particle or cell that contains it. Viral genomes are quite diverse based on the type of nucleic acid-based genome and the proteins that generate the virus particle (Abrescia *et al.*, 2012; Baltimore, 1971). Viruses can have either DNA or RNA genomes that are either linear or circular. The sizes of viral genomes can be constrained by the protein particle that houses the genome (Feiss *et al.*, 1977). In many cases, genes that comprise viral genomes overlap reducing the amount of nucleic acid necessary to encode essential functions. Some viral genomes are segmented into independent pieces of nucleic acids, which can allow for diversification as is seen in the influenza viruses (Pleschka, 2013).

Bacterial genomes are circular DNA-based genomes. The genomes are packaged through supercoiling of DNA and short single-stranded RNA molecules (Pettijohn and Hecht, 1974). Factors such as DNA replication, nucleoid structure, and gene function play a role in how genes are nonrandomly organized in bacterial genomes (Rocha, 2008). Bacterial genomes are replicated from a single origin of replication and genes are encoded on both strands of DNA, but highly expressed genes tend to be near the origin of replication (Rocha, 2008). Genes are arrayed in operons with one or more genes being expressed from a single promoter. Operons lead to the coordinate expression of genes with related functions.

Eukaryotic genomes are organized into linear chromosomes (**Figure 1**). Chromosomes are units of segregation insuring that the genetic material is transmitted accurately each generation. Each chromosome has three fundamental parts a telomere, a centromere, and one or more origins of replication (Tyler-Smith and Willard, 1993). The telomere is the end or cap of the chromosome. Because DNA replicates in 5′ to 3′ direction, the ends of chromosomes would tend to get shorter with each cell division because DNA synthesis on the lagging strand is initiated away from the chromosome end. Telomerase is an enzyme that creates a special sequences at the ends of chromosomes that prevents the loss of DNA from the ends of chromosomes (Zakian, 1996). Centromeres are the regions where the spindle apparatus attaches to chromosomes during cell division and positions the chromosomes at the middle of the cell prior to segregation (Nicklas and Koch, 1969). Two types of chromosomes are found in the plant and animals, monocentric and holocentric. Monocentric chromosomes have a single centralized region that attaches to the spindle fibers while holocentric chromosomes have attachment points for spindle fibers along the entire length of the chromosome. The consequences of genome rearrangements in monocentric and holocentric species are quite different. In monocentric species, crossing over in an inversion heterozygote leads to the formation of acentric and dicentric chromosomes and the formation of unbalanced gametes (Sturtevant and Beadle, 1936). In holocentric species, segregation of chromosomes is unaffected by rearrangements because the entire length of the chromosome is engaged with the spindle apparatus (Dernburg, 2001; Maddox *et al.*, 2004; Melters *et al.*, 2012).

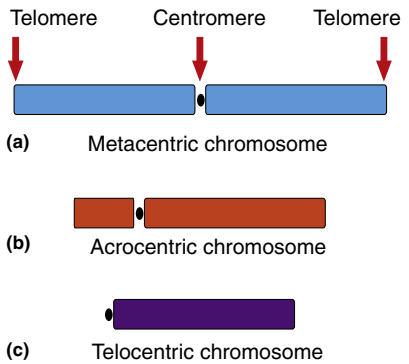

Figure 1 Basic chromosome structures showing the centromere and telomere. The types of chromosome are defined by the location of the centromere. (a) Metacentric chromosome with the centromere in the middle of the chromosome. (b) Acrocentric chromosome with the centromere offset from the middle of the chromosome. (c) Telocentric chromosome with the centromere at the end of the chromosome.

DNA in eukaryotic chromosomes is packaged by the histone proteins that make up the nucleosome particles (Hayes and Hansen, 2001). The DNA associated with the nucleosomes is known as chromatin, which can be found in three different states. Euchromatin occurs in DNA that is always transcriptionally active. Facultative heterochromatin goes between a transcriptionally active and inactive state. Constitutive heterochromatin is always in the transcriptionally inactive state. Giesma stain when applied to chromatin stains heterochromatic regions of DNA allowing one to map transcriptionally inactive state particularly in *Drosophila* polytene chromosomes (Sorsa, 1988a,b). Chromosomes are arranged nonrandomly in the nucleus based on recent data from chromatin conformation mapping, which determine what sequences are in close proximity to one another (Lieberman-Aiden *et al.*, 2009).

Genome Rearrangements

Deletions

Deletions result from the loss of DNA from a chromosome (**Figure 2**). The fitness cost of deletion mutations is not clear. The rapid deletion of DNA associated with recent transposable element insertions suggests that the loss of DNA is not deleterious (Petrov *et al.*, 1996; Petrov and Hartl, 1998). This could suggest that the initial DNA insertion was deleterious such that deletion mutations are favored. On the other hand, analysis of deletions in homologous sequences of DNA suggests that deletions may be deleterious relative to insertions (Pritchard and Schaeffer, 1997; Schaeffer, 2002). It is thought that interstitial deletions from the central regions of chromosomes are more likely to occur than terminal deletions because of the deleterious consequences of shortening the telomeric sequences (Armanios, 2009; Swanson *et al.*, 1981).

Duplications

Duplications result from the gain of DNA in a chromosome resulting from unequal crossing over during meiosis (Takahashi *et al.*, 1982; **Figure 3**). Population differences in

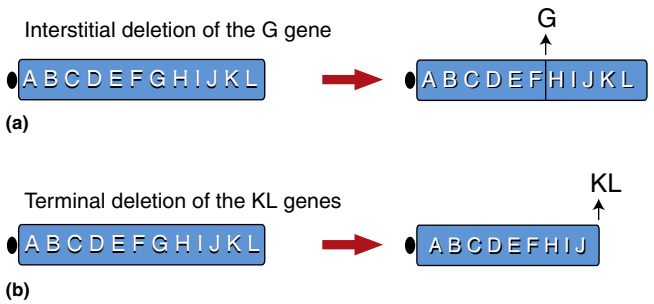

Figure 2 Chromosomal deletions. (a) Interstitial deletion that loses the G gene from the central part of the chromosome. (b) Terminal deletion that removes the KL genes from the telomere of the chromosome.

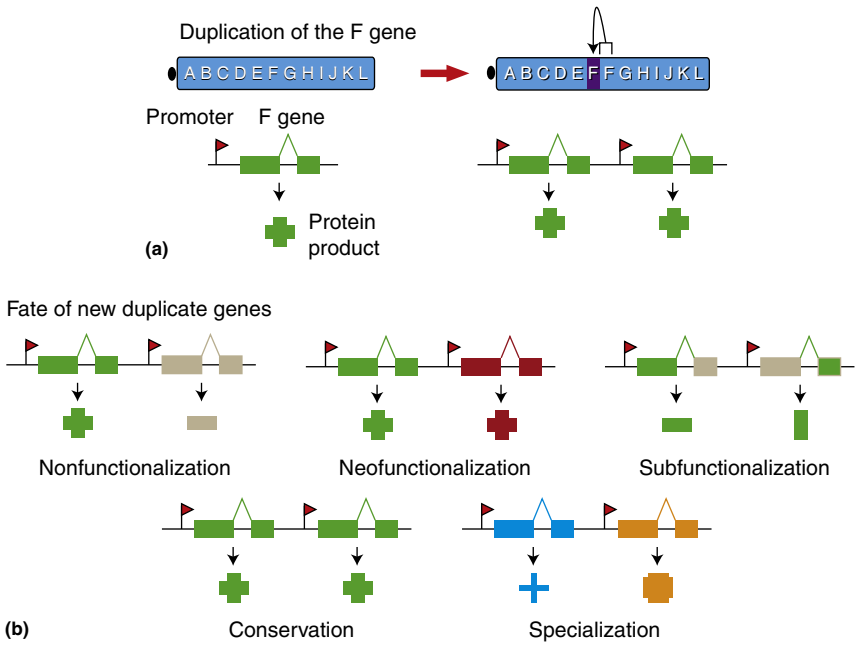

Figure 3 Chromosomal duplication. (a) The chromosome gains a second copy of the F gene. Below the chromosome is a depiction of the F gene duplication at the DNA level including its encoded product where the shape and color symbolizes the confirmation of the protein. (b) The five possible fates of the duplicate genes showing nonfunctionalization, neofunctionalization, subfunctionalization, conservation, and specialization, which are explained in the text.

deletions and duplications are also called copy number variants or CNVs that are of interest because of their potential effects on gene expression, phenotype, and disease (Girirajan *et al.*, 2011; Iskow *et al.*, 2012; Paudel *et al.*, 2013; Völker *et al.*, 2010). The duplication of genetic information especially that of complete protein coding genes has been proposed as a mechanism to generate genes of new functions (Ohno, 1970). There are five possible fates for recently duplicated genes (Assis and Bachtrog, 2013; Lynch and Conery, 2000; **Figure 3**). Nonfunctionalization occurs when one of the copies retains the original function and the second copy loses its function becoming a pseudogene. In this case, selection may favor the loss of one gene if the extra gene dosage is deleterious. Neofunctionalization occurs when one copy retains its original function and the second copy evolves a new function. Subfunctionalization occurs when each gene copy loses a different part of the original gene's function such that both genes are retained for the ancestral gene function. Conservation occurs when both copies of the duplication maintain the original protein function. Selection may favor this type of duplication because the additional gene may provide increased dosage of the protein. Specialization occurs when both copies of the duplication each evolve new functions. Good examples of gene families that resulted from gene duplications are the globin genes in vertebrates and the immunoglobulins of the vertebrate immune system (Efstratiadis *et al.*, 1980; Takahashi *et al.*, 1982).

Gene duplications can also be generated through reverse transcription of messenger RNAs and integration of the complimentary copy of the mRNA back into the genome (Meisel, 2009a,b). For the gene to be functional, the integrated gene must insert near a *cis* regulatory sequence that is capable of transcribing the inserted DNA. Recent data from ribosomal profiling suggests that much of the genome is transcribed at a

low level, which may provide a starting point for selection to act on a newly inserted gene if it is transcribed and translated at a modest level (Carvunis *et al.*, 2012). The integration of reverse transcribed messenger RNAs near preexisting genes can create chimeric genes that can quickly create gene transcripts with new functions for selection to act upon (Long and Langley, 1993; Ponce and Hartl, 2006; Rogers and Hartl, 2012).

Segmental duplications are larger increases of DNA in the genome that are 95% similar to its ancestral copy and comprise 5% of the genome (Eichler, 2001; Girirajan *et al.*, 2011). Segmental duplications were discovered as genomes were assembled from larger cloned sequences such as BAC clones. The use of next-generation short-read technologies has made the detection of segmental duplications difficult because the smaller reads do not assemble well through duplications that are larger than the size of the sequence reads (Alkan *et al.*, 2011; Salzberg *et al.*, 2012). Segmental duplications in mammals are distributed throughout the genome, but tend to be located near centromeres and telomeres (Eichler, 2001). Within chromosomes, these duplications result from unequal crossing over between repeated sequences that flank large segments of unique sequence. Duplications can also occur between chromosomes occur via duplicative transposition. Segmental duplications may have played a role in adaptive evolution in some species (Joshi and Nayak, 2013; Samonte and Eichler, 2002).

Finally, the whole genome can be duplicated. Whole genome duplication is common in plant species and has accompanied the domestication of many crop plants (Coghlan *et al.*, 2005; Renny-Byfield and Wendel, 2014). Evidence for whole genome duplications in animals and fungi is increasing as more complete genomes become available (Albertin and Marullo, 2012; Sémon and Wolfe, 2007).

Inversions

Inversions are generated when two double-stranded breaks are introduced into the chromosome and are rejoined such that the gene order of sequence between the breakpoints is reversed (**Figure 4**). Chromosomal inversions were discovered in *Drosophila melanogaster* when a strain had no cross overs between two loosely linked loci in mapping crosses (Sturtevant, 1917). Sturtevant suggested that this strain carried a 'C-factor' that reduced crossing over. Comparisons of gene order between *D. melanogaster* and *Drosophila simulans* suggested that the C-factors resulted from an inversion that flipped the order of genes (Sturtevant, 1921). Comparison of genetic maps within species suggested that 'C-factors' result from inverted gene orders (Sturtevant, 1926). The examination of polytene chromosomes from larval salivary glands (Painter, 1934) cytologically confirmed that C-factors were due to the reversal of gene order. Cytogenetic examination of a variety of insects has shown a wealth of inversion polymorphism within species (Dobzhansky and Sturtevant, 1938; Krzywinski and Besansky, 2003; Sperlich and Pfriem, 1986).

Two models have been suggested for how inversions are generated in populations based on the study of pairs of inversion breakpoints, nonallelic homologous recombination and staggered cut model (Cáceres *et al.*, 1999; Mathiopoulos and Lanzaro, 1995; Mathiopoulos *et al.*, 1998, 1999; Matzkin *et al.*, 2005; Ranz *et al.*, 2007; Richards *et al.*, 2005).

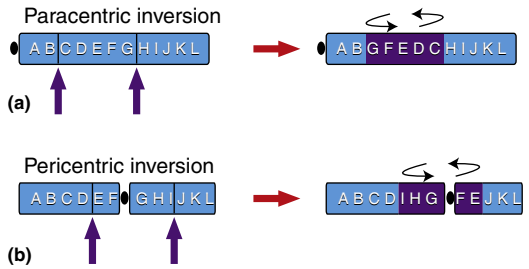

Figure 4 Chromosomal inversions. (a) Paracentric inversion that reverses the order of the CDEFG genes between the two double-stranded DNA breaks (purple arrows). A paracentric inversion does not include the centromere. (b) Pericentric inversion that reverses the order of the EFGHI genes between the two double-stranded DNA breaks (purple arrows). A pericentric inversion includes the centromere.

Several lines of indirect evidence suggest that inversions are selected in natural populations. First, gene arrangements form clines or gradients in frequency in natural populations that in some cases form in parallel in the northern and southern hemispheres (Cheng *et al.*, 2012; Dobzhansky, 1944; Hoffmann *et al.*, 2004; Hoffmann and Weeks, 2007; Kennington and Hoffmann, 2013; Kennington *et al.*, 2006, 2007). Second, arrangements cycle seasonally and form altitudinal clines (Dobzhansky, 1943, 1948). Third, gene arrangement frequencies reach stable equilibria when reared in population cages (Wright and Dobzhansky, 1946).

There are two types of inversion, paracentric and pericentric, with the difference being whether the centromere is involved in the rearrangement. A pericentric inversion includes the centromere in the inverted segment, while a paracentric inversion does not. Paracentric inversions are common in Dipterans, while pericentric inversions are rare typically being observed between species (Lemeunier and Ashburner, 1976; Sperlich and Pfriem, 1986). The situation in mammals is the opposite where paracentric inversions are rare and pericentric inversions are more common (for a review see Dobigny *et al.*, 2015).

Translocations

Translocations involve the movement of genes to new locations within a chromosome or to different chromosomes (**Figure 5**). Robertsonian translocations are a special case where two acrocentric chromosomes recombine to form a metacentric chromosome (Slijepcevic, 1998; **Figure 5(c)**). Polymorphic translocations are not as frequent as chromosomal inversions in natural populations (Dobigny *et al.*, 2015), but have played an important role in the restructuring of chromosomes. Chimpanzees and gorillas have 24 pairs of chromosomes while humans have 23 pairs. Evidence suggests that the reduction in the number of human chromosomes was the result of a Robertsonian translocation caused by a telomeric fusion (IJdo *et al.*, 1991). In *Drosophila*, one tends to observe translocations as fixed differences between species, but there are some cases of polymorphic translocations (McAllister and Charlesworth,

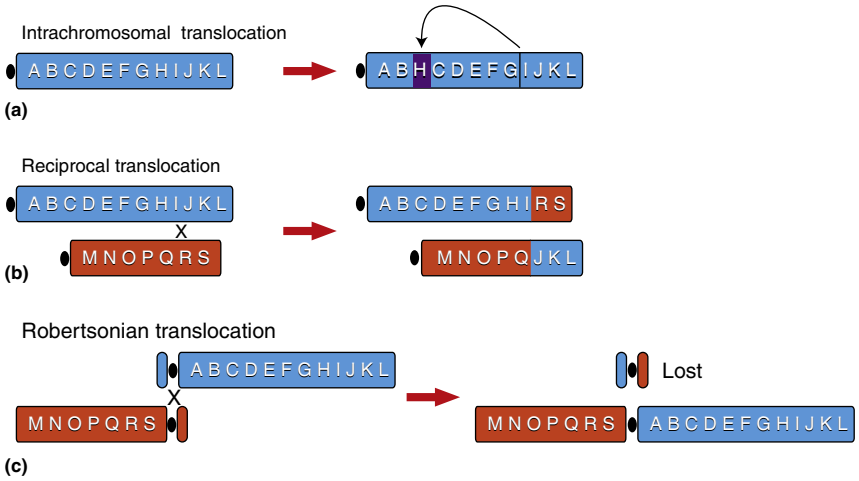

Figure 5 Chromosomal translocations. (a) Intrachromosomal translocation where the H gene moves to a new location within the chromosome. (b) Reciprocal translocation where the ends of two chromosomes are swapped. (c) Robertsonian translocation where exchange between the centromeres of two chromosomes generates a fusion product between two different chromosome arm.

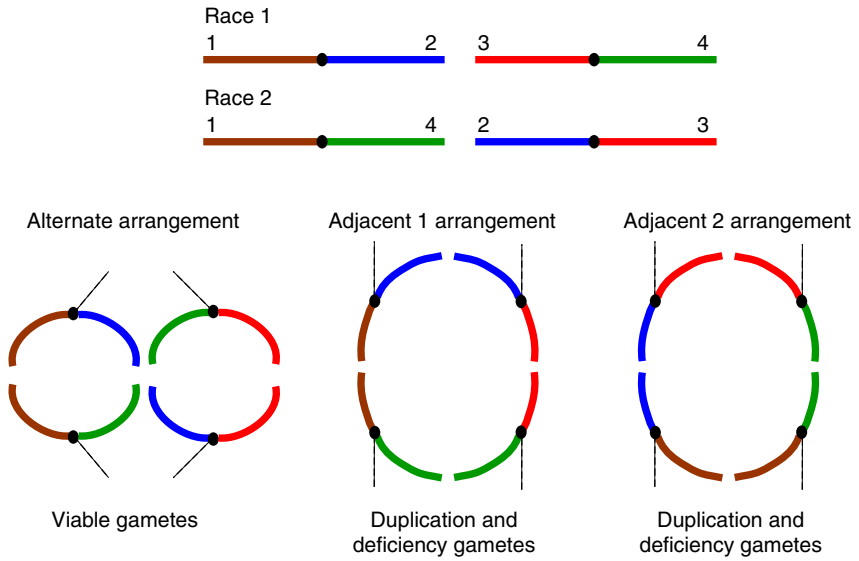

Figure 6 Segregation of chromosomes in a species hybrid that carries different chromosomal fusions.

1999; McAllister, 2002, 2003; McAllister and Evans, 2006; McAllister *et al.*, 2008). The plant genus *Oenothera* has a variety of species that differ in the chromosomal translocations that they carry. Homologous chromosomes in hybrids between *Oenothera* species form rings during meiosis based on the pairing of the translocated chromosomal arms (Darlington, 1929; Golczyk *et al.*, 2014; **Figure 6**). The rate of karyotypic evolution driven by translocation mutations can vary widely among closely related species (Carbone *et al.*, 2014).

Establishment of Rearrangements in Populations

There is no overall consensus for the evolutionary mechanisms responsible for the establishment of inversions in populations and this is an open area for research. Theoretical studies have established conditions for the invasion of new inversions into a population (Charlesworth and Charlesworth, 1973). Kirkpatrick and Barton (2006) summarized four major classes of hypotheses that can allow a new inversion to invade in a population. In the first class of models, the inversion mutation at either site of chromosomal breakpoints creates variation for selection to act on so called position effects. This could result from changes in the structure genes (Calvete *et al.*, 2012) or the alteration of gene expression of adjacent genes (Puig *et al.*, 2004). The second type of model involves the indirect effect due to recombination suppression. Reduced recombination within the inverted region can hold large combinations of alleles together in different chromosomal backgrounds either because the inversion captures epistatically interacting genes (Dobzhansky, 1950; Wallace, 1968), captures a chromosome relatively free of deleterious recessive alleles (Nei *et al.*, 1967;

Ohta and Kojima, 1968), or captures a set of genes involved in local adaptation (Guerrero and Kirkpatrick, 2014; Kirkpatrick and Barton, 2006). The pattern of linkage disequilibrium within inverted regions supports the indirect effect of recombination hypothesis because one sees long-range LD and short-range linkage equilibrium (Schaeffer *et al.*, 2003; Wallace *et al.*, 2013). The local adaptation model is supported in a numerical analysis of selection–migration balance is used to explain the differences in inversion frequencies among *Drosophila pseudoobscura* populations (Schaeffer, 2008). The third class of model assumes that the inversion captures a single positively selected gene that carries the gene arrangement to high frequency (Schaeffer and Aguadé, 2000). The final type of model is genetic drift, which suggests that the gene arrangement increases in frequency by neutral processes (Lande, 1984).

Rearrangements Effect on Nucleotide Variation in Populations

Once established in populations, rearrangements can shape the pattern and organization of nucleotide diversity along the chromosome (Navarro *et al.*, 1997, 2000). Chromosomal rearrangements lead to the suppression of recombination in heterozygotes, which acts as an isolating mechanism to restrict genetic exchange between chromosomes (Hasson and Eanes, 1996). Theoretical studies on how inversions restrict genetic exchange in heterozygotes has shown that the size of the paracentric inversion and its position relative to the centromere dictates divergence between inverted chromosomes (Navarro *et al.*, 1997). Divergence is expected to be greatest near inversion breakpoints (Navarro *et al.*, 2000). Data from inversions within and between species of *Drosophila* are largely consistent with these predictions (Andolfatto *et al.*, 1999; Andolfatto and Kreitman, 2000; Hasson and Eanes, 1996; Noor *et al.*, 2007; Wallace *et al.*, 2013).

Rearrangements as Contributors to the Speciation Process

Once established in populations, genome rearrangements can promote the formation of new species especially in cases where the dominant arrangement is fixed in different environments. White (1973) proposed that underdominance in rearrangement heterozygotes in species hybrids might serve as a reproductive barrier between species. The problem with this model is that experimental estimates of fitness sets do not show underdominance for inversion heterozygotes (Coyne *et al.*, 1991). The discovery of reproductive incompatibility genes in inverted regions of chromosomes led to a reevaluation of the role that rearrangements play in the speciation process (Noor *et al.*, 2001). Navarro and Barton (2003) suggested that rearrangements could isolate genomes through reduced genetic exchange rather than the traditional geographic isolation model. The emergence of a rearrangement frequency cline driven by local adaptation could provide fertile ground for the formation of new species. Different allelic combinations can be fixed within different rearrangement backgrounds, but are less likely to spread among the different chromosomal backgrounds because of suppressed recombination. If any of these fixed allelic differences include incompatibility genes that limit viability or fertility, then this process can lead to the initial phases of the formation of new biological species. *Drosophila* species have been identified based on differences in their karyotypes, which suggests that chromosomal rearrangements may have helped to drive the formation of new species throughout the genus *Drosophila* (Carson, 1992; Lemeunier and Ashburner, 1976; Wasserman, 1992).

Rearrangements and Sex Chromosome Evolution

Chromosomal rearrangements have played a role in the evolution of sex chromosomes (Charlesworth *et al.*, 2005). Sex chromosomes are thought to evolve from autosomal progenitors where one homologue acquires a sex determination gene. Later, the chromosome picks up a negative recombination modifiers in the form of DNA rearrangements that limit genetic exchange between the pair of homologues (Lahn and Page, 1999). In humans, reduced recombination prevents the movement of the sex determination factor between the two sex chromosomes limiting sex reversal mutations, i.e., XX males or XY females (Ferguson-Smith, 1966; Page *et al.*, 1987). In *Drosophila*, new sex chromosomes have evolved multiple times via chromosomal fusions (Bachtrog, 2013).

Computational Methods to Infer Phylogenies from Genome Rearrangements

Muller, Sturtevant, and their colleagues used comparative genetic mapping in *Drosophila* to show that genes tended to be conserved on the same chromosomal arms between different species, yet the gene order was different presumably because of chromosomal rearrangements (Muller, 1940; Sturtevant and Tan, 1937; Sturtevant and Novitski, 1941). Genes on the same chromosomal arm in different species are defined as being in synteny, while syntenic genes also found in the same order on the chromosome among different species are defined as being in conserved linkage or syntenic blocks (Ehrlich *et al.*, 1997; **Figure 7**). Comparisons of complete genomes among different species has confirmed that there is extensive synteny among species and that there has been extensive shuffling of gene order as well (Bhutkar *et al.*, 2008; Hillier *et al.*, 2007; Pevzner and Tesler, 2003a,b). The availability of complete genomes among different species has led to the development of computational approaches to infer the number of rearrangements between two genomes (Bergeron *et al.*, 2006; Durrett *et al.*, 2004; Moret *et al.*, 2001; Sankoff, 2003; Tang and Moret, 2003).

Summary and Future Prospects

Chromosomal rearrangements are just beginning to be appreciated as an important source of genetic diversity that can shape the evolution of genomes. Prior to the use of genomic technologies, the best systems for the study of chromosomal rearrangements were limited to species where cytogenetic techniques were sufficient to detect large-scale changes such as in *Drosophila* (Painter, 1934) and some mammalian systems

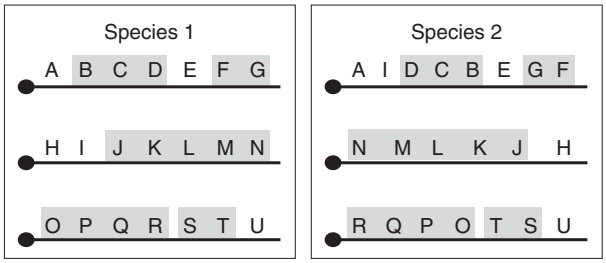

Figure 7 Synteny of genes in chromosomes between two different species. Two related species have genes found on three chromosomes. Each chromosome is indicated with a dot for the centromere and a line. The letters indicate genes for each chromosome. Genes A, B, C, D, E, F, and G are syntenic on the top chromosomes in the two species with two conserved linkage blocks (BCD and FG). Genes H, J, K, L, M, and N are syntenic on the middle chromosomes in the two species with the one conserved linkage block (JKLMN). Genes O, P, Q, R, S, and T are syntenic on the bottom chromosomes in the two species with the two conserved linkage blocks (OPQR and ST).

(for a review see Dobigny *et al.*, 2015). With the aid of genomic approaches, the extent of naturally occurring chromosomal rearrangements is becoming apparent raising new questions about how chromosomes evolve. Of particular interest is taxon specific differences in the rate of one type of rearrangement versus another. For instance, why are paracentric inversion so prevalent in *Drosophila* (Sperlich and Pfriem, 1986), while pericentric inversion more prevalent in mammals (Dobigny *et al.*, 2015). Perhaps the answer lies in differences in population structure. *Drosophila* populations tend to be large with extensive migration between multiple climatic niches which may favor inversions to prevent exchange between chromosomes that have been selected for a particular local environment (Kirkpatrick and Barton, 2006; Schaeffer, 2008). On the other hand, mammalian populations tend to be small, which may lead to the fixation of chromosomal rearrangements due to genetic drift (Dobigny *et al.*, 2015; Lande, 1984). Next-generation sequencing technologies offer the hope that chromosomal rearrangements can be detected in more diverse set of taxa that will help to generate a more general picture of how chromosomes evolve in natural populations.

See also: Gene Interactions in Evolution. Gene Origin, Sex Chromosomes and. Genome Size and Structure, Bacterial. Molecular Evolution, Functional Synthesis of. Mutation and Genome Evolution. Sensory Systems: Molecular Evolution in Vertebrates. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth

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Genome Plasticity, Bacterial

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Glossary

Conjugation Transfer of plasmid DNA from a donor to a recipient cell by direct cell-to-cell contact.
Genetic recombination A process by which a DNA molecule is broken and the fragments are rejoined in a different combination. By homologous recombination different DNA molecules with highly similar (homologous) nucleotide sequence are combined. In case of site-specific recombination, extensive nucleotide sequence homology is not required for recombination.
Horizontal gene transfer Transfer of genetic information between distinct members of a species and beyond species barriers that is independent of sexual reproduction.
Indels A class of mutation that includes insertions, deletions, or the combination of both.
Nucleoid The region within a bacterial cell comprising all or most of the cell's genetic material and DNA-binding proteins.
Pan-genome The pan-genome comprises all genes present in a species. The pan-genome includes (1) the core genome containing the genes, which are shared by all the species members and (2) the flexible genome containing genes present only in some strains.
Synteny Physical co-localization (linkage) of genes and gene clusters on the chromosome of different individuals.
Transduction Virus-mediated transfer of bacterial DNA from a bacterial donor to a bacterial recipient cell.
Transformation Uptake/transfer of free DNA into competent recipient cells.

Mechanisms and Constraints of Bacterial Genome Plasticity

There is large variation in size and content of bacterial genomes between different genera and species, but also among strains of the same species. Bacterial versatility can be directly correlated with genome size. For example, intracellular parasites, which live in constant and rich environments, have rather small genomes compared to environmental bacteria, which are often exposed to variable and changing growth conditions. The Darwinian theory of evolution (Darwin, 1859) relies on the generation of genetic diversity and natural selection. This holds also true for bacteria, and bacterial genome plasticity mirrors the mechanisms and constraints, which shape bacterial genomes and are thus involved in bacterial evolution.

When the chromosomes of related bacterial species are compared, it can be seen that the overall synteny is stable. A typical bacterial genome consists of a core gene pool containing the genes that encode essential cellular functions, and a flexible gene pool encompassing genes that are advantageous under certain environmental conditions. The core genome represents the stable genomic backbone and defines the basic metabolic capacities of a species. In related species it is largely co-linear with only few rearrangements detectable. In contrast, the flexible genome is responsible for the phenotypic diversity within a bacterial species and for its adaptability (Medini *et al.*, 2005; Segerman, 2012). Components of the flexible genome often exhibit marked nucleotide sequence variability and are frequently located on mobile genetic elements (MGEs) or in variable chromosomal regions. They can be horizontally transferred by transformation, transduction, or conjugation (see **Table 1**; for review see Darmon and Leach (2014)).

Table 1 Properties of mobile genetic elements

Type of element	*Example*	*Autonomous replication*	*Autonomous transfer*	*Mobilization*	*Integration*	*Excision*
Large plasmid	RP4	+	+	+	+/−	+/−
Small plasmid	RSF1010	+	−	+/−	(−)	(−)
Prophage	λ	−[a]	(+)	+	+	+
Conjugative transposon[b]	SXT	−	+	+	+	+
Integron, super-integron	In1	−	−	−	+	+
PAIs/GEIs	PAI II_{536}	−	−	(+)	(+)	+
Transposon	Tn*10*	−	−	−	+	+
Insertion sequence element	IS*1*	−	−	−	+	+

[a]Most prophages are integrated into the chromosome with the exception of few phages (e.g., P1) that are maintained as extrachromosomal replicons.
[b]including elements designated as integrative and conjugative elements or conjugative genomic islands.

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Successful horizontal gene transfer (HGT) is characterized by the transfer of genetic material from a donor to a recipient by the stable incorporation into the recipient's genome and its successful expression by the recipient. The acquisition and exchange of mobile and accessory genetic elements by HGT is one of the driving forces of bacterial genome plasticity and evolution (**Figure 1;** Ochman and Jones (2000)).

Acquisition of foreign genetic information is, of course, not unlimited. There are several limitations, which affect the uptake and successful incorporation of MGEs (see **Table 2**). Obviously, strategies exist, which distinguish 'foreign' from 'own' DNA or which recognize closely related MGEs. One well-known defense mechanism of incoming foreign DNA is restriction-modification (R–M). By degrading incoming foreign genetic information, R–M systems help to limit the integration of invasive DNA elements (Vasu and Nagaraja, 2013). Similarly, the 'clustered regularly interspaced short palindromic repeats' (CRISPRs) help the host cell to identify previously encountered bacteriophage or plasmids and mediate their degradation (Sorek *et al.*, 2013; Westra *et al.*, 2014). Also the coexistence of closely related plasmids within a host cell is controlled on the basis of their incompatibility (*inc*) systems, which affect plasmid replication. The stability of MGEs can be controlled by MGE-encoded R–M systems and so-called toxin–antitoxin (TA) systems. R–M as well as TA systems prevent the loss of extrachromosomal plasmids and in case of R–M systems also of some chromosomally inserted MGEs due to postsegregational killing of daughter cells, which lost these

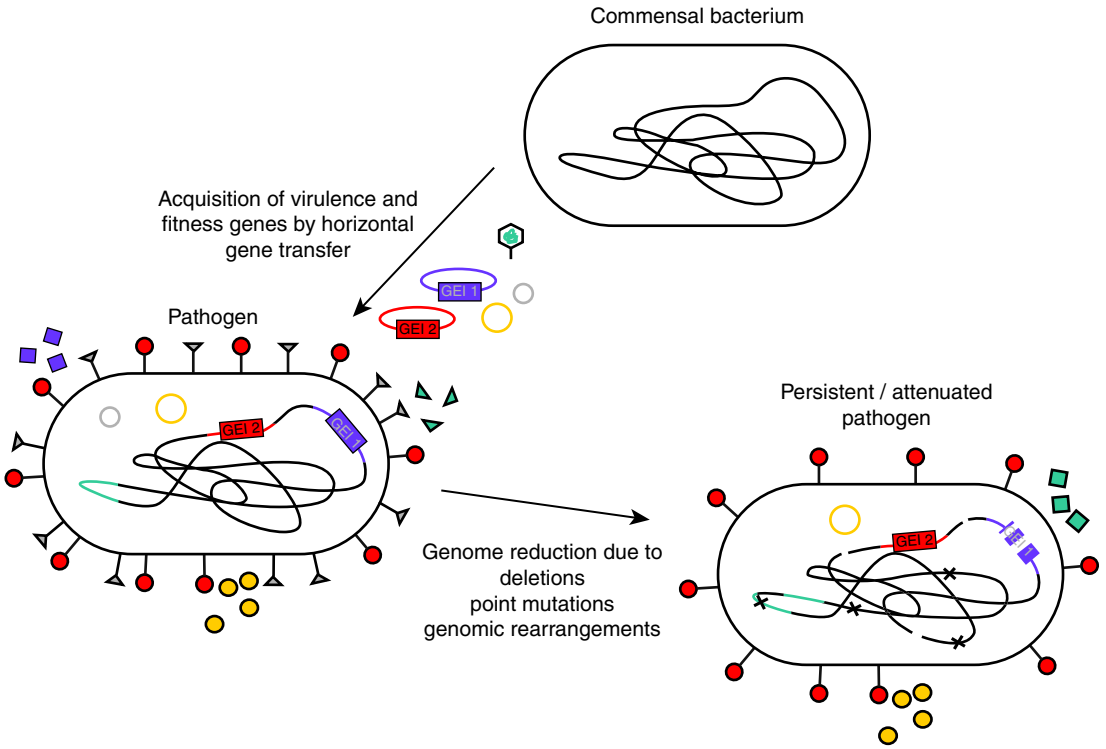

Figure 1 Influence of genome plasticity on bacterial evolution. Horizontal gene transfer by means of transformation, transduction, and conjugation plays an important role during bacterial evolution, for example for the evolution from a commensal to a pathogenic or attenuated bacterium. Commensal bacteria acquire mobile genetic elements such as plasmids, bacteriophages, and genomic islands that can encode fitness and virulence factors and render the strain pathogenic. During further adaptation to the host, gene deletions, point mutations, and genetic rearrangements might occur resulting in altered gene expression and possible loss of certain virulence traits. Those pathogens can evolve into attenuated strains that are able to persist in the host. GEI: genomic island.

Table 2 Limitations of genome plasticity

Trait	*Effect*
Niche restrictions	Availability of foreign DNA or interaction partners for HGT
Availability of phage receptors	Exclusion/limitation of bacteriophage infection
Restriction/modification (R–M) systems	Exclusion/limitation of DNA uptake, genome stability, improved recombination efficiency
CRISPR	Exclusion/limitation of DNA uptake
Plasmid incompatibility	Exclusion/limitation of plasmid uptake
Toxin/antitoxin (TA) systems	Genome stability, maintenance of plasmids
Nucleotide sequence homology	Recombination efficiency
Toxic or lethal mutations	Genome fidelity, gene functionality

elements (for reviews see Unterholzner *et al.* (2013) and Vasu and Nagaraja (2013)). Furthermore, the acquisition and exchange of foreign DNA can be limited by the presence/absence of surface receptors required for bacteriophage infection, by the general availability of foreign DNA (e.g., microorganisms in isolated niches have rarely access to foreign DNA), and by missing nucleotide sequence homology required for recombination and MGE integration into the host genome.

Besides HGT, point mutations and DNA rearrangements contribute to constant evolution of bacterial genomes (**Figure 1**). The accumulation of point mutations and indels can result in gene diversification as well as in gene inactivation. DNA rearrangements may cause relocation or deletion of genomic regions as well as gene duplication. Duplicated genes can then be further shaped by divergent evolution. Also the loss of genetic information contributes to genome plasticity. Non-functional or deleterious genes are frequently lost from bacterial genomes. The inactivation or deletion of genes, which are no longer required, has been observed especially during adaptation to physiologically stable environments (Lawrence, 1999; Maurelli *et al.*, 1998), or as one important aspect of within-host evolution and attenuation of bacterial pathogens causing persistent or chronic infection. *Escherichia coli* (*E. coli*) isolates from asymptomatic bacteriuria often exhibit smaller genome sizes than closely related commensals or uropathogens, because many virulence genes have been inactivated or lost due to the above-mentioned mechanisms (Lawrence, 1999; Maurelli *et al.*, 1998; Roos *et al.*, 2006; Zdziarski *et al.*, 2010; Zdziarski *et al.*, 2008). Another example of such within-host evolution of uropathogenic *E. coli* has been reported for consecutive *E. coli* isolates from recurrent bacteraemia cases (Olesen *et al.*, 1998). Similarly, attenuation and inactivation of virulence-associated genes during persistent asymptomatic infection occurred in *Burkholderia pseudomallei* as well as in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients (Marvig *et al.*, 2015; Price *et al.*, 2013). It is tempting to speculate that *in vivo* growth may be accompanied with increased genome plasticity as a result of increased mutation rates and/or efficient selection for attenuated or best adapted variants.

The variable bacterial genome organization and content mainly originates from transposition and homologous recombination events. For this, repeated and redundant DNA sequences play an important role, because they allow for homologous recombination. Most of the genomic variation can be attributed to insertions or deletions in so-called chromosomal hotspots (Tenaillon *et al.*, 2010). However, the organization and the function of bacterial chromosomes are strongly interconnected with cellular processes such as replication, segregation, transcription, and translation. Furthermore, the insertion or deletion of DNA sequences can disrupt operon structures or regulatory networks and should therefore only be favorable at genomic sites, which are not restricted by such structural or organizational constraints. It has been suggested that chromosomal integration hotspots may result from the insertion of larger DNA stretches into permissive chromosomal regions, thereby supporting future recombination events and additional HGT in this less perfectly adapted region (Touchon *et al.*, 2009b). Whereas point mutations, genome rearrangements and deletions are stochastic processes contributing to relatively slowly occurring evolutionary processes (Ahmed *et al.*, 2008; Dobrindt *et al.*, 2004) (**Figure 2**), HGT allows for faster changes of the genomic content, thus contributing to significantly accelerated evolution, the so-called microevolution.

The Mosaic Continuum of Mobile and Accessory Genetic Elements as a Toolbox of Genome Plasticity

During the last years, the wealth of prokaryotic genome sequencing data made it possible to study bacterial genome evolution from a new point of view and to demonstrate that the genome plasticity of some bacterial species is higher than expected. The model organism *E. coli*, for example, is known to exist as a harmless commensal in the gut, but several pathotypes are also able to cause severe disease. The phenotypic diversity between commensal and pathogenic strains is reflected by their genomic context and structure. The comparison of various *E. coli* genome sequences demonstrated that the *E. coli* pan-genome is an 'open' genome, i.e., this species is still evolving by gene acquisition and diversification, rendering the flexible genome a dynamic system (Rasko *et al.*, 2008).

MGEs, such as plasmids, bacteriophages, and genomic islands, and also conjugative transposons, integrative and conjugative elements (ICE) represent key elements for the exchange of genetic information by conjugation and transduction. They facilitate DNA exchange between the chromosome and MGEs and have the capacity to integrate into and excise from the bacterial chromosome by site-specific or simply by homologous recombination (Wozniak and Waldor, 2010). Accordingly, several identical or closely related determinants can often be found on the chromosome or on mobile DNA elements. If they do not provide a selective advantage to the host, these elements are frequently lost. They can also excise and translocate within the bacterial host genome by re-integration or they can be transferred to another recipient cell. Furthermore, insertion sequence (IS) elements and transposons contribute to genome plasticity due to transposition events and the fact that they can mediate DNA rearrangements by homologous recombination between multiple identical or similar copies within the genome. Furthermore, the transposition of IS elements and transposons can modulate the functionality of genes (Craig, 1996). (Super-) Integrons can be defined as a natural genetic engineering system: they capture and incorporate open reading frames by site-specific recombination and they express them (**Table 1**). They are frequently associated with transposons and conjugative plasmids that are required for their spread (Hall, 2012; Rowe-Magnus and Mazel, 2001). The high mobility and constant transfer, transposition, and recombination events can result in an ongoing generation of new MGEs with overlapping features (Darmon and Leach, 2014).

Plasmids are usually circular and self-replicating DNA elements. Although these autonomously replicating molecules exist as extrachromosomal genomes, some plasmids can be chromosomally inserted. Examples for the role of transferable elements as vectors and the constantly ongoing recombination between different mobilizable and transferable DNA elements are a large virulence plasmid of multi drug-resistant *Salmonella*

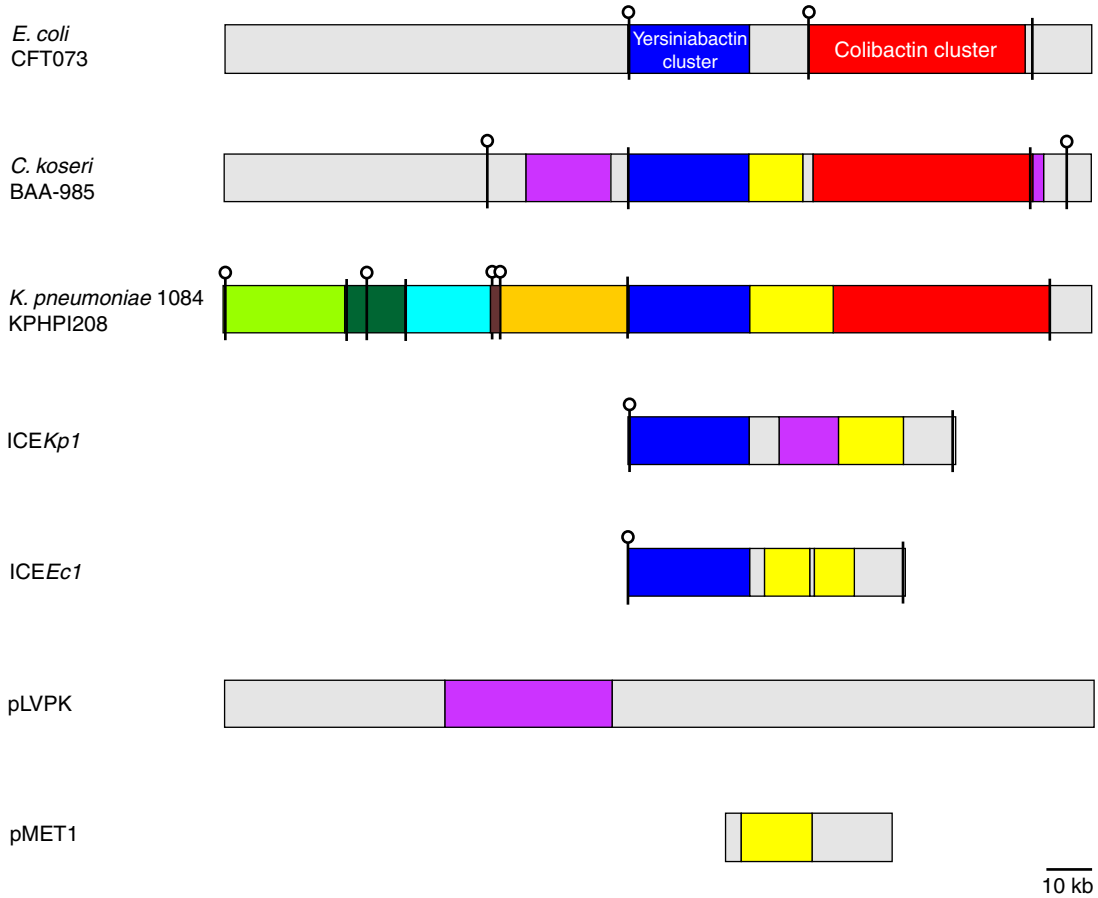

Figure 2 Genetic context of the *E. coli* yersiniabactin and colibactin determinants in comparison to other enterobacterial genomic regions. Modules with high homology to each other are shown in boxes of certain colors: the yersiniabactin cluster is represented in blue and the colibactin cluster in red, the DNA mobilization and transfer regions with high homology to DNA sequences of pMET-1 (GenBank accession number EU383016) are indicated in yellow, related sequences to parts of plasmid pLVPK (GenBank accession number AY378100) are shown in purple; orange and brown boxes represent regions of unknown function similar to *Eh*GM1 and *Eh*GM5 of ICE*Eh*1 (GenBank accession number FN297818), the light blue box represents a microcin module and with similarity to the microcin genomic island of strain *K. pneumoniae* RYC492 (GenBank Accession number AF063590).The green boxes are modules of unknown function. Gray boxes show regions that do not exhibit sequence similarities. Direct repeats are indicated by vertical black bars; tRNA genes are represented by vertical black bars with open circles (GenBank accession numbers for ICE*Kp*1 and ICE*Ec*1 are AB298504 and AY233333, respectively).

enterica serotype Typhimurium that carries several virulence genes and two class 1 integrons (Guerra *et al.*, 2002) as well as a multi-resistance plasmid of *Klebsiella pneumoniae* that resembles enterobacterial ICEs as well as plasmids found in *Yersinia pestis* (Soler Bistué *et al.*, 2008).

Genomic islands (GEIs) and pathogenicity islands (PAIs) represent genomic elements in bacteria, that often result from repeated gene acquisition, insertion, and recombination events (for reviews, see Dobrindt *et al.*, 2004; Hacker and Kaper, 2000; Juhas *et al.*, 2009). Strikingly, they represent a major part of the flexible genome, thus playing a tremendous role in genome plasticity and rapid bacterial evolution. GEIs and PAIs are horizontally acquired DNA stretches. They are often chromosomally inserted at tRNA loci, which serve as chromosomal insertion hotspots for foreign DNA, and flanked by identical or almost identical direct repeats as a result of site-specific genome integration. These islands are considered to originate from the repeated chromosomal insertion and recombination of multiple inserted MGEs and accessory genetic elements. Consequently, they typically carry functional or cryptic genes of plasmid and/or bacteriophage origin, which may encode integrases and conjugation systems, that were or still can be involved in island mobilization and transfer (Dobrindt *et al.*, 2004; Hacker and Kaper, 2000; Juhas *et al.*, 2009). As traits encoded by islands increase bacterial fitness, many islands have already been stabilized in the bacterial chromosome and lost their mobile character due to inactivation or deletion of the required mobility and transfer genes. Self-mobilizable and self-transferable islands often exist as (part of) integrative or conjugative elements (ICEs) (Dobrindt *et al.*, 2004; Juhas *et al.*, 2009). ICEs represent a diverse group of MGEs found in both Gram-positive and Gram-negative bacteria, which primarily reside in the host chromosome, but they retained the ability to excise and to transfer by conjugation due to the presence of a self-encoded transposase and conjugation system (Burrus and Waldor, 2004; Wozniak and Waldor, 2010). There is evidence that many GEIs have originated from ICEs (Bellanger *et al.*, 2014; Boyd *et al.*, 2009).

The acquisition and the exchange of mobile and accessory genetic elements together with repeated transposition and recombination events within the genome result in the continuous generation of genomic variability, thus mainly affecting the content and organization of the flexible gene pool. A good example of a genetic element displaying strain- or species-dependent variability is the *pks* (colibactin) island of *E. coli* and closely related *Enterobacteriaceae*. This 54 kb gene cluster encodes multiple polyketide synthases, non-ribosomal peptide synthetases and accessory as well as tailoring enzymes involved in the biosynthesis of the hybrid non-ribosomal peptide-polyketide colibactin (Nougayrède *et al.*, 2006). Colibactin is considered a bacterial fitness and virulence factor. This polyketide acts as a genotoxin in eukaryotes as it causes DNA double strand breaks (Nougayrède *et al.*, 2006), induces genomic instability in mammalian cells (Cuevas-Ramos *et al.*, 2010) and promotes colorectal cancer (Cougnoux *et al.*, 2014; Raisch *et al.*, 2014). The colibactin island has only been detected in commensal and extraintestinal pathogenic *E. coli* (ExPEC) strains of phylogenetic lineage B2 and in a few lineage B1 isolates (Nougayrède *et al.*, 2006). Interestingly, it was also identified in other *Enterobacteriaceae* like *K. pneumoniae*, *Citrobacter koseri* and *Enterobacter aerogenes* (Putze *et al.*, 2009). The genes of the colibactin determinant are highly conserved and are located in close proximity to another enterobacterial hybrid non-ribosomal peptide-polyketide determinant, the so-called high pathogenicity island (HPI) (Putze *et al.*, 2009; Schubert *et al.*, 2004b). Despite these similarities, remarkable species- and strain-dependent differences with regard to the genomic localization and organization of the colibactin island exist. While in *E. coli* phylogroup B2 strains the colibactin island was found to be chromosomally inserted into the *asnW* tRNA gene, the *asnU* tRNA locus served as integration site in the *E. coli* strains of the phylogenetic lineage B1. Even different *asn* tRNA genes were identified as insertion site in the *K. pneumoniae* strains tested (Putze *et al.*, 2009). In *E. coli* strains of phylogroup B2, both the colibactin and yersiniabactin determinants represent individual islands, which harbor an integrase gene and are flanked by direct repeats (Nougayrède *et al.*, 2006; Putze *et al.*, 2009). In contrast, in phylogroup B1 *E. coli* strains as well as in the *K. pneumoniae*, *E. aerogenes*, and *C. koseri* isolates the colibactin determinant is part of an ICE-like element with similarity to ICE*Ec*1 and ICE*Kp*1 and to several enterobacterial plasmids. This organizational similarity between different chromosomally inserted genetic entities and plasmid-encoded sequences indicates how efficiently recombination and interaction between different MGEs can occur (Nougayrède *et al.*, 2006; Putze *et al.*, 2009; Schubert *et al.*, 2004a; Soler Bistué *et al.*, 2008). Just recently, a 208 kb genetic element, designated KPHPI208, was discovered in a newly sequenced *K. pneumoniae* isolate from a patient with pyogenic liver abscess. This element did not only carry the colibactin and yersiniabactin modules and showed further sequence similarities to ICE*Kp*1, but also contained a microcin module as well as a new module with unknown function (Lai *et al.*, 2014). The fact that this element comprises four *asn* tRNA genes, eight direct repeats, and four integrase genes (Lai *et al.*, 2014) nicely demonstrates how independent mobile elements can cluster together and form a new mobile genetic entity. The example of the colibactin and yersiniabactin determinants illustrates that mobile elements can cross species borders. In addition, it demonstrates that they also display variability regarding their composition, organization, and genomic context (Putze *et al.*, 2009). The genetic flexibility of GEIs points to the fact that they are unstable and often undergo repeated rearrangements, deletions, and insertions underlining their impact on bacterial genome plasticity and evolution.

Impact of Nucleoid-Associated Proteins on Genome Plasticity

Besides the availability of foreign DNA elements and their uptake by suitable recipients, the acquired genetic information has to be stably incorporated into the recipient`s genome and successfully integrated into the gene regulatory networks of the new host cell. In the natural habitat of a bacterial species, the expression of horizontally acquired genes is much more likely to result in a reduced than in an increased fitness when compared to an isogenic population that did not take up heterologous DNA. Especially under the environmental conditions to which a bacterial species is adapted, energetic costs for maintenance and expression of heterologous genes in individual bacteria within a population should result in selective pressure favoring the wild type bacteria. However, new sets of horizontally acquired genes could apparently also provide a 'test toolbox' for a bacterium trying, for example, to cope with stress. Therefore, a regulatory mechanism that results in repression of heterologous genes under normal growth conditions, but allows for the activation of these genes under survival conditions could apparently constitute an evolutionary advantage for a bacterial species. However, it is unlikely that the recipient bacterial species possesses the specific regulators that are required to directly activate or repress foreign genes. Moreover, even in the case of mobile plasmids that might contain the required transcription factors for the appropriate plasmid gene regulation in the donor organism, it appears unlikely that these factors would work in the same manner in combination with the enzymatic machinery of the new host. Therefore, it might not be surprising that a class of abundant bacterial host proteins that bind DNA in a rather unspecific manner, the so-called nucleoid-associated proteins (NAPs), was found to play an important role in transcriptional repression of horizontally acquired DNA. The first NAP that was identified to selectively silence DNA with low GC content was histone-like nucleoid structuring protein (H-NS) in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) (Lucchini *et al.*, 2006; Navarre *et al.*, 2006). Subsequently, the MvaT-like and the Lsr2 proteins were shown to serve a similar function in *Pseudomonadaceae* and *Actinobacteria*, respectively (Ali *et al.*, 2012). As DNA architectural proteins, NAPs are also known to play important roles in other cellular functions that are essential for HGT, like replication, recombination, and transposition.

Nucleoid-Associated Proteins and Their Importance for Genome Architecture

Due to their molecular size, biochemical properties, and abundance the NAPs were initially termed bacterial 'histone-like'

proteins (Drlica and Rouviere-Yaniv, 1987). NAPs are conserved to various degrees in Bacteria and Archaea and are the most abundant DNA-binding proteins in bacteria (Dillon and Dorman, 2010). *E. coli* K-12, for example, possesses at least 12 NAPs with varying intracellular composition, dependent on growth condition and growth phase (Talukder, 1999). During growth of *E. coli* in batch culture, the most abundant NAPs in exponential growth phase are DnaA (DNA-binding protein A), CbpB (Rob) Fis (Factor for inversion stimulation), H-NS, HU, and StpA (suppressor of *td* mutant phenotype A), whereas CbpA (curved DNA-binding protein A), Dps (DNA-binding protein from starved cells), and IHF (integration host factor) are most abundant in stationary phase (Ishihama *et al.*, 2014; Talukder, 1999). NAPs are important for the structural organization of the bacterial nucleoid and differ in their localization within the nucleoid. Dps, HU, IHF, and StpA are evenly distributed, whereas CbpA and CbpB are found at specific loci in the chromosome. Fis has been localized to specific chromosomal regions and H-NS was reported to be evenly scattered throughout the chromosome, whereas a recent study using super-resolution imaging suggested the opposite (Talukder *et al.*, 2000; Wang *et al.*, 2011). As they affect DNA topology and flexibility, NAPs play pleiotropic roles in very basic cellular functions like replication and recombination that are also essential for genome plasticity and the stable integration of foreign DNA into the recipient's chromosome. NAP binding alters the local structural genome organization, thereby affecting the accessibility of the chromosomal DNA to recombination processes and, thus, the recombination frequency. HU, for example, plays an important role in replication of both the chromosomal origin and plasmids (Bahloul *et al.*, 2001; Dixon and Kornberg, 1984; Yasukawa *et al.*, 1997). Fis was first identified as a factor stimulating G inversion in bacteriophage Mu (Kahmann *et al.*, 1985), but plays also a role in replication (Wold *et al.*, 1996). Similarly, IHF is required for site-specific recombination of bacteriophage λ (Friedman, 1988; Kikuchi *et al.*, 1985), but is also important for replication at OriC and at plasmid origins of replication (Fekete *et al.*, 2006; Skarstad *et al.*, 1990).

Nucleoid-Associated Proteins and Gene Regulation

In addition to their often redundant functions in replication and recombination, NAPs regulate the expression of substantial gene sets. Fis is an important regulator of virulence-associated genes and was shown to bind and regulate hundreds of operons in *E. coli* and *S.* Typhimurium (Kahramanoglou *et al.*, 2011; Wang *et al.*, 2013). *In vitro*, Fis binds with affinities that vary by several orders of magnitude to sites with a degenerated 15-bp core sequence, and introduces bends ranging from 50° to 90° (Pan *et al.*, 1996; Schneider *et al.*, 2001). This property in combination with the strong fluctuations in intracellular Fis concentrations and binding site competition with NAPs and other DNA-binding proteins is resulting in a highly dynamic *in vivo* situation that can be utilized to fine-tune gene expression. In the exponential growth phase, the NAP gene *dps* was shown to be repressed by Fis-dependent trapping of RNA polymerase (RNAP) containing the housekeeping sigma factor σ^{70} at the *dps* promoter and by H-NS preventing binding of RNAP σ^{70}. With decreasing Fis levels toward stationary phase, RNAP σ^{70} is no longer trapped at the *dps* promoter and can be bound and transcribed by RNAP containing the stationary phase sigma factor σ^{38}, which is not blocked by H-NS (Grainger *et al.*, 2008). In addition to gene regulation by such rather classical mechanisms based on local protein–DNA interactions at gene promoters, NAPs like Fis, HU, and H-NS also regulate transcription by modulating DNA topology. Fis binding to upstream activating sequences of stable RNA promoters, for example, constrains writhe that can then be either used to facilitate wrapping of DNA around RNAP, or for untwisting of the promoter for transcription initiation (Travers and Muskhelishvili, 2005). Moreover, the absence of Fis and H-NS is resulting in alterations of the supercoiling sensitivity of genomic transcription in *E. coli*. Therefore, these proteins are coordinating growth phase-dependent transcription, at least in part, by locally constraining the torsional energy of negatively supercoiled DNA, thereby directing the stored energy to gene promoters (Blot *et al.*, 2006). Changes in DNA supercoiling and NAP expression do not only occur during regular growth transitions, for example, from logarithmic to stationary phase of growth, but also during intracellular growth of *S.* Typhimurium in a murine macrophage, suggesting that such mechanisms play also an important role in the regulation of horizontally acquired virulence genes (Mangan *et al.*, 2006).

NAPs in Transcriptional Silencing of Horizontally Acquired DNA

Silencing of horizontally acquired DNA by H-NS was first described in *S.* Typhimurium (Lucchini *et al.*, 2006; Navarre *et al.*, 2006). By analyzing the transcriptional profiles of wild type and *hns* mutants of *S.* Typhimurium, the authors could show that most genes repressed by H-NS were likely to be acquired by HGT, including the *Salmonella* pathogenicity islands (SPI) SPI-2, SPI-3, and SPI-5 (Navarre *et al.*, 2006). The genes that were found to be repressed by H-NS in this study displayed a lower G + C content than the average genome, which led to the hypothesis that H-NS would preferentially bind to AT-rich DNA sequences and thereby repress transcription. This suggested that the mechanism by which H-NS is repressing the transcription of horizontally acquired genes is different from mechanisms that are based on repressor interactions with promoter proximal binding sites. To distinguish the mechanistically distinct transcriptional repression of AT-rich sequences by H-NS from classical H-NS-dependent gene promoter regulation, the term 'xenogenic silencing,' i.e., silencing of DNA that originated from a distinct species was introduced (Navarre *et al.*, 2007). As H-NS-mediated xenogenic silencing affects chromosomal regions rather than individual operons, a higher order nucleoprotein structure formed by H-NS and silenced DNA was proposed to result in transcriptional repression. According to this model, H-NS dimers are binding to AT-rich DNA with C-terminal DNA-binding domains and polymerizing by N-terminal H-NS–H-NS interactions. Depending on the binding mode, two different types of H-NS–DNA complexes were proposed (**Figure 3**), even though the exact molecular mechanism of binding and bridging by H-NS is yet to determined (Navarre *et al.*, 2007) (for review see Ali *et al.* (2012)). Specificity of transcriptional silencing can be achieved by high-affinity binding sites, which serve as nucleation site for H-NS polymerization.

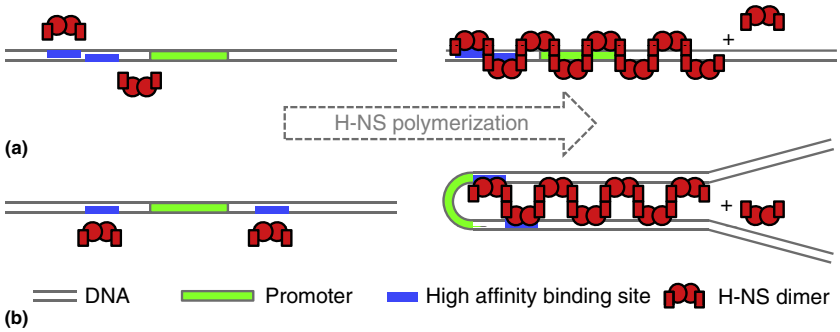

Figure 3 Different polymerization modes of H-NS affect the nucleoid architecture and thus recombination as well as gene expression. (a) High-affinity binding sites on opposite sides of the DNA double helix. Initial binding of H-NS dimers to opposite sides of the DNA double helix results in H-NS polymerization on the same DNA double helix. (b) High-affinity binding sites on the same side of the DNA double helix. Initial binding of H-NS dimers to the same side of the DNA double helix results in polymerization by bridge formation between two DNA helices. The nucleoprotein complex formed by H-NS may depend on the exact positions of high-affinity binding sites that serve as starting point for H-NS polymerization.

High-affinity binding sites for H-NS were shown to be clustered within operons of horizontally acquired virulence related GEIs in pathogenic *E. coli*, which further corroborated the idea that initial binding to high-affinity binding sites and subsequent cooperative binding of H-NS from these sites – eventually in combination with H-NS-dependent bridging of two bound DNA helices – is the mechanistic basis for H-NS-dependent repression of AT-rich DNA regions (Lang *et al.*, 2007). The finding that H-NS homologues are often encoded on large and especially AT-rich plasmids, for example, Sfh and others (Doyle *et al.*, 2007; Takeda *et al.*, 2011), further confirms the importance of this transcriptional repression mechanism for HGT.

The Role of NAPs in Anti-Silencing of Horizontally Acquired DNA

There is, however, no doubt that the silenced regions, for example, the PAIs of *S.* Typhimurium or the 'locus of enterocyte effacement' (LEE) of enteropathogenic or enterhemorrhagic *E. coli* are expressed under specific environmental conditions. Therefore, mechanisms that counteract transcriptional silencing by H-NS and its homologues have to exist. As described above, H-NS-mediated silencing is believed to be rather dependent on the formation of higher order nucleoprotein structures than on local interactions with binding sites on individual promoters. This implies that the activation of H-NS silenced genes is mechanistically distinct from the activation of genes that are repressed by classical H-NS promoter interactions. A recent study showed that the activation kinetics of genes, which are activated by the PhoP master regulator in *S.* Typhimurium depends on gene ancestry (Zwir *et al.*, 2014). Ancestral genes of *S.* Typhimurium are activated by PhoP first, whereas genes that were acquired by HGT are activated later. This expression pattern was not due to differences in PhoP binding site affinities, but dependent on H-NS. Therefore, the delay in activation of silenced genes most likely reflects the more time-consuming requirement for the disintegration of the higher order H-NS-dependent nucleoprotein structure at horizontally acquired genes (Zwir *et al.*, 2014). The additional observation that some of these late genes are also expressed to higher levels than early genes may be explained by individual promoter strength in the absence of H-NS-mediated silencing (Zwir *et al.*, 2014), or the prolonged time requirement for reestablishing the silencing nucleoprotein complex. The molecular anti-silencing mechanisms are diverse and encompass topological changes in DNA structure or the formation of heteromeric protein complexes of H-NS with other H-NS-like proteins (for review see Stoebel *et al.* (2008)). However, recent insights in the activation of the *S.* Typhimurium SPI-1 and SPI-2 by DNA topology changes, mediated by changing DNA supercoiling levels during the infection process and NAPs, suggests a general and in evolutionary terms old mechanism for the initial steps in the activation of horizontally acquired chromosomal regions (Cameron and Dorman, 2012). The regulatory genes *ssrA* of SPI-2 and *hilC* of SPI-1 are induced by DNA relaxation and OmpR, whereas *hilD* of SPI-1 is repressed by DNA relaxation and OmpR. DNA relaxation results in increased OmpR binding and decreased binding of the NAP Fis. Fis interaction with the same DNA sequence was reduced with DNA relaxation, whereas OmpR binding increased with reductions in superhelical density (Cameron and Dorman, 2012; Dorman, 2013a,b). Further evidence for the hypothesis that changes in DNA topology mediated by NAPs are a general mechanism for anti-silencing of horizontally acquired DNA regions comes from the recent finding that Ler, the activator of the H-NS repressed LEE PAI, forms toroidal DNA complexes at LEE promoters (Mellies *et al.*, 2011). This HU-dependent nucleoprotein complex is similar to the complexes known to be required for osmotic induction of the supercoiling sensitive *proU* operon in *E. coli* and S. Typhimurium as well as the complex involved in activation of the virulence gene regulatory gene *hilA* in *S. Typhimurium* (Higgins *et al.*, 1988; Manna and Gowrishankar, 1994; Maurer *et al.*, 2009; Schechter *et al.*, 2003). The facts that Ler expression in turn is dependent on the NAP Fis and that *fis* mutants of *E. coli* and *S.* Typhimurium are attenuated in their virulence, whereas an *hns* mutant of *S.* Typhimurium is not viable, underscore the importance of NAPs for the functional integration of horizontally acquired DNA into bacterial systems (Goldberg *et al.*, 2001; Navarre *et al.*, 2006; Wilson *et al.*, 2001).

Taken together, bacterial evolution depends on the ability to adapt to different environments. Bacterial genome plasticity is a prerequisite for adaptability and results from the

generation of genetic variability without impairing genome fidelity. Various often interrelated processes are involved, which act on genome architecture, genome content and genome organization as well as on gene expression regulation and gene function. These processes enable the constantly ongoing generation of new bacterial variants, some of which will be able to conquer and stably colonize new ecological niches.

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See also: Genome Size and Structure, Bacterial. Plasmid Driven Evolution of Bacteria. Transposable Elements, Population Genetics of

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Genome Size and Structure, Bacterial

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Glossary

Effective population size The size of the population that makes a genetic contribution to the subsequent generation – usually much smaller than the actual population size.
Genetic drift The change in gene frequencies in a population due to random sampling.
Okazaki fragments Short DNA fragments synthesized on the lagging strand during DNA replication.
Paralogs Genes whose shared ancestry can be traced to a duplication event.
Plasmids Small, circular, autonomously replicating, double-stranded DNA molecules that are distinct from the cell's chromosome.
Pseudogenes Previously functional regions that have been inactivated by mutation.
Xenologs Genes whose shared ancestry can be traced to a horizontal transfer event.

Abbreviations

bp basepair
kb kilobase
Mb megabase
sRNA Small RNA

The Architecture of Bacterial Genomes

Early work on diverse model systems, such as *Escherichia coli* and *Bacillus subtilis*, led to the view that all bacteria possessed a single circular chromosome. However, physical mapping techniques revealed that species from divergent bacterial phyla have linear chromosomes, including *Borrelia burgdorferi* (a spirochete), *Agrobacterium tumefaciens* (a proteobacterium), and *Streptomyces coelicolor* (an actinomycete), indicating that this chromosome structure arose multiple times independently (Casjens, 1998; Ochman, 2002). The prevalence of circular chromosomes seems to reside in the fact that linear chromosomes must solve the problem of fully replicating their chromosome ends. Otherwise, the advantage of one configuration over the other is unknown (Marri *et al.*, 2008): in experiments in which the normally circular chromosome of *E. coli* was linearized synthetically, showed no obvious changes in growth rate, gene expression, or cell morphology (Cui *et al.*, 2007).

Several bacterial species have genomes that are partitioned into multiple chromosomes, including at least one instance, that of *A. tumefaciens*, where the genome contains one linear and one circular chromosome. The presence of multiple chromosomes of different genetic content within a cell – as opposed to those bacteria that maintain multiple copies of their single chromosome – is not surprising given that bacteria often harbor additional replicons in the form of plasmids. Distinguishing between chromosomes and plasmids is not always straightforward, and differentiation between the two has been based on size, contents, copy number, dispensability, transmissibility, and mode of replication (Ochman, 2002). In the majority of cases, the origins of a second chromosome in a bacterial genome – and indeed, it appears that a single circular chromosome is the ancestral state – can be traced to an extrachromosomal accessory element that enlarged and acquired essential genes as opposed to the duplication or dissolution of the single ancestral chromosome.

Bacteria Have Compact Genomes

Bacterial genomes are notable in that they are tightly packed with protein-coding genes (Figure 1), which average about 1 kb in length and do not contain introns. Typically, 80–90% of a bacterial chromosome encodes proteins, and a large fraction of intergenic DNA is devoted to regulatory sequences and large numbers of other functional noncoding elements, such as sRNAs (as well as the structural RNAs required for protein assembly). This greatly contrasts the situation in the human genome, in which protein-coding regions are nearly a hundred times longer (owing mostly to the presence of introns) and noncoding regions constituted nearly 98% of the genome (Ahnert *et al.*, 2008). The paucity of nonfunctional DNA is one of the hallmarks of bacterial genomes, and their high-coding densities means that there is a strong association between genome size and gene number (Mira *et al.*, 2001).

Insights into the basis for the high density of functional sequences within bacterial genomes came from several sources, but particularly from those genomes that were anomalous in that they contained large numbers of pseudogenes. The first sequenced genomes shown to have substantial numbers of inactivated genes was *Mycobacterium leprae* (the etiological agent of leprosy), in which over half of its encoded sequences were pseudogenes, which had intact counterparts in its congener *Mycobacterium tuberculosis* (Cole *et al.*, 2001). When comparing nonfunctional regions, such as pseudogenes, to their functional counterparts across a wide array of bacterial lineages, the nonfunctional regions display an excess of deletional events, implying that the mutational process in bacteria

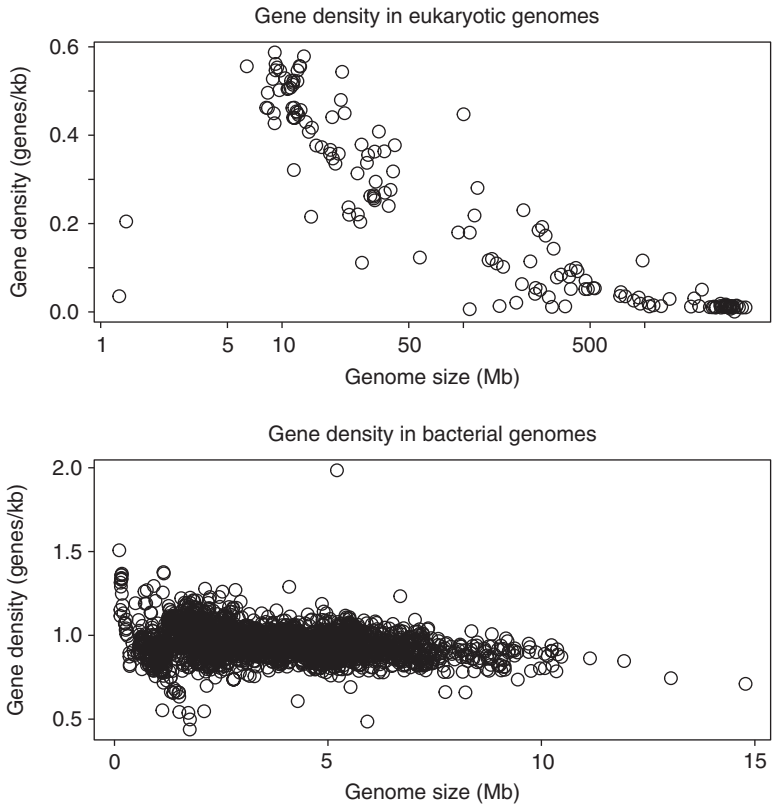

Figure 1 Relationship between gene density and genome size. Gene densities are calculated as the number of genes (in a genome of a given size) per kilobase. Note that gene densities in bacteria are similar regardless of genome size (such that gene numbers increase with genome size), whereas in eukaryotes, there is an inverse relationship between genome size and gene density (such that larger genomes do not necessarily encode additional genes).

is biased toward deletions over insertions. This mutational bias has also been observed in experimental populations, in which evolved strains can harbor individual deletions up to 200 kb in length (Nilsson *et al.*, 2005).

The deletional bias maintains the high density of functional genes observed in bacterial genomes: when inactivating mutations occur in genes that are no longer required, the nonfunctional regions gradually erode through deletions and are eventually eliminated (Andersson and Andersson, 2001; Mira *et al.*, 2001). The presence of a deletional process that removes nonfunctional sequences implies that virtually all genes in a bacterial genome are functional and maintained in the genome by natural selection (Kuo and Ochman, 2009).

Determinants of Genome Size in Bacteria

Among bacteria, genome sizes varies over two orders of magnitude, which, due to the relationship between genome size and gene contents in bacteria means that lineages can differ by as much as a 100-fold in their gene numbers. (In contrast, humans and the budding yeast, *Saccharomyces cerevisiae*, differ by only fourfold in gene number.) Even after the sequencing of only a dozen bacterial genomes, there was a notable association between genome size and bacterial lifestyle: those bacteria possessing small genomes were host-associated pathogens and symbionts, whereas those bacteria with large genomes were either free-living or environmental isolates. Currently, the bacteria with the smallest genomes are obligate symbionts of insects (Moran and Bennett, 2014), with the tiniest genome yet sequenced – only 112 kb – belonging to *Nasuia deltocephalinicola*, a symbiont of leafhoppers (Bennett and Moran, 2013). At the other end of spectrum are free-living bacteria, and a soil-associated species, *Ktedonobacter racimifer*, which at 15.6 Mb has the largest sequenced genome to date (Chang *et al.*, 2011).

Phylogenetic analyses indicate that host-associated bacterial lineages are derived from ancestors with larger genomes, which is not surprising considering that bacteria as a group are much more ancient than their potential eukaryotic hosts. Pathogen and symbiont genomes can possess fewer functional genes because many nutrients and biochemical pathways are supplied by their hosts. Considering all of these factors together, it is possible to trace the evolutionary progression toward highly reduced genomes and to account for the variation in the gene repertoires observed among contemporary species. Initially, (ancestral) free-living bacteria become associated with a (nutrient-rich) eukaryotic host. This association renders many bacterial genes superfluous in the host environment, and these unnecessary genes accumulate mutations, becoming pseudogenes. The inactivated genes are eventually removed by the pervasive mutational bias toward deletions, resulting in a compact genome harboring only functional genes. Note that this scenario accounts not only for the high gene densities

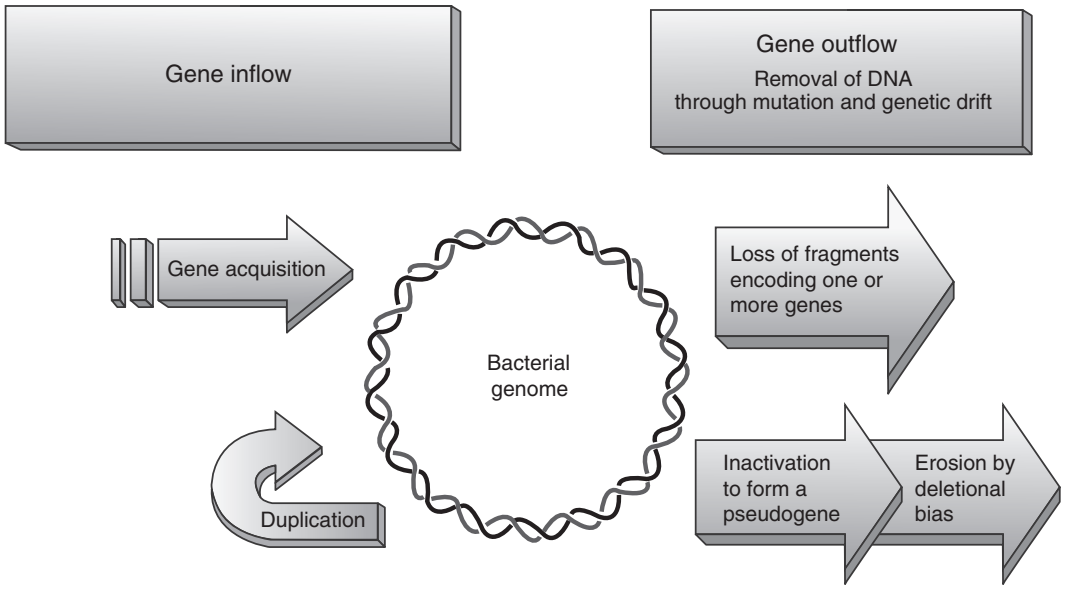

Figure 2 Interplay of factors impacting bacterial genome size. New sequences are acquired by gene transfer and gene duplication, whereas DNA loss occurs by large deletions that remove multiple genes in a single event and by the erosion of pseudogenes and other nonfunctional sequences. Modified from Mira, A., Ochman, H., Moran, N.A., 2001. Deletional bias and the evolution of bacterial genomes. Trends in Genetics 10, 589–596.

observed in the largest and smallest genomes but also for the presence of large numbers of pseudogenes observed in recent pathogens and other host-associated lineages.

The compactness of bacterial genomes has traditionally been thought to result from 'streamlining' and considered to be an adaptation to increase replication speed and growth rates. For the most part, this view is incorrect. When looking across bacteria, there is no clear association between generation time and genome size (Mira *et al.*, 2001). In tests within species, natural strains of *E. coli* that differed by 15% in genome size did not differ in growth rates (Bergthorsson and Ochman, 1998), and isogenic strains of *Salmonella* engineered to harbor duplications up to 700 bp in length did not replicate significantly slower than the parental strain in either minimal or nutrient-rich media (Matthews and Maloy, 2010). Although bacterial genome size is not generally driven by selection for replication efficiency, a few species in nutritionally poor environments have reduced genomes in order to decrease the metabolic burden associated with replicating extra DNA. The marine planktonic bacteria, *Prochlorococcus* (Dufresne *et al.*, 2005) and *Pelagibacter ubique* (Giovannoni *et al.*, 2005) occupy environments where nitrogen and phosphorous concentration are limited, and both possess the smallest and most gene-dense genomes among free-living bacteria.

The contrasting view is that bacterial genome size is governed by a nonadaptive process (Kuo *et al.*, 2009). Due to the association between genome size and gene number in bacteria, the evolutionary forces that act on individual genes will have profound effects on overall genome size. As a result, those species with small effective population sizes, such as those whose populations are severely restricted during transmission between hosts, are subject to genetic drift, which reduces the efficacy of selection and allows the accumulation of deleterious mutations, even in useful genes. Thus, bacteria with small effective population sizes, such as symbionts and pathogens, will have the smallest genomes because they are more susceptible to gene decay and loss, even for genes that are usually considered beneficial.

The view that small genome size in bacteria is largely a consequence of a nonadaptive process, i.e., genetic drift, runs counter to the situation in eukaryotes. Lynch (2007) has posited that reductions in the efficacy of selection in eukaryotic species with small effective population sizes have allowed the proliferation of deleterious sequences in the form of introns and transposable elements, both of which serve to increase genome size. Therefore, increased genetic drift has caused the size of eukaryotic genomes to increase but bacterial genomes to decrease.

Offsetting the deletional processes that serve to reduce genome size, bacterial genomes can grow by gene duplication and gene acquisition (*a.k.a.* lateral/horizontal gene transfer) (**Figure 2**). To determine the relative contributions of these two processes to the emergence of new bacterial genes, the numbers of paralogs (genes arising by duplication) and xenologs (genes acquired by transfer) were compared in bacterial groups across a wide range of genome sizes (Treangen and Rocha, 2011). In general, gene transfer played a larger role in the expansion of protein families and provided bacteria with genes of new function, whereas gene duplication usually led to an increase in gene dosage.

Genome Size Variation within Species

DNA content was considered to be invariant within a species, hence the application of the term 'C(onstant)-value' to denote the amount of DNA contained in a gamete or genome. Whereas the sexual system of eukaryotes has a homogenizing effect on chromosome size, no such constraints are imposed on organisms that reproduce by binary fission. But because the

Figure 3 Genome size variation within bacterial species. Size ranges are shown for species for which complete genome sequences are available for at least 10 strains.

members of a bacterial species are metabolically and phenotypically similar – in fact, species assignment and differentiation were based largely on metabolic capabilities before DNA typing became routine – the genomes of bacteria typed to the same species were thought to be alike in their sizes and contents, with variation, if any, attributable to the sporadic distribution of extrachromosomal elements among genomes.

There is early evidence, based on DNA reassociation experiments, that there was substantial variation in the genome sizes among clinical isolates of *E. coli* – variation could not be attributed to extrachromosomal sequences since many strains had smaller genomes than a control strain known to lack plasmids (Brenner *et al.*, 1972). However, it was not until the broad-scale application of pulsed-field gel electrophoresis – a method that allows resolution of very large DNA fragments – that the true extent of genome size diversity within bacterial species was fully appreciated (Cole and Saint Girons, 1994).

The sequencing and assembly of complete bacterial genomes has led to comparisons of the contents of genomes among closely related bacteria, and scrutiny of the extent of variation and the specific changes that contributed to variation in genome size (**Figure 3**). The first in-depth analysis of multiple sequenced members of bacterial species was of strains of *E. coli* that differed in their pathogenic potential (Welch *et al.*, 2002). The most pronounced source of the differences among these strains of *E. coli* was the integration of large DNA segments, mostly by phage-mediated events, forming islands of genes that were unique to a particular strain. This seems to be a common theme among bacteria: although changes in the numbers of repetitive and translocatable elements can inflate and deflate genomes, the acquisition of large regions by horizontal gene transfer is the major contributor to the genome size variation among members of a bacterial species. The within-species variation in genome size and contents has led

to the concept of the 'core-genome,' which comprises those genes that are present in (virtually) all members of a species, and the 'pan-genome,' which is the entire set of genes encoded by the species (Tettelin *et al.*, 2005).

Genome Organization

Genes encoded are not positioned at random along the bacterial chromosome. It has long been known that certain sets of genes are situated in proximity because they constitute a single functional unit (i.e., an operon); however, the location, arrangement, and distribution of genes in bacterial genome are influenced by several gene- and genome-level factors, many of which result from the manner in which replication occurs. Recall that in bacteria, there is a single replication origin, and that replication of the circular chromosome proceeds bidirectionally to a terminus that is situated transversely, at approximately 180°, from the origin. This replication process is asymmetric, generating leading strands, which are synthesized continuously from the origin to the terminus, and lagging strands, which are replicated discontinuously through the synthesis and joining of short Okazaki fragments.

Maintenance of Chromosome Balance

Early comparisons of the genetic maps of *E. coli* and *Salmonella typhimurium*, related enteric species thought to have diverged about hundred million years ago, displayed a high degree of concordance but differ with respect to a large inversion that spanned the replication terminus (Krawiec and Riley, 1990). Additionally, other *Salmonella* strains possess different inversions that were similarly symmetric with respect to the replication terminus, suggesting that inversions that offset the positions of the origin and terminus relative to one another are deleterious (Liu *et al.*, 1993). Although experimentally disrupting the positions of the origin and terminus through the introduction of large duplications on one side of the chromosome showed no appreciable effect on growth rates (Matthews and Maloy, 2010), there is evidence that selection acts to maintain chromosome balance over evolutionary timescales. Using physical maps to reconstruct the history of the *Salmonella paratyphi* genome exposed that an insertion that disrupted the balance between origin and the terminus was succeeded by an inversion that restored their positions (Liu and Sanderson, 1995).

Gene Location and Orientation

Inversions that maintain chromosome balance, i.e., those that are symmetric with respect to replication origin and terminus, preserve not only the positions of the origin and terminus but also the distances of all genes relative to the origin and terminus. All other inversions alter the positions of some genes relative to the origin. Moreover, those inversions that do not include the replication origin or terminus reverse gene orientation, such that genes that were originally encoded on the leading become lagging-strand genes and vice versa. Comparing the relative positions of genes in pairs of related genomes revealed a remarkable pattern: most genes retain the same orientation and the same relative distance to the replication origin despite the occurrence of numerous rearrangement events, yielding whole-genome alignment plots that display a characteristic X-shaped pattern (Eisen *et al.*, 2000; Tillier and Collins, 2000; Suyama and Bork, 2001; **Figure 4**).

How can proximity to the replication origin exert a selective effect on genes and chromosome organization? This can occur in two ways: Firstly, both mutation rates (Mira and Ochman, 2002) and recombination events (Louarn *et al.*, 1994) increase with distance from the replication origin, so it is possible that selection might favor the positioning of highly conserved, essential genes nearer to the replication origin as is observed in *B. subtilis* and *E. coli* (Rocha and Danchin, 2003). Secondly, because new rounds of replication can be initiated before the previous round is complete, genes closer to the replication origin exist in higher dosage and are more highly expressed than those near the replication terminus (Segall *et al.*, 1988; Liu and Sanderson, 1996). Therefore, the relocation of genes or operons by inversions or transpositions can alter their expression patterns and have a detrimental effect on the cell.

How can strand orientation exert a selective effect on gene and chromosome organization? The difference in the processes

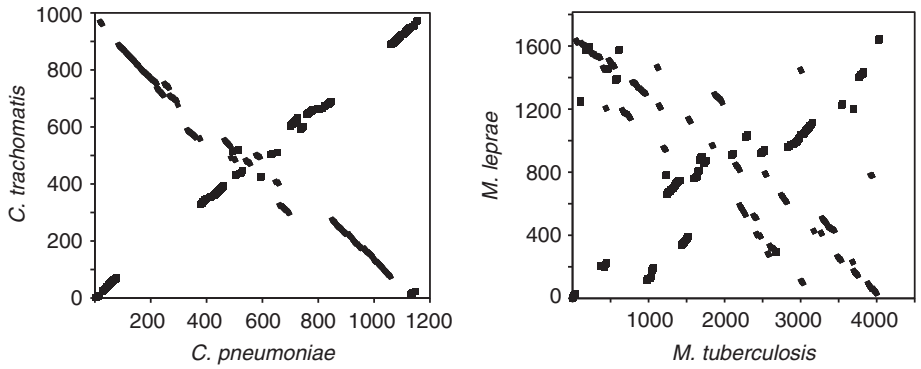

Figure 4 Correspondence of positions of homologous genes in pairs of sequenced genomes in *Chlamydia* (*C. pneumonia*e and *C. trachomatis*) and *Mycobacterium* (*M. tuberculosis* and *M. leprae*). Genomes are represented linearly beginning at the replication origin (position 0) with gene positions, indicated in kb from the replication origin, proceeding clockwise around the chromosome. Scatterplots redrawn from Tillier, E.R., Collins, R.A., 2000. Genome rearrangement by replication-directed translocation. Nature Genetics 26, 195–197 and Eisen, J.A., Heidelberg, J.F., White, O., Salzberg, S.L., 2000. Evidence for symmetric chromosomal inversions around the replication origin in bacteria. Genome Biology 1, research0011.1–0011.9.

used to replicate the leading and lagging strands causes asymmetries in the rates and patterns of mutations, and hence, the nucleotide contents of each strand (Francino and Ochman, 1997; Frank and Lobry, 1999). In general, there is an excess of guanine residues on the leading strand – termed 'GC skew,' which calculated as degree of the difference in the number of guanines and cytosines on a given strand relative to the total number of these guanines and cytosine residues (i.e., G − C/G + C) – and many genome sequences show an abrupt change in the direction of skew at the replication origin and terminus (Lobry, 1996).

Because the same DNA strands are used as templates for replication and transcription, both processes will occur simultaneously; however, DNA polymerase proceeds at over 10 times the speed of RNA polymerase causing collisions between the two polymerization machineries. On the leading strand, replication and transcription proceed in the same direction, such that collisions between DNA polymerase and RNA polymerase are co-oriented and produce fully formed transcripts. On the lagging strand, however, DNA polymerase and RNA polymerase have head-on collisions that abort transcription (Rocha and Danchin, 2003; Merrikh *et al.*, 2012). Owing to the deleterious nature of head-on collisions, there is an asymmetric distribution of genes between the two strands. In most genomes, the majority of genes are preferentially encoded on the leading strand (Mao *et al.*, 2012; Chen and Zhang, 2013), indicating that there is selection against inversions and translocations that alter the orientation of genes or operons.

Genomic Base Composition

One of the most highly variable features of bacterial genomes is overall base composition, which among sequenced genomes ranges from 13% to 75% G + C. The fact base composition is relatively homogeneous within a genome but varies greatly among genomes led to the view, developed in the 1960s, that the variation was caused by differences in the underlying patterns of mutations, such that high G + C and low G + C organisms differed in their spectra of replication errors (Freese, 1962; Sueoka, 1962). (It is interesting to note that this attribution of molecular variation to a strictly mutational process preceded Kimura's neutral theory of molecular evolution by several years.) An alternative explanation is that the variation in genomic base compositions is adaptive, such that the differences are due to a selective force that favors certain base compositions under some particular environment conditions (McEwan *et al.*, 1998; Naya *et al.*, 2002; Rocha and Danchin, 2002; Romero *et al.*, 2009). In that G/C basepairing is more thermally stable than A/T basepairing, it has been suggested repeatedly that genome base compositions reflect growth conditions; however, there is no association between growth temperature and genomic base composition when analyzed across diverse taxa (and in fact, many thermophiles have genomic G + C contents that are substantially lower than those of mesophiles) (Galtier and Lobry, 1997; Hurst and Merchant, 2001; Wang *et al.*, 2006).

To date, no fewer than a dozen factors have been proposed as the source of the base-compositional variation in bacteria (Rocha and Feil, 2010); however, the lack of a single unifying explanation has led many to adopt the decades-old view that the variation is neutral and caused by differences in the mutational process. Recent analyses performed on multiple taxa suggest that, even in bacterial groups with intermediate and high genomic G + C contents, the mutations are biased toward A + T (Hershberg and Petrov, 2010; Hildebrand *et al.*, 2010). These results, based on sequence comparisons, are most readily explained by the action of natural selection favoring individual base substitutions that increase the G + C composition of the genome; however, the manner in which selection is operating is still unknown.

See also: Genome Organization, Evolution of. Genome Plasticity, Bacterial. Mutation and Genome Evolution

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Genotype-by-Environment Interaction

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Glossary

Across-environment genetic correlations Genetic correlations (see below in glossary) between traits expressed in different environments. Most typically, this refers to the same trait expressed in two or more environments, but it also encompasses correlations among different traits expressed in different environments.

Co-gradient variation A geographic pattern of phenotypic variation in which the direction of trait plasticity elicited by an environment is in the same direction as genetic differentiation in the trait caused by local adaptation to that environment (as opposed to 'counter-gradient variation' in which the direction of plasticity is opposite to that of genetic differentiation).

Cost of plasticity The decrease in fitness caused by the ability to be plastic, independent of fitness consequences of the expressed phenotypes. This can theoretically be caused by costs of maintaining the behavioral or biochemical capacity (environmental sensors, signal transduction pathways) to respond to environmental variation. A cost of plasticity can be detected by comparing the fitness of plastic versus nonplastic genotypes that exhibit the same phenotype in a given environment: if two genotypes have identical phenotypes in a given environment, but one of them exhibits plasticity (changes its phenotype in a different environment) and the other does not, the nonplastic genotype would have the higher fitness if there is a cost to being plastic.

Discontinuous trait A trait that exhibits distinct phenotypes, instead of continuously varying phenotypes, in response to continuous environmental factors.

Function-valued trait A trait that exhibits incremental plasticity in response to a continuous environment (contrasted to a discontinuous trait, see glossary). Its reaction norm is depicted as a continuous function of the phenotype in response to the continuous environmental factor.

Genetic accommodation A type of reaction-norm evolution whereby a phenotype that was originally induced by the environment contributes to adaptive evolution in that new environment and can cause the phenotype to be expressed even without the environmental cue. Genetic accommodation includes several types of reaction-norm evolution, including 'genetic assimilation' (see glossary below), as well as an increase in the mean trait value across all environments when the function of response to the environment (e.g., the slope of the reaction norm) remains the same.

Genetic assimilation A type of reaction-norm evolution whereby a phenotype that was originally induced by the environment becomes expressed even when the environmental cue is absent. Genetic assimilation entails the reaction-norm evolving from that of a plastic trait to a nonplastic trait; that is, the trait becomes 'canalized.'

Genetic correlation The degree to which two traits are correlated because of a shared genetic basis or linkage disequilibrium. Genetic correlations cause direct selection on one trait to result in indirect selection on correlated traits. As a consequence, a trait can evolve in response to selection even if it is not the target of selection.

Heritability (narrow-sense) Denoted h^2, heritability is the proportion of total phenotypic variation (Vp) that is additive genetic variation (Va): $h^2 = Va/Vp$. Heritability is proportional to the expected response to selection, with larger heritability leading to greater response to selection.

Maternal effect More narrowly specified, a 'maternal environmental effect' is phenotypic plasticity across generations, such that the environment experienced by the maternal parent influences the phenotype expressed by progeny. Parental environmental effects occur when the environment experienced (or provided) by either parent – maternal or paternal – influences the phenotype expressed by progeny.

Phenology The timing of biological events, such as the timing of developmental transitions, including germination, flowering, leaf-out, senescence in plants, or hatching, metamorphosis, and reproduction in animals.

Phenotypic plasticity The ability of a genotype to alter its phenotype in response to the environment.

Polyphenism Discrete phenotypic states expressed by an organism in response to environmental conditions. Examples include different color morphs of insects that eat different host plants and different color morphs of insects that develop at different times of year.

QTL analysis Quantitative trait locus (QTL) analysis is a form of genetic analysis of quantitative traits, or traits that are controlled by many genes. The analysis uses lineages that have been genotyped at many markers throughout the genome, and tests for associations between the alleles at each marker and the phenotype that is expressed by individuals with those alleles. An association between a phenotype and a marker allele is evidence that a locus near the marker contributes to genetic variation in the phenotype.

Reaction norm The graphical depiction of phenotypic plasticity, with the phenotype on the y axis and the environmental factor on the x axis. A line connects the phenotypes expressed by one genotype in each environment.

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General Concepts

The ability of a genotype to alter its phenotype in response to the environment is called 'phenotypic plasticity.' Examples of phenotypic plasticity include the elongation of plants growing in shade (Smith, 1982; Ballaré *et al.*, 1990), the increase in a snail's shell thickness when grown in the presence of predators (DeWitt, 1998), or the polyphenism of coloration or form of some insects depending on the season of their development or the host plants they consume (e.g., Kingsolver, 1995). Phenotypic plasticity can occur not only in morphology, but also in behavior, life-history expression, and phenology (the timing of biological/developmental events). Phenotypic plasticity can also occur across generations, such that the environment experienced by parents influences the phenotype expressed by progeny, resulting in 'parental effects' (or commonly 'maternal effects'; Mousseau and Fox, 1998).

When different genotypes exhibit different responses to an environmental factor, there is a 'genotype-by-environment interaction.' Phenotypic plasticity of a given genotype can be represented graphically as a 'norm of reaction' or 'reaction norm.' A reaction norm represents the mean phenotype expressed by a genotype in two or more environments. The environments can be discrete, such as distinct host plants; or continuous, such as temperature. Genotype-by-environment interaction is manifest when genotypes differ in their reaction norms (**Figure 1**).

Genotype-by-environment interaction has consequences for trait evolution because it influences the expression of genetic variation and covariation for traits and because it enables the evolution of phenotypic plasticity, as reviewed below.

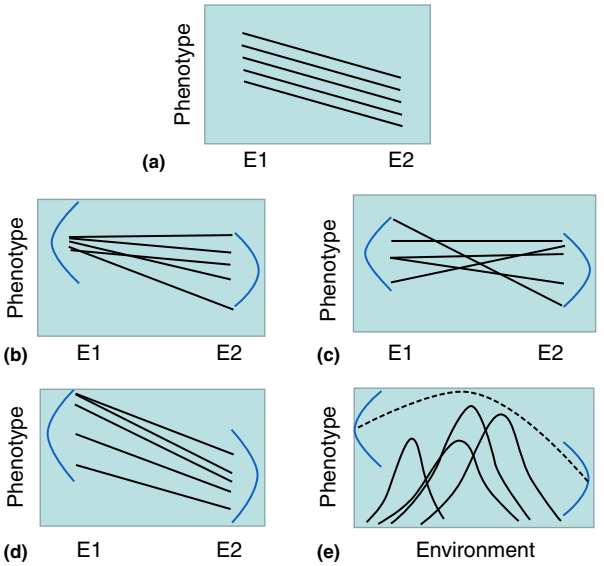

Figure 1 Norms of reaction depicting genotype-by-environment interaction and adaptive phenotypic plasticity. Within each panel, each line ('reaction norm') depicts the mean phenotype expressed by a given genotype in each of two discrete environments, E1 or E2 ((a)–(d)), or in a continuously variable environment (e). The curves on each side of each panel indicate stabilizing selection on the phenotype, whereby the optimal phenotype occurs at the peak of the curve. The optimal phenotype changes across environments. (a) Norms of reaction depicting plasticity, but no genotype-by-environment interaction, because all reaction norms are parallel. (b) Norms of reaction showing a genotype-by-environment interaction that results in the masking of genetic variation in E1 and the expression of genetic variation in E2. (c) Crossing norms of reaction that result in changes in the rank-order of genotypes across environments. The degree of genetic variation does not change across environments, but different genotypes have different relative phenotypes in each environment, and different genotypes are closest to the optimum in different environments. (d) Norms of reaction in which all genotypes exhibit phenotypic plasticity, and in which genotypes do not change rank across environments. In all cases of (b)–(c), some genotypes exhibit adaptive plasticity, such that plasticity results in the phenotype being closer to the optimum in each environment, but other genotypes do not. (e) Norms of reaction that show a 'function-valued trait,' in which the expressed phenotype varies continuously as a function of the environment. The dotted line indicates the optimal phenotype across the environmental gradient.

Predicting Genotype-by-Environment Interaction

Predicting genotype-by-environment interaction entails predicting the phenotype expressed by different genotypes in different environments. When a reaction norm is a continuous function, the plastic trait is referred to as a 'function-valued trait' (Stinchcombe *et al.*, 2012), and the reaction norm is described by the function of the response of the phenotype to the environmental factor (**Figure 1(d)**). In contrast, a discontinuous trait exhibits distinct phenotypes, or polyphenisms, even if the environment varies continuously. Functions can describe both sorts of phenotypic plasticity (with discontinuous traits being modeled by an underlying, continuously labile component combined with a threshold for switching from one phenotype to the next). Such functions can be estimated empirically for different genotypes.

A major challenge is to be able to predict environment-dependent phenotypes based on knowledge of physiological and developmental processes, as opposed to simply describing, interpolating, or extrapolating them based on empirical measurements. Process-based models have been applied to describe physiological responses to specific environmental factors, and then combine those functions to predict phenotypes in complex multi-factorial environments (e.g., Bradford, 2005; Morin *et al.*, 2007; Wilczek *et al.*, 2009; Andreini *et al.*, 2014). For example, enhanced photothermal models have been used to predict the timing of flowering or budburst; functions are combined, specifically those describing developmental rates in response to photoperiod, temperature, and 'vernalization' (exposure to cold temperatures, which switches off repressors of flowering or budburst), and the time required to attain a specific developmental threshold, such as competency for flowering or budburst, is calculated. Such process-based models are flexible because developmental progress is calculated at small time intervals; even if environments change over time, the developmental progress can be calculated for each time interval and summed over time. Thus, phenotypes that are the outcome of environment-dependent physiological or developmental processes can be predicted even in nonconstant environments.

Genotypes may differ in one or more physiological parameters in process-based models (e.g., plants may differ in the amount of cold required to induce flowering or in the optimum temperature for germination). When they do, the phenotypic consequence of genetic differences in each parameter can be assessed (Wilczek *et al.*, 2009). In this manner, not only individual phenotypes can be predicted under different environmental scenarios, but the differences among genotypes can also be predicted in each environmental scenario (Burghardt *et al.*, 2015). By providing predictions of the phenotypes expressed by specific genotypes under different environmental scenarios, these methods permit predictions of genotype-by-environment interactions.

In some cases, the genes that determine specific physiological parameters are known. Process-based models that use parameter values that represent known genetic variants in particular genes have been able to predict the phenotypic expression of known alleles. For instance, mutants in photoperiod-sensing genes are unresponsive to inductive long days and have predictably longer times to flower (Wilczek *et al.*, 2009). An ongoing goal is to be able to translate how complex genetic pathways translate into physiological parameters in such process-based models (Welch *et al.*, 2003; Promislow, 2005; Chew *et al.*, 2014).

Causes of Phenotypic Plasticity and Genotype-by-Environment Interaction

Environment-dependent phenotypes can be caused by environment-dependent gene expression, environment-dependent posttranscriptional modifications of RNA, and environment-dependent gene-product activity (West-Eberhard, 2003; Bossdorf *et al.*, 2008). If certain genes are expressed, or their gene products are active, only in some environmental conditions, then specific phenotypes will be manifest only under some conditions. Genetic (including quantitative trait locus (QTL)) studies conducted under multiple environments have clearly shown that genes associated with trait variation differ across environments, indicating some independent genetic basis of phenotypic expression in different environments (e.g., Ungerer *et al.*, 2003; Lacaze *et al.*, 2009; reviewed in Mitchell-Olds, 1995).

Environment-dependent gene expression is widely documented and contributes to phenotypic plasticity (reviewed in Aubin-Horth and Renn, 2009). Genome-wide expression studies have documented that entire genetic pathways are variously expressed according to the environment that an organism is exposed to. Transcriptional regulation of gene expression occurs via the recruitment of specific transcription factors and through chromatin remodeling (Russo *et al.*, 1996; Jaenisch and Bird, 2003; Grant-Downton and Dickinson, 2005; Berger, 2007; Mattick *et al.*, 2009). Chromatin remodeling can determine how accessible DNA regions are to transcription. Methylation, for example, alters chromatin structure (Bender, 2004) and typically converts cytosine to 5-methylcytosine. When methyl groups are added to nucleotides at the regulatory regions of genes, those genes are less transcriptionally active; the more highly methylated those regions are, the less transcriptionally active is the gene. Chromatin remodeling also occurs through changes in the histones (Grant-Downton and Dickinson, 2005; Berger, 2007). As the structure of the histone molecules is altered, the manner in which the specific DNA region it associates with is also altered, changing the accessibility of that particular sequence to transcription. Histones are modified in a variety of ways, including acetylation, phosphorylation, methylation, ubiquitylation, sumoylation, and isomerization. Acetylation of lysine is frequently associated with gene activation and de-acetylation with the repression of gene expression. However, it has been proposed that it is the combinations of modifications that determine specific responses in gene expression (Berger, 2007).

Posttranscriptional regulation includes alternative splicing (which determines the translated mRNA sequence itself), stability of the mRNA strand (which can be actively degraded in a regulated manner), transport of the mRNA to the ribosome, and binding of mRNA to the ribosome. Some of these processes depend on the production of other gene products, and as such have the potential to be regulated in an environment-dependent manner and therefore involved in phenotypic plasticity. For most of them, however, their environment-dependence has not been well elucidated, especially in regard to ecologically relevant environmental variation.

Environment-dependent gene-product activity can be a major contribution to phenotypic plasticity. Some gene products are direct environmental sensors and initiate the process of signal transduction by changing conformation in response to environmental stimuli. Photoreceptors, for example, change conformation in response to light, enabling them to bind to their interactors to initiate changes in gene expression; in contrast, other gene products might be degraded in light. Environmental factors such as temperature can alter the kinetic activity of proteins, binding properties, or propensity to denature. This could result in a continuous modulation of phenotype, or if the downstream response requires a threshold level of substrate, it could result in a discontinuous switch of phenotype (Pigliucci, 2001).

The environment itself may directly interact with enzymatic reactions by determining the presence of a substrate. If a substrate is supplied by the external environment, then the reaction will be initiated only under conditions in which the substrate is present, and the resulting phenotype will be environment-dependent. Phenotypes that vary with nutritional status, such as size or diet-dependent coloration, are examples. Other elicitors that resemble immune responses, such as insect herbivores or pathogens (reviewed in Howe and Jander, 2008), can initiate complex reactions such as the production of secondary compounds, trichomes or hairs, or other modifications of leaf form in plants.

Although the genetic basis of phenotypic plasticity can entail all of the above mechanisms – transcriptional regulation, posttranscriptional modification, gene-product activity, and contributions of substrates – the basis of genotype-by-environment interaction depends on which of those processes are genetically variable in natural populations. Genotype-by-environment interaction does not exist unless the genes that regulate plasticity harbor allelic variation. Variation in gene expression could be caused by variation in transcription factors or other *trans*-regulating factors, or variation in *cis*-regulatory

regions. Variation in posttranscriptional regulation could involve variation in splice sites or DNA sequence variation in the genes that regulate mRNA stability and transport. Genetic variation in gene-product stability would be determined by variation in coding sequences of those genes. Characterizing which genes that regulate phenotypic plasticity also vary in or among natural populations is important for understanding which genes have contributed to its adaptive or maladaptive evolution.

Quantitative-Genetic Consequences of Genotype-by-Environment Interaction

Genotype-by-environment interaction can influence the expression of genetic variation, the nature of genetic correlations among characters, and the ability of phenotypic plasticity itself to evolve.

When different genotypes exhibit different patterns of phenotypic plasticity, the magnitude of differences among genotypes can change with the environment (Falconer, 1952; Via and Lande, 1985). That is, genetic variation can be less ('masked') in one environment and greater ('released,' or 'expressed') in another (**Figure 1(a)**). Genetic variation is a key parameter of evolutionary potential and heritability: unless variation in a trait is genetically based, natural selection cannot cause a change in the mean trait from one generation to the next. The greater the genetic variation, all else being equal, the greater is the heritability and the greater the potential for an evolutionary response to selection (Falconer and Mackay, 1996). Therefore, genotype-by-environment interaction can influence which environments can produce evolutionary responses to selection and how large those evolutionary responses can be.

Because genotype-by-environment interaction can mask genetic variation in some environments, such that many genotypes have the same phenotype, genetic variation can persist in those environments (Via and Lande, 1985). In environments in which genetic variation is expressed, in contrast, natural selection can cull genotypes that produce less adapted phenotypes and deplete genetic variation. Masking and its consequence for the persistence of genetic variation can also allow the opportunity for the expression of genetic variation in a novel environment that has not yet experienced natural selection. Genotypes that exhibited similar phenotypes in one environment may differ in a novel environment, and thereby provide genetic variation upon which natural selection can act. In this manner, genetic masking can contribute to adaptation in novel environments.

Genotype-by-environment interaction can also influence genetic correlations among characters expressed in different environments. If different traits have different patterns of plasticity, then genetic correlations between those traits can be environment-dependent (**Figure 2(a)**). Genetic correlations among traits influence trait evolution (Falconer and Mackay, 1996). A trait (e.g., tall stems) can directly increase fitness, and that trait can increase in frequency as a consequence. If a second trait is correlated with that trait (e.g., large leaves), it too can change in frequency even though it did not influence fitness favorably (**Figure 2(b)**). It is said to be under 'indirect' or 'correlated' selection. If selection on the first trait is strong, and the correlation between traits is large, then indirect selection can actually be stronger than direct selection on the second trait, even causing that second trait to evolve maladaptively. When the strength (or direction) of the correlation among traits changes with the environment, the strength (or direction) of indirect selection also changes. Therefore, environment-dependent genetic correlations can influence whether and how much a trait evolves adaptively or maladaptively in a given environment.

Genotype-by-environment interaction may also change the phenotypic rank-order of genotypes in different environments (**Figure 1(b)**). This is the case when reaction norms cross. A genotype that has the greatest value of a phenotype in one environment may have an intermediate or small value in another environment. If those phenotypes are under natural selection, genotype-by-environment interaction determines which genotype has the highest fitness in each environment.

If different genotypes have different responses to the environment, then genetic variation for plasticity exists, and it can therefore respond to natural selection. Genotype-by-environment interaction is a necessary condition for the evolution of phenotypic plasticity. Greater genotype-by-environment interaction indicates more genetic variation for plasticity, and consequently a greater opportunity for responses to selection on plasticity.

The Evolution and Adaptive Value of Phenotypic Plasticity

The evolution of phenotypic plasticity depends on the adaptive value of expressed phenotypes within each environment, costs of being able to exhibit plasticity, and constraints on abilities to attain optimal phenotypes in any given environment (**Figure 3**).

The adaptive value of the expressed phenotypes depends on the strength and mode of natural selection in each environment. The original models of the evolution of phenotypic plasticity are formulated such that individuals of a given genotype are distributed across a heterogeneous environment and experience each environment with a given probability (Falconer, 1952, 1990; Via and Lande, 1985; Via, 1987). The optimum phenotype is considered to differ among environments. Barring any constraints or costs, the optimum level of plasticity would be that which results in the expression of the optimum phenotype in each environment.

Constraints do occur, however. The optimal degree of plasticity, considering these constraints, depends on the probability of experiencing each environment, the strength and mode of natural selection on the phenotypes in each environment, additional costs of the ability to alter one's phenotype (aka 'costs of plasticity') that are independent of selection on the expressed phenotypes per se (Via and Lande, 1985; van Tienderen, 1991; Dewitt *et al.*, 1998), and genetic constraints on the ability to express optimal phenotypes.

If a genotype experiences only one environment, there is no adaptive advantage to phenotypic plasticity, and the constraints and costs of plasticity would favor a specialist to that one environment. Likewise, if a genotype experiences one

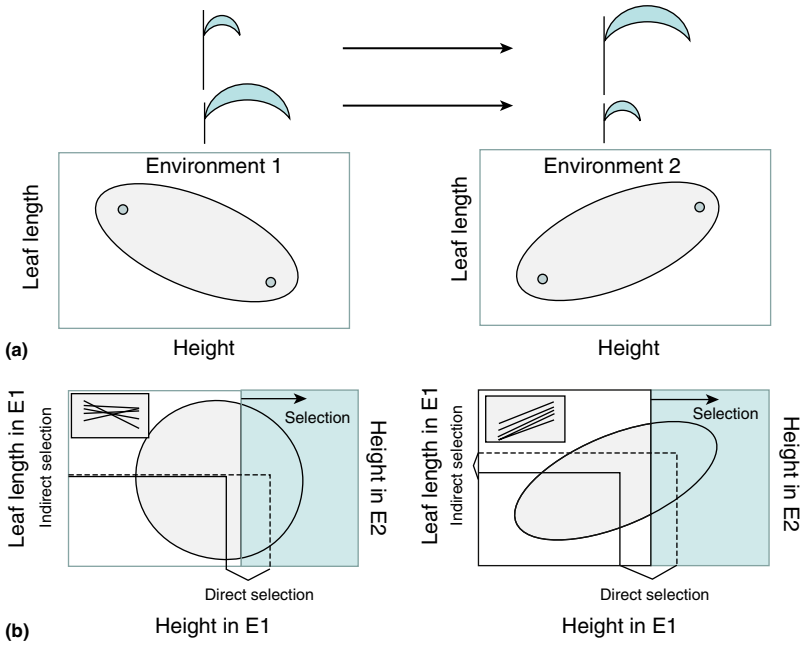

Figure 2 Environment-dependent genetic correlations caused by genotype-by-environment interaction, and their evolutionary consequences. (a) The cartoon shows two genotypes of plants in each of two environments. Stem height does not exhibit plasticity from one environment to the other, but leaf length does. When different traits exhibit different degrees of phenotypic plasticity, the correlation between them can change across environments. The correlation in Environment 1 is negative (long stem and short leaf, or short stem and long leaf), indicated in the graph below it; the two points represent the genotypes illustrated in the cartoon, and the ellipse represents a cloud of points from a sample of individuals that show a negative correlation between the traits. The correlation is positive in Environment 2. (b) Selection and response to selection with correlated characters. The left-hand graph shows an example in which the correlation between two characters (height and leaf length, both expressed within any given environment, E1) is zero, and the right-hand graph shows an example in which the correlation between the two characters is positive. Ellipses indicate a cloud of points from a sample of individuals, as above. The solid line intersecting each axis indicates the mean phenotype of the population for each trait. In each case, selection favors taller plants, and the shaded portion represents the proportion of the population that survives selection. The dotted line intersecting each axis represents the mean phenotype of the surviving population after selection. In the case of no correlation among traits (left), selection on height changes the population mean value of height (direct selection on height), but has no effect on the population mean value of stem length after selection (no indirect selection). In the case of a positive correlation between traits (right), selection on height changes the population mean value of height (direct selection on height), and also changes the population mean value of stem length after selection (indirect selection). Stem length therefore can evolve even if selection does not act directly on it, if it is correlated with a trait that is the direct target of selection (height in this example). Insets: The evolutionary consequences of correlations among traits also applies to correlations of the same trait expressed in different environment, in this case, height in E1 and height in E2 (indicated on the *y* axis on the right-hand side of graphs). The inset shows examples of reaction norms that result in zero correlations across environment (left) and positive correlations across environments (right). In the case of positive correlations across environment (right), selection on the trait in E1 can cause correlated selection of the phenotype that is expressed in E2.

environment much more frequently than another, all else being equal, its evolved phenotype should be closer to the optimum in the more frequent environment, presuming it cannot attain the optimum in both. Similarly, if natural selection is much stronger in one environment than another, all else being equal, the evolved phenotype would more quickly attain the optimum in the environment with stronger selection. The outcome, however, also depends on differences in the quality of each environment (and thereby whether natural selection is 'hard' or 'soft'): if one environment is so harsh that even highly adapted individuals produce very few offspring ('hard selection'), whereas a small phenotypic adjustment might increase fitness greatly in a high-quality environment, then phenotypes would evolve toward the optimum in the high-quality environment, in which they can attain a proportionally higher fitness advantage. The overall outcome therefore depends on the balance between the frequency of the different environments that are experienced, the strength of selection in each environment, and the quality of each environment (Via and Lande, 1985; van Tienderen, 1991; Gavrilets and Scheiner, 1993; Scheiner, 1993; DeWitt and Scheiner, 2004).

If a cost of being plastic exists, a plastic individual will have lower fitness in a given environment than a nonplastic individual even if it expresses the exact same phenotype in that environment as the nonplastic individual (van Tienderen, 1991). Such costs may be caused by the requirement to maintain sensory systems and signal transduction mechanisms that would not be maintained in nonplastic individuals who do not respond to environmental conditions (DeWitt *et al.*, 1998). The magnitude of that cost influences the adaptive value of plasticity directly; if large, it can favor a nonplastic specialist to one environment, or a nonplastic generalist with an intermediate suboptimal phenotype, depending on the

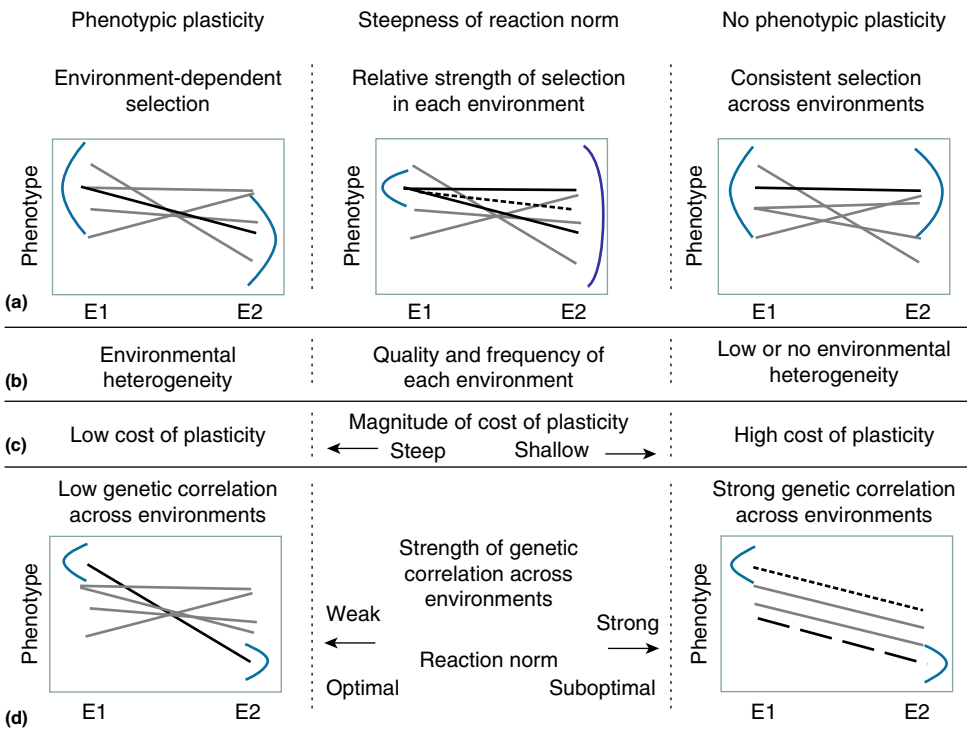

Figure 3 Factors that influence the evolution of plasticity. (a) Norms of reaction in response to two environments. The curves on each side of each panel indicate stabilizing selection on the phenotype, whereby the optimal phenotype occurs at the peak of the curve. Black lines show the reaction norm that produces the adaptive phenotype in both environments. Environment-dependent selection (left) favors plasticity, while consistent selection across environments (right) does not. The relative strength of selection in each environment (center) influences the slope of the evolved reaction norm. Selection is stronger in environment 1 (E1) than in E2. Two reaction norms give adaptive phenotypes in both environments, while four give equivalently adaptive phenotypes in the second environment. The mean adaptive reaction norm (dotted line) has a slope that is shallower than that in the left-hand panel, and it expresses a phenotype that is closer to the phenotype that is favored in the environment that has stronger selection (E1). (b) Plasticity is favored when heterogeneity exists in the selective environment. The slope of the evolved reaction norm is influenced by the frequency and quality of selective environments, favoring phenotypes that are adaptive in the more frequent and higher-quality environment. (c) Costs of plasticity disfavor the evolution of plasticity, and the magnitude of the cost influences the slope of the optimal reaction norm. (d) Low genetic correlations across environments (left) indicate genotype-by-environment interaction and genetic variation for phenotypic plasticity. They permit the evolution of phenotypic plasticity. High genetic correlations across environments (right) prevent the evolution of plasticity. The dotted reaction norm expresses the adaptive phenotype in E1 but not E2; the dashed line expresses the adaptive phenotype in E2 but not E1. No genotype expresses the optimum phenotype in both environments. More generally, strong genetic correlations across environments impede the evolution of an optimal reaction norm (center).

frequency of environments experienced and the strength of selection in each environment as discussed above.

Genetic constraints on the evolution of plasticity include a lack of genetic variance for a trait within any given environment; if no genetic variant exists that can produce the optimal phenotype in any given environment, then the phenotype cannot evolve to the optimum. Therefore, genetic variation must be expressed for the trait in each of the environments it experiences.

Genetic constraints on the evolution of plasticity also include genetic correlations. As with any set of correlated characters under selection, selection on one trait can constrain adaptive evolution of correlated traits, as discussed above. In the case of phenotypic plasticity, one must consider not only correlations among multiple traits expressed in any given environment, as in standard quantitative-genetic models of trait evolution, but also the correlations among traits expressed in different environments (Falconer, 1952, 1990; Via and Lande, 1985; Via, 1987; van Tienderen and Koelewijn, 1994) or 'across-environment genetic correlations' (**Figure 2(b)**). For example, if genotypes that are the tallest in one environment are also the tallest in the other environment, then height is positively genetically correlated across environments. If selection varies across environments, favoring tall individuals in the first environment but short in the second, tall genotypes would have high relative fitness in the first environment but low relative fitness in the second. The across-environment genetic correlations also influence the degree to which selection imposed by one environment can influence the expression of that trait in other environments (**Figure 2(b)**).

With such across-environment genetic correlations, possible outcomes include the evolution of a specialist genotype (which expresses a phenotype that is optimal in one environment but maladaptive in the other), a plastic generalist (which expresses suboptimal but different phenotypes in each environment), or a nonplastic generalist (which expresses a

constant but suboptimal phenotype in both environments; Bradshaw, 1965; Via and Lande, 1985; Via, 1987; Whitlock, 1996). The outcome depends on the degree to which plasticity is adaptive, as discussed above (including the strength and mode of natural selection on phenotypes in each environment and costs of plasticity), and on the strength of the genetic correlations within (and across) environments.

Ecological and Evolutionary Outcomes of Genotype-by-Environment Interaction

Genotype-by-environment interaction enables the evolution of plasticity. The evolution of phenotypic plasticity, in turn, has important ecological and evolutionary consequences including the ability to maintain fitness in variable environments (discussed above), the facilitation of adaptation to novel environments and the evolution of novel specialist phenotypes, and the persistence of populations in the face of environmental change (Figure 4).

Plasticity has been implicated in adaptation to novel environments. It has been proposed that phenotypic plasticity can enable the expression of a phenotype that is closer to the optimum in a novel environment than would be the case if plasticity were absent. While the trait need not express the actual optimum at first, a trait that is closer to the optimum would nonetheless provide a demographic advantage by slowing the rate of, or preventing, extinction long enough for the population to adapt (Baldwin, 1896; Bradshaw, 1965; Sultan, 1987; West-Eberhardt, 1989, 2003; Schlichting and Pigliucci, 1998; Price *et al.*, 2003; Yeh and Price, 2004). The success of plasticity in improving fitness in novel environments depends on how similar the novel environment is to the ancestral environment that elicits plasticity – both in terms of the cues that elicit plasticity and how well those cues predict fitness in that environment. That is, the direction of plasticity must be adaptive in the new environment. Some evidence that plasticity has contributed to local adaptation in this manner is in the form of 'co-gradient variation,' in which the direction of trait plasticity elicited by an environment is in the same direction as genetic differentiation in the trait (as opposed to 'counter-gradient variation' in which the direction of plasticity is opposite to that of genetic differentiation).

Plasticity is hypothesized to contribute to genetic adaptation to novel environments through a process called 'genetic accommodation' (Figure 4), a form of reaction-norm evolution whereby a phenotype that was originally induced by the environment contributes to adaptive evolution in that new environment and can cause the phenotype to be expressed even without the environmental cue (Waddington, 1953, 1961; West-Eberhard, 2003; Crispo, 2007; Braendle and Flatt, 2006; Schlichting and Wund, 2014). Genetic accommodation includes several types of reaction-norm evolution, but generally entails the evolutionary change of a plastic phenotype in the adaptive direction in the novel environment. This could occur through an initial increase in plasticity to attain the optimal phenotype in the novel environment, while the phenotype expressed in the ancestral environment remains the same (Figure 4(a)). Subsequently (or alternatively), the trait expressed in the ancestral environment can also change, recovering (or maintaining) the original function of response to the environment (e.g., the slope of the reaction norm). When this occurs, the trait expressed in the ancestral

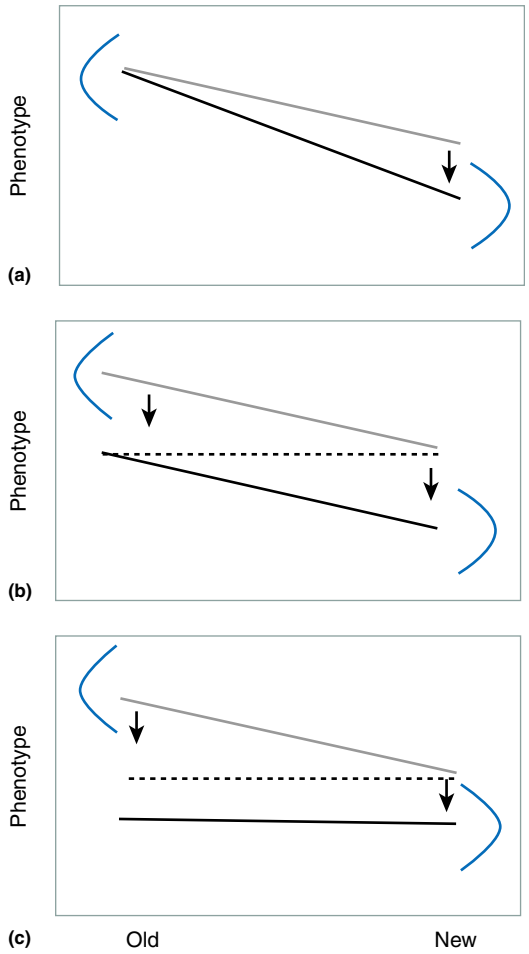

Figure 4 Scenarios of reaction-norm evolution leading to adaptation to novel environments. Mean reaction norms of a population in an 'Old' or ancestral environment and a 'New' environment. Gray lines indicate the reaction norm before evolution (the ancestral reaction norm), and black lines indicate the newly evolved reaction norm after colonization of the new environment. Arrows indicate the direction of evolution of the reaction norm. The curves on each side of each panel indicate stabilizing selection on the phenotype, whereby the optimal phenotype occurs at the peak of the curve. The optimal phenotype changes across environments. (a) The evolution of increased phenotypic plasticity caused by adaptation of the phenotype to the optimum in the new environment, with no change in the phenotype expressed in the ancestral environment. (b) Evolution toward the optimum phenotype in the new environment with no change in the slope of the reaction norm. The phenotype expressed in the ancestral environment also decreases. (c) Genetic assimilation: Evolution toward the optimum phenotype in the new environment, with a loss of plasticity, or 'canalization.' Dotted lines in (b) and (c) indicate the phenotype that was formerly expressed by the ancestral reaction norm in the new environment, in comparison to the phenotype expressed by the newly evolved reaction norm in the old environment; the phenotype that was expressed by the ancestral reaction norm only after exposure to the new environment is now expressed by the newly evolved reaction norm even without exposure to the environmental stimulus.

environment is closer to the phenotype that was originally only induced by the novel environmental stimulus (**Figure 4(b)**).

A more extreme manifestation of this sort of reaction-norm evolution is the process of 'genetic assimilation,' whereby a phenotype that was originally induced by the environment becomes expressed in a consistent (or 'canalized') manner in all environments, even when the environmental cue is absent (**Figure 4(c)**). That is, the phenotype, formerly plastic, becomes constitutively expressed in one form, nonplastic (Waddington, 1953, 1961; Schlichting and Pigliucci, 1998; Pigliucci *et al.*, 2006; Crispo, 2007; Lande, 2009; Schlichting and Wund, 2014). The first phase in this scenario is that plasticity facilitates adaptation to a novel environment, while the second phase is the constitutive expression of the adaptive phenotype in its new environment. As such, plasticity is hypothesized to contribute not only to local adaptation but actually to specialization to novel environments. Only a small number of experimental studies have documented this phenomenon (Waddington, 1953; Rutherford and Lindquist, 1998; Queitsch *et al.*, 2002). While it is generally accepted that adaptive plasticity can increase fitness in novel environments, what is more controversial is how plasticity would be lost, to result in canalization and specialization.

The ability of plasticity to improve fitness in novel environments has been also shown theoretically to increase the probability of persistence of populations under scenarios of environmental change (Lande, 2009; Chevin *et al.*, 2010). Plasticity to new environments, if it is in an adaptive direction, can increase the demographic performance of populations and reduce their risk of extinction in changing environments. It can also allow populations to persist under more rapid environmental change than would be possible without plasticity. Therefore, the role of phenotypic plasticity in mitigating adverse effects of rapid environmental change is a topic of great current interest.

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See also: Climate Change, Quantitative Genetics and. Epigenetic Inheritance. Maternal Effects

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Genotype to Phenotype: Insights from Evo-Devo

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Glossary

Canalization Evolution of development to a state that minimizes the variation in phenotype.
Epistasis Nonadditive interactions between alleles at different loci.
Phenotype Any measurable property of an organism.
Phenotype landscape A function, potentially of many dimensions, mapping phenotype to underlying genetic and environmental factors.
Pliotropy A situation in which one genetic locus influences multiple phenotypic traits.

Nearly all questions about the mechanics of evolution come down to variation and transmission – how deterministic and stochastic processes shape the distribution of phenotypic variation, and how this variation is transmitted across generations? To the extent that genes are what is transmitted across generations, all theories of phenotypic evolution therefore must involve some sort of model, implicit or explicit, of how phenotype is related to genotype.

This key relationship is often described as the 'genotype–phenotype map' (Landry and Rifkin, 2012), though this term is somewhat misleading – since environmental factors that are not part of the genome play important roles in development. Thus, a particular genotype does not actually map to a particular phenotype, but rather may produce different phenotypes in different environments.

Genotype–phenotype maps are often represented as a network linking different genes to different phenotypic traits. This is a good way to capture pliotropy (Wagner and Zhang, 2011), but it does not allow us to describe the inherently nonlinear aspects of developmental processes. Another approach to visualization of the relationship between genotype, environment, and phenotype (which further allows us to construct mathematical models of developmental evolution) is to describe phenotype as a function of the expression of underlying genetic and environmental factors (hereafter referred to as underlying factors). Such a function is a phenotype landscape (Figure 1). Though the name implies a two-dimensional surface, we can apply the concept to any number of underlying factors. Given a phenotype landscape (in as many dimensions as are important) and a distribution of variation of underlying factors, we can solve analytically for how a population will evolve in response to any given selection regime. (Note that, properly, any measurable property of an organism is part of its phenotype – including its genotype.)

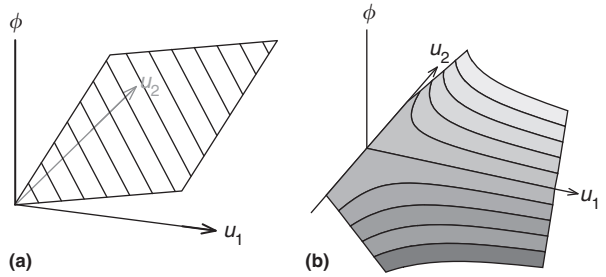

Figure 1 Phenotype landscapes. ϕ is a phenotypic trait. u_1 and u_2 are underlying genetic or environmental factors that influence ϕ. (a) A case in which the underlying factors contribute additively to phenotype. Additive landscapes are always uncurved. (b) A case in which u_1 and u_2 interact nonadditively (i.e., there is epistasis).

The various mechanisms by which genetic and environmental variation generate phenotypic variation are the subject of developmental biology, so one might expect development to be an explicit part of most evolutionary models. This is not the case. Though all evolutionary theories must involve some model of genotype–phenotype relationships, this model need not be explicit. In many cases, it is folded into simplifying assumptions. The most common such simplifying assumption is that phenotype is a linear function of genotype. This assumption is nearly universal in those fields, such as quantitative genetics and evolutionary game theory, that study morphological or behavioral evolution.

We can visualize the assumption of additive gene action as an uncurved phenotype landscape (Figure 1(a)). In this case, the landscape looks the same for all possible values of underlying genetic or environmental factors. Any nonlinear interaction between underlying factors induces epistasis (nonadditive gene action), which causes the landscape to curve (Figure 1(b)), and makes it so that the local geometry of the landscape is different at different places on it.

Taken literally, the assumption of linearity would mean that different genetic elements each make some contribution (generally assumed to be small), and that we could predict phenotype by simply adding up all of these contributions (as well as the contribution of the environment). In fact, nobody takes this assumption literally. Everything that we know about development, from the regulation of gene expression to tissue differentiation, suggests that underlying genetic and environmental factors interact nonadditively in the development of adult phenotype.

The assumption of additive gene effects is, however, sometimes a reasonable approximation. This is because of the fact that even traits that develop through highly nonlinear processes may look approximately additive if we follow their evolution over only a short time. Figure 2 illustrates this using a nonlinear phenotype landscape. At any given time, the distribution of variation in a population covers only a small region of the landscape. If this region is small enough, the

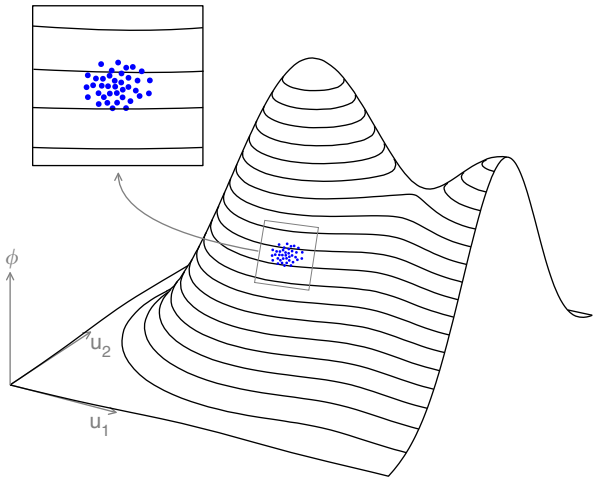

Figure 2 A nonadditive landscape may appear approximately additive to a population that covers only a small part of it. In this case, an additive model would be a reasonable approximation for a few generations.

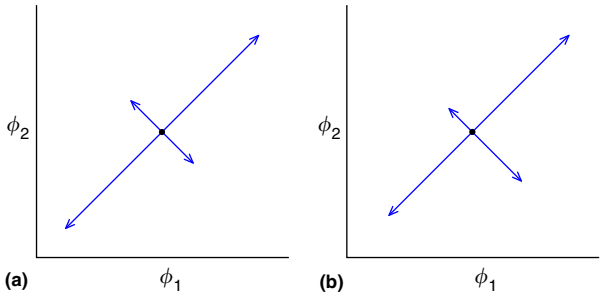

Figure 3 Responses to selection (arrows) on two traits that exhibit strong additive genetic covariance. (a) The prediction of additive models is that the response in opposite directions will be of the same magnitude. (b) We often see asymmetrical responses to selection in opposite directions. This can result from slight differences in patterns of epistasis for the two traits.

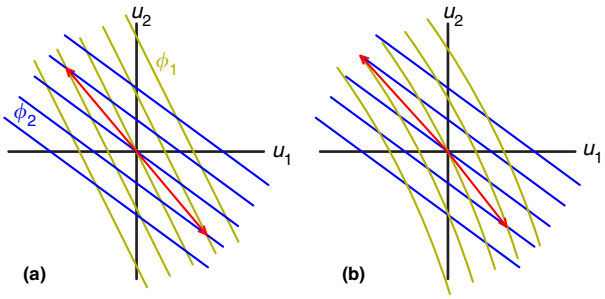

Figure 4 Phenotype landscapes (as contour lines) for two traits that are genetically correlated. The red arrows show the change in the underlying factors that would be necessary to increase one trait by one unit while decreasing the other trait by one unit. (a) In a completely additive case, the responses in opposite directions are symmetrical; it is just as easy to increase ϕ_1 (yellow contours) while decreasing ϕ_2 (blue contours) as to move in the opposite direction, increasing ϕ_2 while decreasing ϕ_1. (b) When the landscapes have slightly different curvature (corresponding to slightly different patterns of epistasis), the responses become asymmetrical. The direction of the asymmetry depends on the exact patterns of epistasis.

landscape will appear approximately linear to the population. In such a case, it will appear that most of the phenotypic variation in the population is due to the additive effects of alleles (Hill *et al.*, 2008). This is why quantitative genetics often works well on a local scale.

So, why should we be concerned with the complexities of development if models that ignore them often suffice? First, models that assume additive gene action are, at best, approximations. Sometimes they are acceptable approximations, but sometimes not. Second, some important questions lie outside the purview of additive models that focus on the variation currently existing in natural populations. These include questions about the evolution of quantitative genetic parameters, such as heritability, as well as why we do not see certain phenotypes in certain groups of organisms. Finally, we sometimes want to study the evolution of developmental processes in their own right, which we cannot do using models that assume that those processes are so simple that they can be ignored. I consider examples of each of these cases below.

When the Linear Approximation Does Not Suffice

Models based on the assumption of additivity are often good at predicting the general direction of evolution, given a selection regime and the current pattern of genetic variation and covariation between traits (Falconer and Mackay, 1996; Roff, 1997). For example, quantitative genetics theory predicts that when there is a nonzero genetic covariance between two traits, the population will be able to evolve more quickly in response to selection that goes with the genetic covariation, than in response to selection against it. Thus, if height and weight show a positive genetic covariance, then selection to increase both height and weight should produce a larger response than selection to increase height while decreasing weight. This prediction has been born out in both laboratory and agricultural studies.

Some other predictions, though, have not fared so well. For example, the standard theory (based on additive gene action) predicts that the response to selection in opposite directions should be symmetrical (Figure 3). Selection for decreased height and decreased weight should produce the same magnitude of change (though in the opposite direction) as does selection to increase both height and weight. The same should hold in selection against the correlation; selection for increased height and decreased weight should produce the same magnitude of change, in the opposite direction, as does selection for decreased height and increased weight. In fact, though, a number of studies have found that selection in opposite directions tends to yield different magnitudes of response (Frankham, 1990; Rice, 2008). In some cases, this asymmetry can be quite pronounced, making the population essentially unresponsive to selection in certain directions.

If genes really did contribute additively to phenotype, we would not expect such asymmetries. They are expected, though, when we allow for even weak epistatic interactions between genes (Rice, 2008). Figure 4 illustrates this. Two different traits correspond to two phenotype landscapes over

the same set of underlying factors. If the covariation between the traits results from shared developmental processes (being influenced in similar ways by many of the same loci or environmental factors), then the two landscapes nearly line up, as shown in Figure 4. (Covariation between traits can also result from covariation between the underlying factors, though this is more transient as it is subject to being broken up by recombination.) Because the two landscapes nearly line up, a slight change in the curvature of one relative to the other – corresponding to slightly different patterns of epistasis due to different developmental processes – can result in substantial asymmetry in the joint evolution of the two traits.

Note that this effect should be much less pronounced for landscapes that slope at different angles (corresponding to lower genetic covariance between the traits). In a sense, highly correlated traits amplify the effects of local epistasis, making the assumption of additivity a poor approximation for such traits.

Development and Morphological Diversity

One of the early goals of research in evolutionary developmental biology (evo-devo) was to explain why certain traits that might seem to be adaptive fail to evolve in particular groups of organisms because of 'developmental constraints.' The idea that developmental constraints impose hard boundaries on adaptive change was behind many of the early arguments for expanding evolutionary theory to more fully encompass development (Goldschmidt, 1940; Gould and Eldredge, 1977; Riedl, 1978). Unfortunately, some authors tended to underestimate the efficacy of local adaptation, and rejection of developmental constraints as a ubiquitous phenomenon was behind some of the early skepticism of evo-devo research (Charlesworth *et al.*, 1982; Maynard Smith *et al.*, 1985).

In fact, nearly all quantitative traits in natural populations exhibit enough heritable variation to respond quickly to directional selection. However, the fact that developmental constraints rarely impose hard limits on adaptive evolution does not mean that understanding development is not important for a full understanding of patterns of phenotypic diversity. We will consider two examples that illustrate how understanding development is important in determining why certain traits are not observed in certain groups of organisms. In one case, development itself provides the explanation. In the other case, understanding development allows us to rule out developmental constraint as an explanation.

Image forming eyes have arisen many times and in a number of metazoan phyla (Salvini-Plawn and Mayr, 1977; Land and Fernald, 1992; Ogura *et al.*, 2004). Since such eyes involve focusing an image on a field of receptors, it is tempting to think of them as analogous to cameras. Since a particularly simple camera uses a pinhole, with no lens, to focus the image, we might expect to see pinhole eyes arising in the evolution of image forming organs.

In fact, pinhole eyes have arisen at least twice in mollusks (in the chambered nautilus (*Nautilus pompilius*) and in the giant clam (*Tridacna gigas*) (Land, 2003)). By contrast, they do not appear to have ever arisen in vertebrates. One might speculate that a pinhole focusing mechanism was simply never selected for in vertebrates, but this would only make it more mysterious that this type of eye has arisen multiple times in mollusks.

This pattern makes sense when we consider the developmental differences between the molluskan eye and the vertebrate eye. The optic cup of mollusks develops as an inpocketing of the epithelium. In species with a lens, the lens forms as the outer part of the optic cup begins to close. If no lens forms, though, the closing optic cup naturally passes through a state in which there is a small opening – essentially acting like a pinhole camera (Figure 5).

By contrast, the vertebrate optic cup forms as an outpocketing from the developing brain. This neural-derived tissue ultimately induces the formation of a lens in the overlying ectoderm. Even if no lens formed, though, there would still be a layer of tissue overlying the presumptive optic cup. The vertebrate eye thus does not naturally pass through a pinhole stage. Note that this does not mean that a pinhole eye could not possibly evolve in vertebrates, only that it is unlikely in the absence of just the right kind of directional selection for that particular morphology.

Recent work by Ogura *et al.* (2013) has shown that the six 3/6 regulatory pathway, which is associated with lens formation in other organisms, is not active in *Nautilus*. Knocking out the homologous regulatory system in a vertebrate would produce a nonimage forming eye. Doing so in *Nautilus* produces a functional pinhole eye. It thus appears that the presence of pinhole eyes in mollusks, and their absence in vertebrates, is best explained in terms of the likelihood of that morphology arising through a relatively minor developmental change.

In some cases, an understanding of development can allow us to rule out developmental constraint as an explanation for an observed distribution of phenotypes. Ammonites were a group of shelled cephalopod mollusks that were highly diverse but went extinct during the end cretaceous mass extinction 66 mya. Most ammonites had planispitral shells – coiling in a single plane like modern Nautilus (to which they were not closely related). A few ammonite species coiled along an axis, like most modern snails, but some – called heteromorphs – had shell forms unlike any other mollusk. These included some that coiled in one manner then abruptly changed, as well as some that did not coil around any single axis at all (Figure 6). Because no modern mollusk exhibits such a strange shell form, whereas a number of ammonites did, we might be tempted to suspect that the developmental processes that allowed for diverse heteromorph shells went extinct with the rest of the ammonites.

In fact, though, it appears that the developmental potential to build heteromorph shells is present in modern gastropods. Using a model of shell growth, Rice (1998) showed that some of the most odd-looking heteromorph ammonite shells could be produced by a very simple mechanisms – having the animal rotate within the shell as it is producing new shell material (Figure 7). That this is what some heteromorphs were actually doing is suggested by the pattern of sculpture on the outside of the shell of *Dydimoceras* (Figure 7).

We know that the potential to rotate the mantle within the growing shell is present in modern day shelled mollusks. For

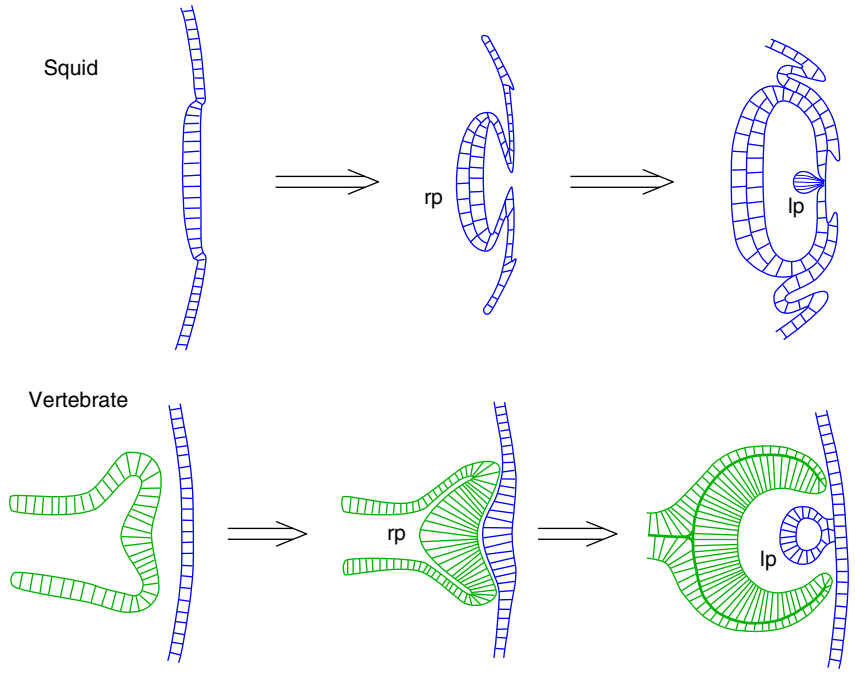

Figure 5 Development of image forming eyes in squid and vertebrates. rp = retinal primordium, lp = lens primordium. In the squid, both the retina and lens develop from a single epithelial optic placode. In vertebrates, the retina is derived from neuroectoderm (green), while the lens is induced to form in the overlying ectoderm (blue). Note that the squid eye passes through what is effectively a pinhole camera stage, whereas the vertebrate eye never does so, since the retina is always covered by layers of tissue.

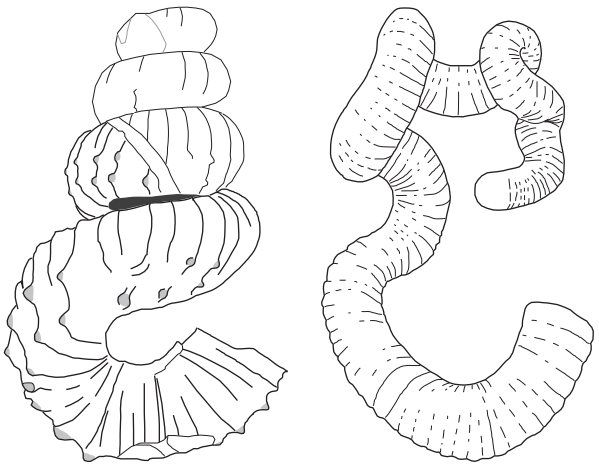

Figure 6 Two heteromorph ammonites (redrawn from Rice, S.H., 1998. The bio-geometry of mollusc shells. Paleobiology 24, 133–149). Left: *Didymoceras*. Right: *Nipponites*. Note the rows of sculptural bumps on *Didymoceras* (highlighted in gray).

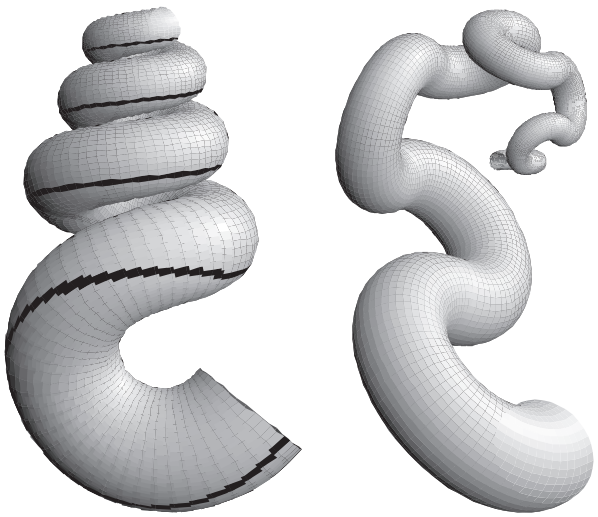

Figure 7 Models of *Didymoceras* and *Nipponites* produced by having the mantle rotate back and forth as the shell grows – with one cycle of rotation for *Didymoceras* and repeated cycles for *Nipponites*. The black line on the simulated *Didymoceras* traces the path followed by a particular point on the mantle. Compare with the trajectory of the sculptural bumps in **Figure 6**.

example, Liew *et al.* (2014) recently showed that the modern irregularly coiled gastropod *Plectostoma concinnum*, for which the last coil is upturned, achieves its odd shape by rotating the mantle within the growing shell. The same trick, though less pronounced, is seen in the snail appropriately named *Distorsio*, in which the axis of coiling changes slightly in alternate whorls (Checa and Aguado, 1992). Though none of these modern species produces a shell as spectacularly irregular as *Nipponites*, the difference is only a matter of degree – involving only the amount and timing of rotation. We thus conclude that the lack of heteromorph forms in modern mollusks is likely due to these morphologies not being selected for, rather than lack of variation on which such selection could act.

Note that though it seems that we can rule out 'developmental constraint' as an explanation for the lack of heteromorph

phenotypes in modern mollusks, we can conclude this only because we have a model of development of this trait. Understanding how a trait develops is thus sometimes essential even to confirming that observed phenotype distributions are structured by selection.

The Evolution of Development

Though much of the early interest in evolutionary developmental biology centered around explaining how development might constrain or facilitate different sorts of phenotypic evolution, a substantial amount of recent research focuses instead on how developmental processes themselves evolve. Additive models are of little use here, since they assume that the phenomenon under study – complex interactions between gene products – does not exist. We are thus forced to consider evolution on curved phenotype landscapes.

On an uncurved (additive) landscape, evolution along a contour is completely neutral with respect to selection, since moving along a contour does not change the distribution of phenotypic variation. On a curved landscape, though, things are different. The slope of the landscape changes as a population moves along a contour, and as it does so the amount of phenotypic variation that results from a particular amount of underlying genetic or environmental variation changes. In other words, movement along a contour leads to change in the shape of the phenotype distribution, even if the mean phenotype does not change (Rice, 2002).

The best understood kind of selection that acts on the shape, rather than the mean, of the phenotype distribution is stabilizing selection – defined as selection that reduces phenotypic variance (Lande and Arnold, 1983; Rice, 1998). Stabilizing selection favors the evolution of canalization, or phenotypic robustness in the face of underlying genetic or environmental variation.

On a phenotype landscape, the amount of phenotypic variation that results from a given amount of underlying genetic variation is determined in large part by the slope, or gradient, of the landscape. A low slope means that a large amount of underlying genetic variation translates into relatively little phenotypic variation. By contrast, a high slope amplifies the underlying variation – producing high phenotypic variance. We thus expect a population experiencing stabilizing selection to evolve toward points of minimum slope along a contour (the mathematical theory leading to this conclusion is presented in Rice (2002)).

This makes canalization something that we can test for empirically. Since most points on a phenotype landscape are not near a point of minimum slope, demonstrating that a particular population is at or near such a point is evidence that its development was structured in part by stabilizing selection. A more compelling case could be made if we could show that a population, in adapting to a new environment, moves from a point of minimum slope (i.e., maximum canalization) on one contour to a new point of minimum slope on a different contour. This has been demonstrated in at least one case.

Scoville and Pfrender (2010) studied *Daphnia* from mountain lakes with and without predatory fish. In the absence of visual predators, the high UV intensity at high elevations selects for high levels of melanin. However, this also makes individuals more visible; so the presence of predatory fish selects for lower melanin levels than would be optimum in the absence of visual predators.

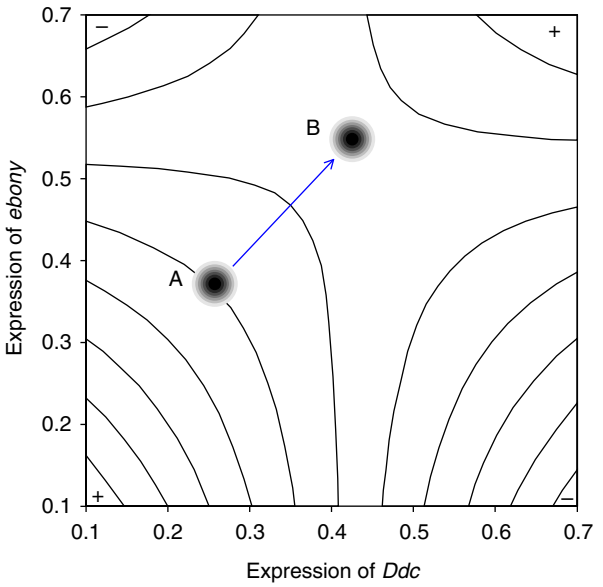

Figure 8 Reconstructed phenotype landscape for melanin production as a function of the expression of two genes that interact epistatically. A and B represent the position of populations in lakes without visual predators (A), and lakes into which visual predators were introduced (B). '+' and '−' represent, respectively, high and low points on the landscape.

In a number of high lakes, fish were introduced for sport fishing in the early to mid-twentieth century. In these lakes, the resident *Daphnia* populations responded by evolving lower melanin levels (lighter coloration) than is seen in similar lakes without introduced predators. Two loci were found to interact nonadditively to influence melanin production. By regressing melanin concentration on the expression of each gene individually, and on their product, the authors constructed a phenotype landscape for these underlying factors (**Figure 8**).

As expected, individual from predator-free lakes are higher up on the landscape – corresponding to higher melanin levels – than those subject to visual predation. Notably, both populations were very close to points of maximum canalization for their respective melanin levels, consistent with the expectation that this developmental system has adapted to maximize robustness. The authors note that, in evolving to lower levels of melanin, the populations followed the gradient of the phenotype landscape, as predicted by previous theory. It thus appears that selection for canalization plays an important role in the evolution of this trait, and that we could not understand the evolution of the two loci influencing the trait if we did not understand how they interact, nonadditively, with one another.

Conclusions

Models of evolution invariably involve assumptions – explicit or implicit – about the translation of genetic variation into

phenotypic variation. For most phenotypic traits, this means making assumptions about development. The simplest such assumption, which produces the easiest models to study, is that gene products contribute additively to phenotype. This is the basis of quantitative genetics, and it is often a reasonable approximation for studying short-term adaptive evolution.

There are a variety of cases, though, in which the assumption of additivity is unsatisfactory. Some of these are situations in which we would expect to have to consider development, such as when the goal is to study the evolution of canalization. In other cases, though, the nonadditivity resulting from development makes its presence known even when we are studying the short-term response of quantitative traits to directional selection. This is likely to be the case whenever we wish to predict the joint evolution of tightly correlated traits, or when we are studying how specific loci respond to selection on a trait that they influence epistatically. Overall, situations in which we can effectively ignore development should be seen as special cases.

See also: Developmental-Genetic Toolkit for Evolutionary Developmental Biology. Macroevolution, Quantitative Genetics and

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Geographic Mosaic of Coevolution

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Glossary

Adaptive radiation The rapid divergence of a single phyletic line into a number of species occupying different ecological niches.

Character displacement The difference in resource use resulting from reciprocal selection to avoid competition between species.

Coevolutionary arms races The continuing escalation of reciprocally selected traits for defense and counter-defense in an antagonistic interaction.

Common garden experiment The transplantation of organisms from a range of different environments into a single environment to determine the contribution of local genetic variation among locations to phenotypic variation.

Horizontal transmission The transfer of a symbiont (parasite or mutualist) between unrelated individuals.

Maladaptation The low fitness of an organism due to the mismatch between its genotype and the environment. This occurs in geographic mosaic of coevolution when genotypes adapted to one local environment are found in another environment.

Negative frequency-dependent selection A mode of selection where the fitness of a genotype is inversely related to the proportion of that genotype in the population.

Reciprocal transplant experiment The movement of organisms from several different environments into each of the alternative environments in order to measure local adaptation.

Trait remixing The gene flow between populations adapted to different environments resulting in reduced fitness due to a mismatch between organism traits and their environment.

Vertical transmission The transfer of a symbiont (parasite or mutualist) from parent to offspring.

Introduction

Coevolution is the reciprocal adaptation among interacting organisms, and it is one of the major forces that organize biodiversity by linking the genomes of interacting species (Thompson and Cunningham, 2002). It is also one of the major forces creating biodiversity because diversifying coevolutionary selection can lead to speciation (Thompson, 2005, 2009). Measuring coevolution is difficult because it involves determining how two species interact through time, and without a time machine it is difficult to measure how traits of interacting species have changed through time. The difficulties in rigorously testing coevolutionary hypotheses led to questions about its importance as an evolutionary force. To solve the problems in testing the coevolutionary hypothesis, Thompson (1999, 2005) developed the geographic mosaic theory of coevolution that examines geographic variation in species interactions to use space as a proxy for time to measure coevolution. A geographic mosaic of coevolution (GMC) occurs when local variation in species interactions produces a spatially variable pattern of reciprocal adaptation in these species.

Coevolution involves at least three steps: first one species evolves a response to a trait of a second species, and this is followed by a response of the second species to the first (Janzen, 1980). These steps can be measured by comparing sites where two species are exerting reciprocal selection on each other that are termed coevolutionary hotspots, and sites where two species are not exerting reciprocal selection on each other that are termed coevolutionary coldspots (**Figure 1**). If coevolution is occurring, then it can be measured by comparing traits in coevolutionary hotspots versus coldspots. Interactions between species may also differ in different environments, with those environments changing the selection on coevolved traits, so coevolution can also be measured by comparing hotspots in different environments (**Figure 1**). The predicted outcome of geographic mosaics of coevolution is that populations of the interacting species should become geographically differentiated and locally adapted due to the presence or absence of diversifying selection, or by different strengths of selection between interacting species in two hotspots. However, if movement of individuals causes gene flow between populations adapted to environments with different coevolutionary forces then this can result in 'trait remixing' and 'maladaptation' (**Figure 1**). For example, if there is migration from a coldspot where the population is not adapted to an interacting species to a hotspot where the interacting species is present then non-coevolved individuals with low fitness may be found in the hotspot.

The geographic mixture of coldspots and hotspots produces a geographic selection mosaic, which consists of a matrix of interactions where the genotypes of one species interact with the genotypes of a second species in a specific environment, or a $G \times G \times E$ interaction. The $G \times G$ interaction that determines coevolution varies geographically from hotspots (**Figure 1**), where species frequently interact resulting in strong reciprocal selection, to coldspots, where interactions are rare or absent and reciprocal selection is weak (**Figure 1**). Geographic variation in the environment can alter the $G \times G \times E$ interaction so that selection differs among hotspots (**Figure 1**). The evolutionary outcomes produced by the divergent geographic selection mosaics can range from transient polymorphisms to speciation and adaptive radiation.

The pervasive patterns of geographic variation in selection and local adaptation that are found among natural populations

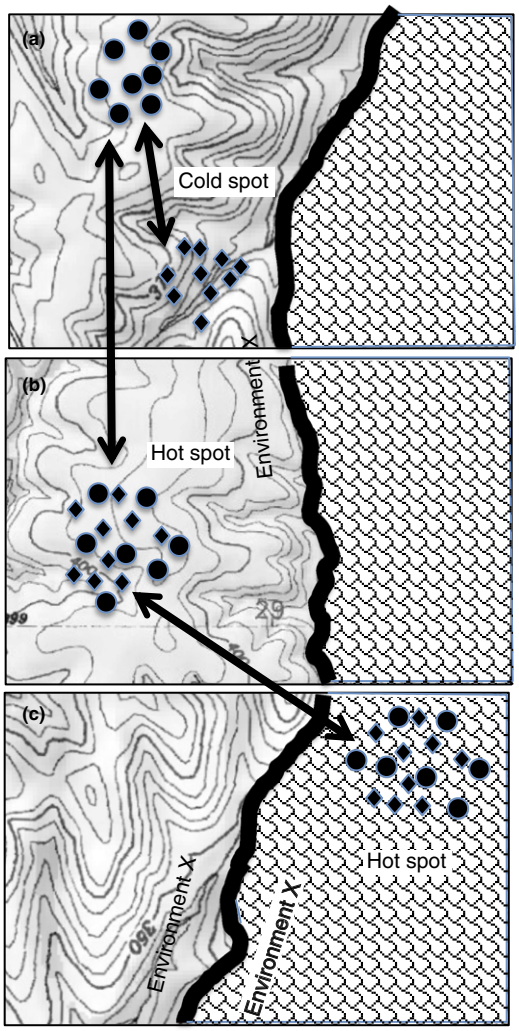

Figure 1 The mosaic of coevolution. (a) Coldspot: Selection in environment X on species 1(•) in the absence of species 2(♦). Coldspot: Selection in environment X on species 2 in the absence of species 1. (b) Hotspot: Reciprocal selection of species 1 and species 2 in environment X. (c) Hotspot: Reciprocal selection of species 1 and 2 in environment Y. Arrows represent gene flow among hotspots and coldspots that can produce maladaptation.

(see Kawecki and Ebert, 2004; Greischar and Koskella, 2008; Hokesema and Forde, 2008 for reviews) are consistent with the geographic mosaic of coevolution theory. However, to demonstrate that geographic population variation between interacting species is due to their reciprocal selection requires separating selection due to genotype × genotype interaction from those of a myriad of other species and abiotic factors represented by the E in the mosaic of G × G × E interactions. To completely describe a GMC it is necessary to measure the G × G × E coevolution mosaic, gene flow among populations, and the mosaic of local adaptation and maladaptation that these processes produce.

One approach to testing hypotheses generated by the GMC theory is determining whether observed ecological patterns are consistent with the predictions of the GMC. Thompson (2005) has made three such predictions, and these predictions are illustrated by the interactions between a predatory fish and three species of freshwater snail (Chaves-Compos *et al.*, 2011). First, Thompson predicts that geographic populations will differ in traits shaped by the interaction. In the fish–snail example, since the success of a fish that feeds on snails depends on snail shell thickness relative to fish crushing ability, then the geographic variation found in these traits indicates that they are coevolving. Thompson's second prediction is that if these species are coevolving, then traits of interacting species will be matched in some, but not all, of the populations (Thompson *et al.*, 2002). Chaves-Compos *et al.* (2011) found snail shell thickness and teeth morphology is matched in some areas as predicted if coevolution produced reciprocal local adaptation, but not matched in others because of gene flow. Finally, Thompson predicts that geographic variation in diversifying selection will result in few coevolutionary traits being fixed in all populations. In the snail–fish example, neither shell thickness nor fish tooth morphology is a fixed trait, but rather varies geographically.

A match with the ecological predictions is consistent with the existence of a GMC, but alternative hypotheses are also consistent with these patterns and additional studies are often needed to distinguish GMC from alternatives. Experimental measurements of the geographic mosaics of selection and local adaptation can provide rigorous tests of the GMC hypotheses. A variety of approaches have been used to measure and partition the effects components of $G_1 \times G_2 \times E_x$ to measure coevolutionary forces. The GMC predictions that the outcome of interactions of species 1, G_1, and species 2, G_2 from different locations will differ due to coevolutionary selection for local adaptation. To test this hypothesis, 'common garden experiments' measure interactions between a range of populations of a species from different areas (i.e., E_A, or E_B) in a single environment (either E_A, or E_B or a laboratory or garden (E_C)) in order to measure the effects of reciprocal selection by comparing the $G_{1A} \times G_{2A}$, $G_{1B} \times G_{2B}$, $G_{1A} \times G_{2B}$ interactions, in the absence of environmental variation (Lively, 1989; Lively and Jokela, 1996; Laine 2005). While these experiments can provide indications of geographic variation in the interaction of two species it cannot accurately measure coevolutionary forces because the crucial G × E interactions are not accounted for.

'Reciprocal transplant experiments' measure local adaptation of populations, a crucial prediction of the GMC theory, by comparing the performance of each population in their local environment and foreign environments, for example, the performance of the population G_{1A} is measured in the local $G_{1A} \times G_{2A} \times E_A$ and in the 'foreign' $G_{1A} \times G_{2B} \times E_B$ environments. Local adaptation is measured by either comparing whether a local population performs better within its own habitat than a foreign population does or whether a local population performs better in its local habitat than in a foreign habitat (Gandon, 1998; Kawecki and Ebert, 2004; Greischar and Koskella, 2008). However, merely demonstrating that populations are locally adapted does not demonstrate that coevolution has occurred between two focal species, because each species could be locally adapted to the abiotic environment or to other non-focal species. To measure coevolutionary forces, Nuismer and Gandon (2008) have developed an experimental design and mathematical analysis that allows full measurement of the G × G × E coevolutionary forces.

It requires a double reciprocal transplant experiment where multiple geographic populations interact in multiple environments.

Geographic Mosaics of Coevolution

Geographic mosaics of coevolution have been studied in a range of interactions allowing for an evaluation of how the characteristics of the interactions shape the outcomes. Interactions differ on several dimensions including: Interaction type (predator–prey, competition, mutualism), geographic scale, the amount of gene flow, the taxa involved, whether hotspot–coldspot interactions or hotspot–hotspot interactions are occurring, what sets the limits in the escalation of coevolving traits, and the time scale on which coevolution occurs. Variation on all of these axes has resulted in a wide range of evolutionary outcomes ranging from transient polymorphisms to adaptive radiations.

Geographic Mosaics of Diversifying Selection in Antagonistic Interactions

Antagonistic (-,-) interactions can result in 'coevolutionary arms races' where offensive and defensive weapons are produced in an escalating sequence. These arms races may continue indefinitely, or they may reach some limit. Alternatively, 'negative frequency-dependent' selection can create a coevolutionary chase in space and time where the predator or parasite becomes adapted to the most common genotypes of a host favoring a less common genotype that increases in frequency until its antagonist adapts to its defenses, and the cycle repeats. Gene flow among areas can produce a geographic mosaic where a genotype that is vulnerable to the adapted antagonists in one area escapes to another area where there are fewer adapted antagonists.

Morphological evolution of pinecones, squirrels, and crossbills

An arms race involving lodgepole pine, *Pinus contorta*, red squirrels, *Tamiasciurus* sp., and red crossbills, *Loxia curvirostra* provides an example of a large-scale GMC that has produced local adaptation and contributed to adaptive radiation in the interacting species. A mosaic of hotspots and coldspots in the interaction of squirrels and pine trees is found on the wet and dry slopes of the Cascade Mountain Range of the US Pacific Northwest. Smith (1970) conducted a classic study of a GMC in this interaction. A coevolutionary hotspot for squirrels and pines exists on the east side of the Cascades where a rain shadow results in periodic drought and fire. Periodic fire selects lodgepole pines to develop serotinous cones, which are cones that only open and disperse their seeds when a fire that creates opportunities for germination. Serotinous cones remain on the trees for extended periods between fires, providing a predictable supply of food for the squirrels, which exert strong selection on the cones to develop defenses. A coevolutionary coldspot in the squirrel–pinecone interaction occurs on the west side of the Cascades where high precipitation prevents frequent fires. In this area pinecones open yearly and drop their seeds, and they are not a dependable seed source for squirrels. As a result there is weak selection for the evolution of pinecone defenses, and counter-defenses by the squirrels. A comparison of pinecone and squirrel traits in the hotspot and the coldspot demonstrated that diversifying selection had resulted in reciprocal local adaptation. In the hotspot pinecones have several traits that inhibit squirrel predation. Cones are strongly attached to branches, and positioned so that they are difficult for squirrels to detach (**Figure 2**). Pinecones have evolved so that it requires the maximum amount of effort to extract the seeds from a cone with few seeds per cone, thick distal bracts, tough bracts, and scales (**Figure 2**). These traits are all lacking in the coevolutionary coldspot where squirrel predation is low. Squirrels in the hotspots are locally adapted to these tree defenses: they are larger, have stronger jaw muscles, and more pronounced sagittal crests which allows for stronger attachment of jaw muscles (**Figure 2**).

Red crossbills are also seed predators of lodgepole pinecones and coevolve with the lodge pole pine in a complex selection mosaic of hotspots and coldspots that depends on the presence or absence of squirrels which has been described by Craig Benkman and his colleagues (Benkman, 1999, 2003; Benkman *et al.*, 2001, 2003). Red squirrels are such effective cone predators that they largely exclude crossbills from utilizing the lodgepole seeds, and mountain ranges with squirrels are coevolutionary coldspots for the crossbill–lodgepole interaction. Pinecones in these ranges are primarily adapted to squirrel predation. Coevolutionary hotspots between crossbills and lodgepole pines are found in isolated mountain ranges in the Northern Rockies which were not colonized by squirrels after the last ice age, but which were colonized by crossbills. Crossbills have specially adapted bills for extracting pine seeds that coevolve with lodgepole pine in the coevolutionary hotspots when squirrels are absent. Crossbill bill shape and cone characteristics evolve differently in hotspots and coldspots resulting in a coevolutionary mosaic (**Figure 2**). Differences in the way that squirrels and crossbills extract seeds have led to different cone morphologies in areas where each is the predominant seed predator. Squirrels remove the entire cone and prefer cones with more seeds so in squirrel hotspots cones are massive, harder to detach, and contain few seeds. In contrast, crossbills attack the cone from the proximal end so in crossbill hotspots cones have thicker proximal scales to protect against crossbills extracting seeds from this end. In response, the bills of the crossbills in hotspots have coevolved with longer more decurved bills that allow them to more easily extract seeds from cones that have evolved these thicker proximal scales. A common-environment experiment demonstrated that the bills are locally adapted to lodgepole cone characters: crossbill populations from areas with and without squirrels were more efficient at attacking their local cones (Benkman *et al.*, 2001).

This GMC has been important in the adaptive radiation of crossbills (Benkman *et al.*, 2010). Reproductively isolated populations of crossbills adapted for feeding on cones of different morphologies have different call types. Coevolutionary diversifying selection to adapt to feeding on cones of different morphologies drives the evolution of this reproductive isolation (Smith and Benkman, 2007). Crossbills have undergone substantial adaptive radiation in sites where

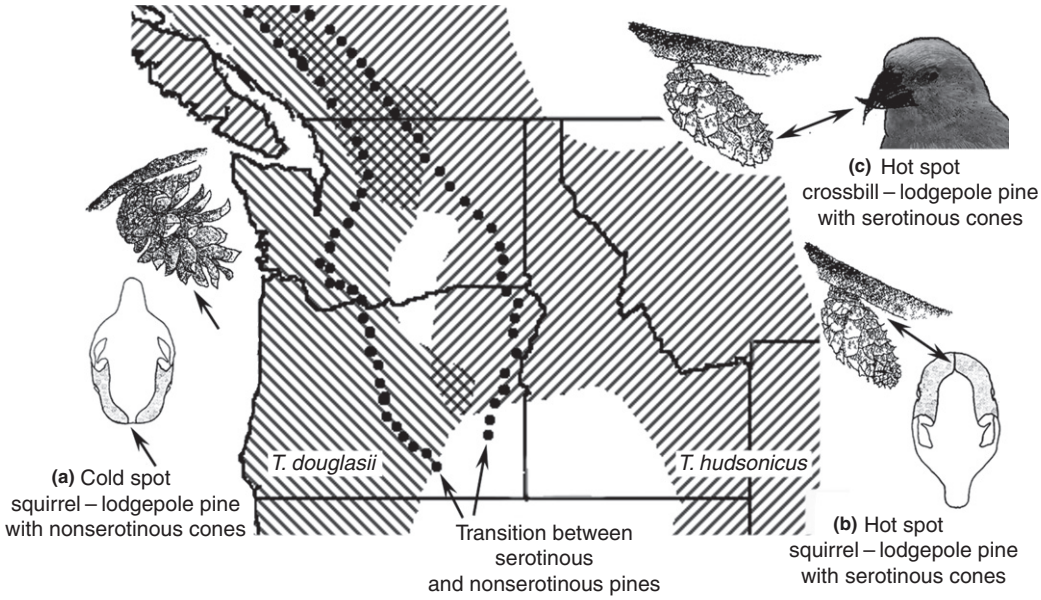

Figure 2 Hotspots and coldspots in the geographic mosaic of coevolution of lodgepole pine *Pinus contorta*, red squirrels, *Tamiasciurus* sp., and red crossbills *Loxia curvirostra*. Redrawn from Smith, C.C., 1970. The coevolution of pine squirrels (*Tamiascirus*) and conifers. Ecological Monographs 40, 349–371 and Benkman, C.W., 1999. The selection mosaic and the diversifying coevolution between crossbills and lodgepole pine. American Naturalist 153, S75–S91. (a) A coevolutionary coldspot in the interaction of pine trees and squirrels occurs where cones are non-serotinous and shed their seeds yearly. In these sites, the cones are not under selection to provide protection for seeds for extended time periods, and squirrels have not evolved strong jaw muscles that are necessary to open heavily defended cones. (b) A coevolutionary hotspot in the pine tree-squirrel interaction occurs where cones are serotinous and retain their seeds for several years necessitating the evolution of numerous seed protection mechanisms against squirrels, and squirrels have evolved strong jaw muscles to counter these defenses. (c) A coevolutionary hotspot in the pine tree-crossbill interaction occurs where pine cones are serotinous and squirrels are absent. Cones evolve seed protection mechanisms specific to defending against crossbill predation, and crossbills have evolved bill characteristics to counter these defenses.

squirrels are absent indicating that coevolution with lodgepoles and squirrels is driving their speciation (Benkman *et al.*, 2010).

Effects of environment on a geographic mosaic of variation in a plant–galler–natural enemies interaction

Antagonistic interactions involving a very different set of interactions involving tall goldenrod, *Solidago altissima*, a gall-inducing fly, *Eurosta solidaginis,* and the natural enemies of the galler, including birds and the parasitoid wasp *Eurytoma gigantea*, have also produced a GMC. The goldenrod–fly interaction has diverged in hotspots where different environments affect the reciprocal selection they exert on each other, and the fly–parasitoid interaction has hotspots and coldspots mediated by their interactions with other natural enemies. The fly larvae develop inside the gall, and they are attacked by the natural enemies, which varies in a geographic mosaic of hot and coldspots (**Figure 3**). In forested areas birds cause high mortality on larvae in large galls, and the parasitoid causes higher mortality on larvae in small galls, resulting in stabilizing selection. Gall size is partially due to heritable genetic variation in the fly, and the combination of these natural enemies exerts stabilizing selection on gall size in forested regions (Weis and Abrahamson, 1986). The tree-dwelling birds that feed on the larvae are absent or at low densities in prairie regions, creating a coldspot in the interaction of birds and the galler (Craig, 2007; Craig *et al.*, 2007). In the absence of birds there is a coevolutionary hotspot in the interaction between the fly and parasitoid resulting in an evolutionary arms race: flies produce larger galls and parasitoids are larger with longer ovipositors (Craig *et al.*, 2007).

The goldenrod plant and fly interaction is also affected by the local environment. Plants grow in both prairie and forest environments, where differences in soils, climate, competition with other plants, and herbivory produce very different selection on the goldenrod. Common garden experiments demonstrate that prairie and forest plants have strong genetically based differences in morphology and drought tolerance. Common garden experiments also showed that *Eurosta* are locally adapted to their host plants: survival was higher for both forest and prairie flies on plants from their local biome (Craig and Itami, 2011). Craig and Itami (2011) hypothesized that diversifying selection on the plant to adapt to the different forest and prairie environments has had cascading effects that select for diversification among the flies. *Eurosta solidaginis* inducing galls on prairie and forest *S. altissima* subspecies are partially reproductively isolated due to host-plant-mediated assortative mating and differences in mating times due to plant phenology. In turn, the plant has also become locally adapted to the fly. Gall production is costly to the plant, and the plant is under selection to minimize resources hijacked by the fly for the gall (Abrahamson and Weis, 1997). Gall size is influenced by both the geographical origin of the plant and the fly (Craig and Itami, 2011). In response to selection by natural enemies forest flies induce relatively small galls, and prairie flies induce large galls.

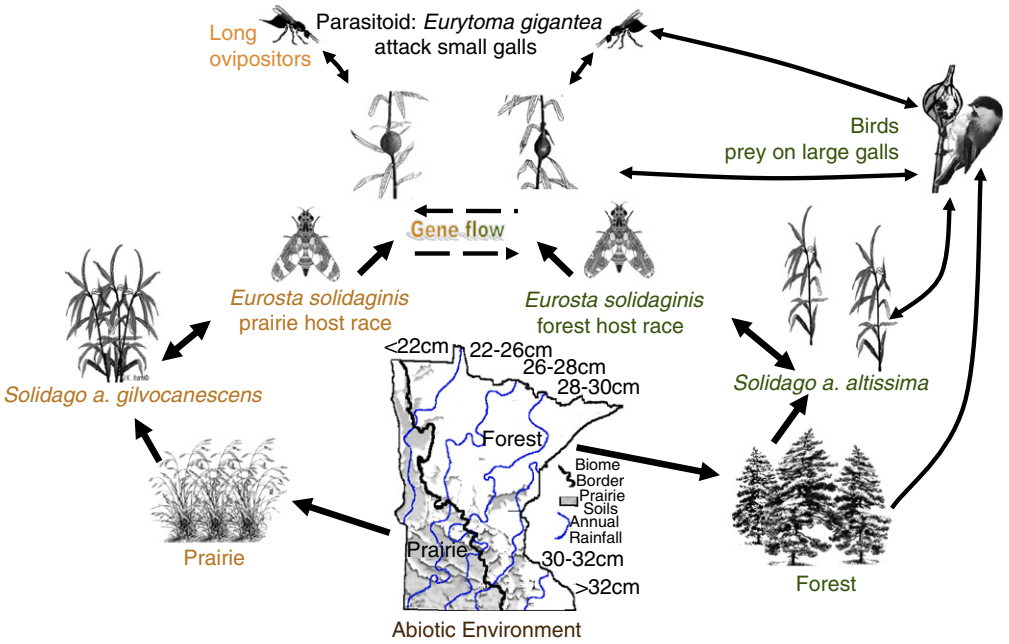

Figure 3 Geographic mosaic of selection in the interaction of *Solidago altissima*, *Eurosta solidaginis*, and natural enemies. The *Eurosta–Solidago* interaction in the prairie is a hotspot as is the *Eurosta–Solidago* interaction in the forest. The hotspot interaction of the *Eurosta–Eurytoma* in the prairie, in the absence of the birds, results in larger galls and longer *Eurytoma* ovipositors. The hotspot interactions of *Eurosta–Eurytoma*–birds in the forests, results in birds selecting for smaller *Eurosta* galls and indirectly for shorter *Eurytoma* ovipositors.

Geographic mosaics of competitive coevolution

Competition can also produce geographic selection mosaics when the presence or intensity of competitors varies among areas. 'Character displacement' is evidence of a competition geographic selection mosaic, and it is demonstrated in interactions where greater differences in a pair species occurs in sympatry, a coevolutionary hotspot, than in allopatry, a coevolutionary coldspot. It has been demonstrated by the coevolution sticklebacks in Canadian lakes (Schluter and McPhail, 1993). Three-spined sticklebacks, *Gasterosteus aculeatus*, were originally a marine species which invaded inland lakes that formed after the end of the last ice age. In each of four lakes that have two species there is a small limnetic species that feeds on plankton, and a larger benthic species that feed on macro-invertebrates (**Figure 4**). In contrast, in lakes with a single species body size is intermediate. The divergence of traits when there is competition is a local adaptation that increases fitness in each species by decreasing competition. Genetic analysis indicates that the divergence into benthic and limnetic forms had independently occurred multiple times following colonization of lakes by the marine species at two different times indicating that coevolution hotspots can produce parallel evolution and speciation (Schluter and McPhail, 1993).

Geographic Mosaics of Mutualistic Coevolution

Mutualists in obligate symbiotic interactions can also form geographic mosaics of coevolution. Mutualisms do not evolve to a point where both interacting species become fixed for optimally mutualistic beneficial traits. This is because while both species in a mutualistic interaction benefit from the interaction, mutualisms always maintain an antagonistic element as each species is trying to obtain the maximum benefit from its mutualist at the lowest cost. The location of a species along the mutualism–antagonism continuum depends upon specific characteristics of the interaction. The mode of transmission has a powerful impact on how an interaction will evolve along this continuum. If a symbiont (parasite or mutualist) is transferred among unrelated individuals, which is termed 'horizontal transmission,' then a reduction of the fitness of its host will have limited consequences for the symbiont. In contrast, when a symbiont is transferred from mother to offspring, which is termed 'vertical transmission,' then the symbiont must insure the survival of its host for it to survive. Vertically transferred parasites or mutualists will be under strong selection to evolve from the antagonistic toward the mutualistic end of the continuum. One prediction is that there will be selection on a host to maintain only a single genotype of their symbiont in order to eliminate the negative consequences of competition among strains. Such a mutualistic GMC is illustrated by the complex interaction of fungus-cultivating ants, *Apterostigma dentigerum*, the cultivated fungus in their gardens, a pathogen that infects the fungus gardens *Escovopsis*, and a bacteria, *Pseudocardia*, that controls the fungus (Caldera and Currie, 2012). *Pseudocardia* is therefore a mutualist of the ants, and it is transmitted vertically by the ants with the queen when she establishes new colonies. A GMC is indicated because each colony has a single strain of *Pseudocadria*, and the populations of the bacteria are strongly differentiated on a small geographic scale with little gene flow among them. The distribution of *Pseudocardia* strains and *Escoposis* strains indicates that there is a GMC among the mutualistic symbionts and their antagonist.

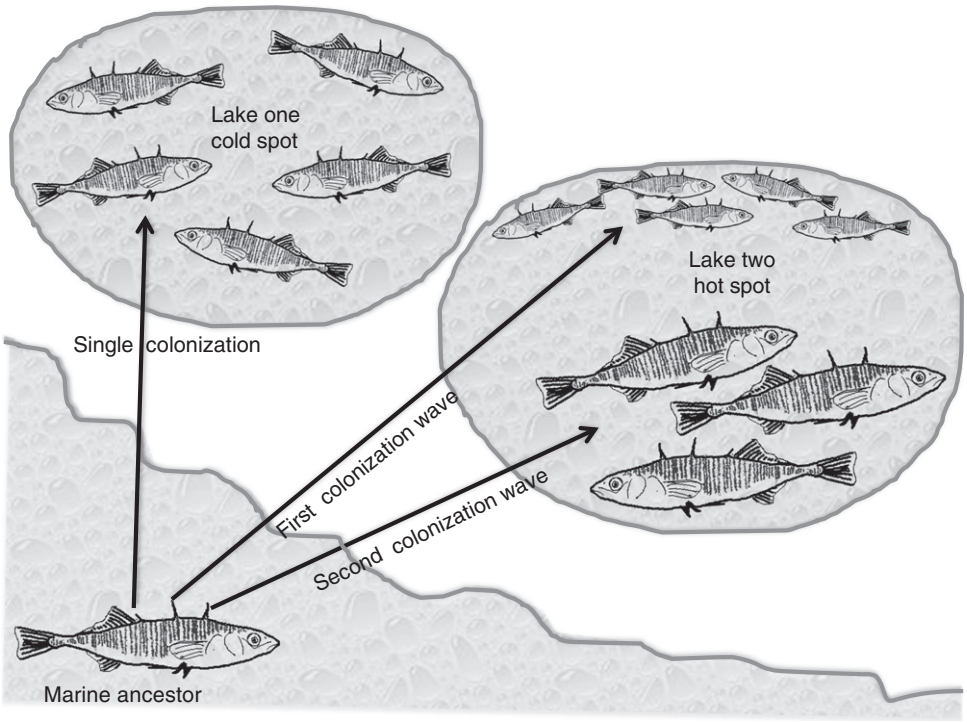

Figure 4 A geographic mosaic of coevolution between three-spined stickleback species resulting from competitive character displacement. (a) A coevolutionary coldspot where only one species colonized a lake and where without competition the species has evolved an intermediate feeding strategy and body size. (b) A coevolutionary hotspot where two species colonized a lake and where interspecific competition has produced character displacement leading to the evolution of a smaller limnetic planktivore and a larger benthic macroinvertebrate feeder.

The Geographic Mosaic of Coevolution in Human-Modified Environments

Humans have altered many ecological systems in ways that provide opportunities for studying the GMC. The extensive movement by humans of species and locally adapted populations to new locations creates many new coevolutionary hotspots and coldspots. Species introduced to new areas can encounter new natural enemies, competitors, and mutualists resulting in a significant change in the evolutionary trajectory of the interaction in a coevolutionary hotspot. Conversely, an introduced species can escape its enemies and competitors sometimes resulting in its evolution into a pest species. Released from selection imposed in its native environment the introduced species may lose adaptations to species it has formerly interacted with, as evidenced by the increased susceptibility of plants to herbivores from its previous habitat.

Solidago altissima is native to North America, and it was introduced to Japan about 100 years ago where it evolved in its new environment in the absence of phytophagous insects native to North America. In 2000, an herbivorous lacebug, *Corythucha marmorata*, was introduced from North America reuniting it with the goldenrod. It rapidly reached higher densities than in North America, but the density rapidly declined. In a common garden experiment, Sakata *et al.* (2014) demonstrated that goldenrod populations that had been exposed to herbivory for 12 years were much more resistant than populations that had not yet been exposed. This provides strong evidence of an intercontinental geographic mosaic of evolution with a hotspot in North America that led to reciprocal coevolution of defense by goldenrod and counter-defense by the lacebug. When selection by the lacebug was relaxed in the evolutionary coldspot established in Japan, goldenrod lost its resistance. When the lacebug was introduced and began to spread across Japan a new and rapidly shifting mosaic of hotspots and coldspots was established exerting geographically diversifying selection on the goldenrod. An as-yet untested hypothesis is that the differences among plants in these hotspots and coldspots are also exerting diversifying selection on the lacebug.

Humans in Geographic Mosaics of Coevolution

Agriculture and forestry practices alter the geographic mosaic of evolution as humans constantly alter the genetic composition of domesticated plants and animals and spatially redistribute this genetic variation in a way that continually alters the coevolutionary interactions with humans. One example is the coevolution of humans and cows. Adult *Homo sapiens* usually lose the ability to produce the enzyme lactase that allows them to metabolize the milk sugar lactose. When humans domesticated cattle, *Bos taurus*, they exerted strong selection that altered a large number of traits (Craig, 1981), and in turn cattle have exerted strong selection on humans producing a geographic mosaic due to local adaptation to milk consumption (Gerbault *et al.*, 2009). Utilizing cow's milk provides access to a significant source of protein that can give a significant fitness advantage to individuals who retain

production of the enzyme lactase. Dairy farming has been adopted in widely geographically isolated areas of northern Europe and Africa, and the distribution of dairy farming is strongly correlated with the frequency of the retention of lactase production in adults. Local adaptation to the use of cow's milk is an example of a GMC.

Conclusion

There is abundant evidence that the GMC is an important and widespread phenomena, and this has provided strong support the importance of coevolution in evolution. It occurs across a wide range of species interactions and kinds of interactions: predator–prey, host–parasite, between mutualists, and between competitors. Diversifying coevolution occurs as the result of both differences between hotspots and coldspots and between hotspots where the environment alters the interaction between species. It has been shown to operate at a range of scales from kilometers to continental scales. It has been shown to produce patterns of both local adaptation and local maladaptation. However, many questions remain to be answered. New experiments need to be conducted in order to measure the strength of coevolutionary forces in local adaptation, and to separate selection for reciprocal coevolution between focal species and selection for on each species from adaptation to other species and the abiotic environment. In addition, the importance of coevolution in complex interactions involving many species needs to be understood so that the importance of coevolution in structuring entire communities can be understood. Finally, there is much work to be done in applying what has been learned from natural systems to systems that are impacted or managed by humans.

See also: Biogeography of Interactions. Mutualism, the Evolutionary Ecology of. Predation and Parasitism

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H

Hardy–Weinberg Equilibrium and Random Mating

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Glossary

Allele One of a number of alternative forms of a gene.
Assortative mating Non-random mating on the basis of phenotype.
Fitness The expected contribution of an individual or genotype to next generation's gene pool.
Genetic drift Changes in allele or genotype frequencies due to random sampling.
Genotype The pair of alleles present at a single locus.
Inbreeding Preferential mating between relatives.
Natural selection Differential survival or reproduction.
Phenotype The set of an organism's observable traits.
Ploidy The number of sets of chromosomes in a cell.
Single nucleotide polymorphism (SNP) DNA sequence variation at a single nucleotide position that differs between individuals.

History

Darwin's theory of evolution transformed biology in the mid-nineteenth century. However, Darwin had an inaccurate understanding of heredity and his belief in blending inheritance was problematic. Because blending inheritance ultimately results in homogenized populations full of intermediate genotypes, it is unable to explain how genetic variation can persist over evolutionary time (Charlesworth and Charlesworth, 2009). It wasn't until the rediscovery of Mendelian genetics in the early twentieth century that a potential solution could be found. Importantly, Mendel treated genes as discrete units, which enabled a mathematical formulation of population genetics. Despite the rediscovery of Mendelian genetics and the pioneering work of biologists and statisticians (Castle, 1903; Pearson, 1904) there was still some confusion, and during a 1908 meeting of the Royal Society of Medicine it was claimed that dominant mutations would increase in frequency until phenotypes reached 3:1 Mendelian ratios (Provine, 2001). Something about this claim seemed wrong to R.C. Punnett, who passed on the question to his cricket-playing friend G.H. Hardy. Hardy was one of the leading mathematicians in Britain and he quickly deduced a solution. In a 1908 letter to the journal *Science*, Hardy began by writing "I should have expected the very simple point that I wish to make to have been familiar to biologists" before using the binomial expansion to demonstrate that genotype frequencies will reach a stable equilibrium after one generation of random mating (Hardy, 1908). Unbeknownst to Hardy, a Germany physician named Wilhelm Weinberg had published a similar paper on the same topic six months earlier. Weinberg derived a general equilibrium principle for a single locus with two segregating alleles (Weinberg, 1908).

The Hardy–Weinberg Principle

The Hardy–Weinberg principle relates allele frequencies to genotype frequencies in a randomly mating population. Imagine that you have a population with two alleles (A and B) that segregate at a single locus. The frequency of allele A is denoted by p and the frequency of allele B is denoted by q. The Hardy–Weinberg principle states that after one generation of random mating genotype frequencies will be p^2, $2pq$, and q^2. In the absence of other evolutionary forces (such as natural selection), genotype frequencies are expected to remain constant and the population is said to be at *Hardy–Weinberg equilibrium*. The Hardy–Weinberg principle relies on a number of assumptions: (1) random mating (i.e, population structure is absent and matings occur in proportion to genotype frequencies), (2) the absence of natural selection, (3) a very large population size (i.e., genetic drift is negligible), (4) no gene flow or migration, (5) no mutation, and (6) the locus is autosomal. When these assumptions are violated, departures from Hardy–Weinberg proportions can result.

One useful way to think about the Hardy–Weinberg principle is to use the metaphor of a gene pool (Crow, 2001). Here, individuals contribute alleles to an infinitely large pool of gametes. In a randomly mating population without natural selection, offspring genotypes are found by randomly sampling two alleles from this gene pool (one from their mother and one from their father). Because the allele that an

 doi:10.1016/B978-0-12-800049-6.00022-6

individual receives from their mother is independent of the allele they receive from their father, the probability of observing a particular genotype is found by multiplying maternal and paternal allele frequencies. Mathematically this involves the binomial expansion: $(p + q)^2 = p^2 + 2pq + q^2$ (see the modified Punnett Square in Figure 1 for a graphical representation). Note that there are two ways that an individual can be an *AB* heterozygote: they can either inherit an *A* allele from their mother and a *B* allele from their father or they can inherit a *B* allele from their mother and an *A* allele from their father.

Additional insight can be found by considering an empirical example (Figure 2). Consider a population that initially contains 18 *AA* homozygotes, 4 *AB* heterozygotes, and 3 *BB* homozygotes. The alleles in the gene pool, 80% are *A* and 20% are *B*. After a single generation of random mating we observe Hardy–Weinberg proportions: 16 *AA* homozygotes, 8 *AB* heterozygotes, and 1 *BB* homozygote. Note that allele frequencies remain unchanged.

There are a number of evolutionary implications of the Hardy–Weinberg principle. Most importantly, genetic variation is conserved in large, randomly mating populations. A second implication is that the Hardy–Weinberg principle allows one to determine the proportion of individuals that are carriers for a recessive allele. Third, it is important to note that dominant alleles are not always the most common alleles in a population. Another implication of the Hardy–Weinberg principle is that rare alleles are more likely to be found in heterozygous individuals than in homozygous individuals. This occurs because q^2 is much smaller than $2pq$ when q is close to zero.

The Hardy–Weinberg principle can be generalized to include polyploid organisms and genes that have more than two segregating alleles. Equilibrium genotype frequencies are found by expanding the multinomial $(p_1 + \ldots + p_k)^n$, where n is the number of sets of chromosomes in a cell and k is the number of segregating alleles. For example, tetraploid organisms ($n = 4$) with two segregating alleles ($k = 2$) are expected to have genotype frequencies of: ${p_1}^4$ (*AAAA*), $4{p_1}^3p_2$ (*AAAB*), $6{p_1}^2{p_2}^2$ (*AABB*), $4p_1{p_2}^3$ (*ABBB*), and p^4 (*BBBB*). Similarly, diploid organisms ($n = 2$) with three segregating alleles ($k = 3$) are expected to have genotype frequencies of: ${p_1}^2$ (*AA*), ${p_2}^2$ (*BB*), ${p_3}^2$ (*CC*), $2p_1p_2$ (*AB*), $2p_1p_3$ (*AC*), and $2p_2p_3$ (*BC*). Genotype frequencies sum to one for each of the above scenarios. Although the Hardy–Weinberg principle can also be generalized to include genes located on sex chromosomes (e.g., X chromosomes in humans), it is important to note that it can take multiple generations for genotype frequencies at sex-linked loci to reach equilibrium values.

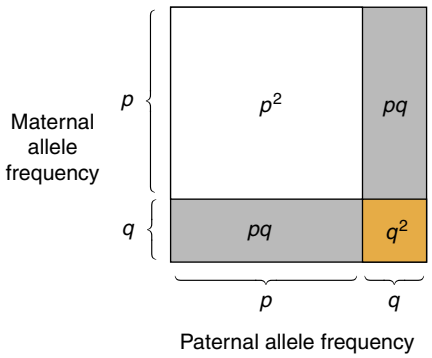

Figure 1 Graphical representation of the Hardy–Weinberg principle. The frequency of *A* alleles is denoted by *p* and the proportion of *B* alleles by *q*. *AA* homozygotes are represented by white, *AB* heterozygotes by gray, and *BB* homozygotes by gold. Shaded areas are proportional to the probability of observing each genotype.

Testing for Hardy–Weinberg Proportions

A Chi-square (χ^2) test with one degree of freedom can be used to determine whether a population is at Hardy–Weinberg equilibrium (Weir, 1996) (see Table 1 for an example). First, the observed numbers of individuals with each genotype are counted. These genotype frequencies are then used to obtain allele frequencies (p and q). The expected numbers of individuals with each genotype are calculated by multiplying the total number of individuals sampled by p^2, $2pq$, and q^2. Once observed and expected genotype counts are known, χ^2 statistics

Table 1 Chi-square test of Hardy–Weinberg proportions. Allele frequencies are $p = 0.7$ and $q = 0.3$. The expected number of individuals with each genotype are calculated by multiplying the total number of individuals by p^2 (*AA*), $2pq$ (*AB*), and q^2 (*BB*). A χ^2 of 8.724 with one degree of freedom yields a *p*-value of 0.00172, indicating that there is a statistically significant departure from Hardy–Weinberg proportions

Genotype	*Observed*	*Expected*	χ^2
AA	117	107.8	0.785
AB	74	92.4	3.664
BB	29	19.8	4.275
Total	220	220	8.724

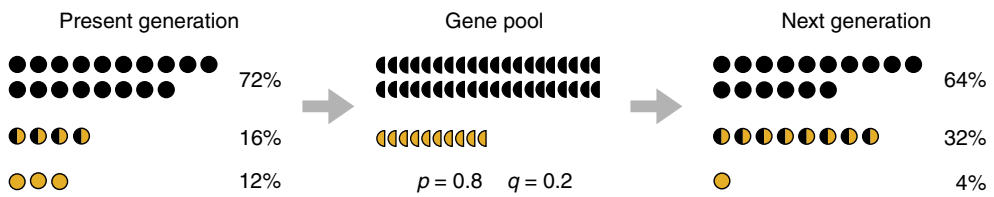

Figure 2 Hardy–Weinberg example. *AA* homozygotes (black circles), AB heterozygotes (black and gold circles), and *BB* homozygotes (gold circles) contribute to the gene pool. *A* alleles are shown as black half-circles and *B* alleles are shown as gold half-circles. After a single generation of random mating Hardy–Weinberg proportions are obtained.

can be calculated for each genotype using the equation $\chi^2 = (observed - expected)^2/expected$. These χ^2 values are summed, and if the overall χ^2 test statistic is greater than 3.84 the null hypothesis that the population is in Hardy–Weinberg equilibrium can be rejected (p-value <0.05). The power of this statistical test is directly related to sample size: departures from Hardy–Weinberg proportions are unlikely to be statistically significant if you have genotype frequency data from a small number of individuals.

Departures from Hardy–Weinberg Proportions

There are a number of reasons why one might fail to observe Hardy–Weinberg proportions, and these departures from Hardy–Weinberg equilibrium can involve either an excess of homozygotes or an excess of heterozygotes.

Genotyping Error

While it may not be the most interesting scenario from an evolutionary perspective, departures from Hardy–Weinberg proportions sometimes occur because of genotyping error. These errors can be due to low quality DNA samples, biochemical artifacts, or human error (Pompanon *et al.*, 2005). For example, low-coverage DNA sequencing can cause genotypes to be incorrectly inferred. To improve data quality, a common practice in genome-wide association studies is to filter out any single nucleotide polymorphisms (SNPs) that show significant departures from Hardy–Weinberg proportions (Turner *et al.*, 2011). However, this runs the risk of eliminating SNPs that have departures from Hardy–Weinberg proportions that are due to some other reason than genotyping error.

Non-Random Mating and Population Structure

Non-random mating leads to departures from Hardy–Weinberg proportions. For example, inbreeding and positive assortative mating (where individuals prefer to mate with phenotypically similar individuals) yield an excess of homozygotes. By contrast, negative assortative mating (where opposites attract and individuals prefer to mate with phenotypically different individuals) results in excess of heterozygotes. Population structure also causes departures from Hardy–Weinberg proportions. For example, consider what happens when samples are drawn from multiple populations instead of a single randomly mating population. If these samples are pooled together and there are allele frequency differences between source populations the resulting mixture will have an excess of homozygotes. This reduction in heterozygosity is known as the *Wahlund effect* (Wahlund, 1928). In practice, departures from Hardy–Weinberg proportions due to non-random mating and population structure tend to be genome-wide (i.e., their effects can be seen at multiple loci).

Natural Selection

Natural selection modifies allele and genotype frequencies and these effects depend on both the magnitude and type of selection present. Selection results in departures from Hardy–Weinberg proportions whenever genotypic fitnesses are non-multiplicative (i.e., $w_{AB}{}^2 \neq w_{AA} \times w_{BB}$) (Lachance, 2008). Not surprisingly, overdominance (heterozygote advantage) results in an excess of heterozygotes compared to Hardy–Weinberg expectations, and underdominance (heterozygote disadvantage) results in an excess of homozygotes. Strong directional selection, such as when one allele is a recessive lethal, leads to marked departures from Hardy–Weinberg expectations. However, weak directional selection has only modest effects on genotype frequencies, and detecting these effects can require sample sizes that are larger than 10 000 individuals (Lachance, 2009). Note that the effects of natural selection tend to be locus-specific rather than genome-wide.

Other Causes

Additional population genetic phenomena that can result in departures from Hardy–Weinberg proportions include genetic drift and mutation. In small populations the proportion of individuals with each genotype often differs from p^2, $2pq$, and q^2. The idea here is that sampling effects due to genetic drift yield a slight excess of homozygotes or heterozygotes. These effects are more pronounced in very small populations. This is akin to what happens when you flip a coin a small number of times and end up with an unequal number of heads and tails. Although mutation can lead to slight departures from Hardy–Weinberg proportions, these departures are difficult to detect. This is because mutation rates tend to be small (on the order of 10^{-8} mutations per base pair per generation in humans).

Ultimately, departures from Hardy–Weinberg proportions reveal the biological complexity of natural populations. By building upon the foundation of the Hardy–Weinberg principle, generations of population geneticists have been able to construct more complex models of evolution. These mathematical models have led to an increased understanding of how evolutionary forces shape natural patterns of genetic diversity.

See also: Genetic Variation in Populations. Linkage Disequilibrium: Population Genetics of Multiple Loci

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Hermaphrodites

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Glossary

Anisogamy Literally meaning 'unequal marriage,' a condition where the fusing gametes are of unequal size, with the male sex by definition producing the smaller and the female sex the larger gametes, leading to an often highly unequal resource contribution to the zygote; more strictly, an additional distinction is sometimes made between anisogamy, where both gametes retain at least some motility, and 'oogamy,' where the larger gamete has lost motility entirely.

Dioecy See Gonochorism.

Gonochorism A term mainly used for animals: a sexual system where individuals only ever exhibit either the male or the female sexual strategy throughout their lives (synonymous with 'dioecy' used for plants and 'heterothallism' used for algae and fungi).

Hermaphroditism A term mainly used for animals: a sexual system where individuals usually exhibit both the male and female sexual strategy, either at different times of their lives (sequential hermaphroditism) or at the same time (simultaneous hermaphroditism) (synonymous with 'monoecy' used for some forms of hermaphroditism in plants and 'homothallism' used for algae and fungi).

Heterothallism See Gonochorism.

Homothallism See Gonochorism.

Isogamy Literally meaning 'equal marriage,' a condition where the fusing gametes are of equal size and make an equal resource contribution to the zygote, although they may exhibit different 'mating types.'

Local sperm competition Results from competition between related sperm, generally from the same sperm donor (or also from related sperm donors), for the fertilization of a given set of ova of a sperm recipient. It can thus be viewed as the mirror image of sperm competition, which represents the 'competition between the sperm from two or more (unrelated) males for the fertilization of a given set of ova.'

Mating conflict It occurs when two potential mating partners have incompatible sex role preferences, owing to asymmetries in the benefits of mating versus its costs between acting as a sperm donor or sperm recipient (so that both individuals may preferentially adopt the same sex role).

Mating types A genetic compatibility system that only permits gametes that have a different mating type to fuse with each other, possibly in order to avoid self-fertilization; often individuals are thought to produce gametes of only one type and in many organisms there are only two mating types, often called plus (+) and minus (−).

Monoecy See Gonochorism.

Oogamy See Anisogamy.

Protandry In sequential hermaphrodites, sex change from male to female; in simultaneous hermaphrodites, when the male function matures before the female function.

Protogyny In sequential hermaphrodites, sex change from female to male; in simultaneous hermaphrodites, when the female function matures before the male function.

Reproductive value Expected future reproductive success of an individual, of key importance in predicting sex-change decisions made by sequential hermaphrodites.

Sex allocation A decision about the amount of investment channeled toward the two sex functions; for example, the number of sons vs. daughters in gonochorists (the sex ratio), the timing of sex change in sequential hermaphrodites, or the investment toward the production of male vs. female gametes in simultaneous hermaphrodites.

Sexual conflict A conflict between the evolutionary interests of a sperm donor and a sperm recipient.

Size-advantage model A model to describe the idea that the sex-specific fecundity (or reproductive value) of an individual might change as it is growing, for example, being higher for the female function when small and higher for the male function when large, thus favoring sequential hermaphroditism with 'protogyny.'

Introduction

Hermaphroditism is common, occurring in >90% of plant genera (Renner and Ricklefs, 1995), >70% of animal phyla (Jarne and Auld, 2006), and being present also in many other multicellular taxa, such as volvocine algae (Coleman, 2012) and arguably also in the fungi (Nieuwenhuis and Aanen, 2012) (see 'Glossary' for related terms used in diverse organismal groups). The key distinguishing feature of hermaphroditism is that each individual can (at least potentially) gain fitness through both male and female reproduction, either by adopting the two sexes sequentially or simultaneously.

We first outline the evolutionary context for thinking about hermaphroditism, focusing on (1) it being a frequent outcome following the evolution of 'anisogamy,' (2) what might be the evolutionary advantages of maintaining two individual routes to fitness, and (3) how local competition drives individual investment decisions between the two sex functions (i.e., 'sex allocation'). We then apply this framework to highlight some of the key differences in the evolutionary biology of sex in hermaphrodites compared to gonochorists, firstly for sequential hermaphrodites and then for simultaneous hermaphrodites. Finally, we provide an outlook, and argue that studying sex in hermaphrodites is important for gaining a

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comprehensive general picture of the consequences of the evolution of anisogamy.

Hermaphroditism in Context

The Evolution of Anisogamy and Its Consequences

Sexual reproduction usually involves the union of two haploid gametes stemming from two different parental individuals, leading to a diploid zygote in which each parent obtains an equal genetic representation. A common, but far from universal, observation among sexually reproducing eukaryotes is that the two fusing gametes differ substantially in size, a condition termed 'anisogamy.' A phylogenetic perspective suggests that anisogamy has evolved from 'isogamy' at least half a dozen times independently (Kirk, 2006), with well-known examples including land plants, some red, brown and green algae, malaria parasites, and animals. These multiple origins of anisogamy point to a fundamental phenomenon linked to sexual reproduction and suggest that common selective pressures are at work. In the following we focus largely on animals, but this simply reflects our own expertise and many of the points we raise have parallels in other organismal groups (see also Charnov, 1982; Lloyd, 1982; Charlesworth and Morgan, 1991; Bernasconi *et al.*, 2004).

The evolution of anisogamy probably results from a primordial 'sexual conflict' over which parent provides more of the resources needed for the successful development of the zygote, given that larger zygotes may often be fitter and develop into fitter individuals (Parker *et al.*, 1972; Lessells *et al.*, 2009; Parker, 2011). By making large gametes a parent can increase its probability of having fit offspring, but given limited resources, it will also make relatively fewer offspring (i.e., gamete size and number trade-off). Conversely, by making many small gametes, a parent may instead aim at finding and (preferentially) fusing with (more) large gametes, so that its genes also end up in large zygotes (thus exploiting other parents' gametic investment). Under broad conditions evolutionary models show that this leads to disruptive selection on gamete size, yielding two fundamentally different sexual strategies (Parker, 2011, 2014). By convention, making many small gametes is called the male strategy and making fewer large gametes is called the female strategy, resulting in a type of sexual reproduction – anisogamous sex – in which there are two fundamentally different routes to fitness, male and female reproduction.

When thinking about sexual reproduction, biologists (and especially zoologists) often presuppose that these two sexual strategies already exist and also that they are stably associated with two particular types of individuals, namely males and females, a sexual system called 'gonochorism.' But an important challenge for a more complete understanding of anisogamous sex is to acknowledge (1) that it has evolved multiple times independently and that the male and female sexual strategies are therefore convergent phenomena in different organismal groups, and (2) that these strategies can be distributed over individuals in a population in diverse ways, often involving 'hermaphroditism.' We therefore argue here that beyond the fact that hermaphrodites show striking sexual adaptations that are interesting in their own right (see below), they are also worth studying because they force us to reevaluate preconceived ideas about what we consider male and female, which – at root – is about anisogamy, and not about courtship dances, huge antlers, or colorful plumage (Schärer *et al.*, 2012).

The Adaptive Significance of Having Two Routes to Fitness

Arguably the biggest difference between gonochorists and hermaphrodites is that in the latter each individual has (at least potentially) access to both the male and female routes to fitness (**Figure 1**). It is currently unclear whether the evolution of anisogamy originally leads to gonochorism or hermaphroditism (Schärer *et al.*, 2014), and the answer may well depend on the specific organismal group. Despite this, many existing models for the evolution of anisogamy make assumptions that necessarily lead to the evolution of gonochorism (e.g., Lehtonen and Kokko, 2011; Parker, 2011), so broadening this theory base remains a significant challenge (as does conducting empirical work in extant groups where ongoing evolution of anisogamy can be studied). From a hermaphrodite perspective, it could be argued that gonochorists are a special case, where some individuals have lost (or given up) their ability to reproduce via one of the two routes, and an important aspect of understanding hermaphroditism is therefore to understand the conditions under which it may or may not be advantageous for individuals to maintain two routes to fitness.

Current thinking about the evolution of sequential hermaphroditism considers that the male and female functions may

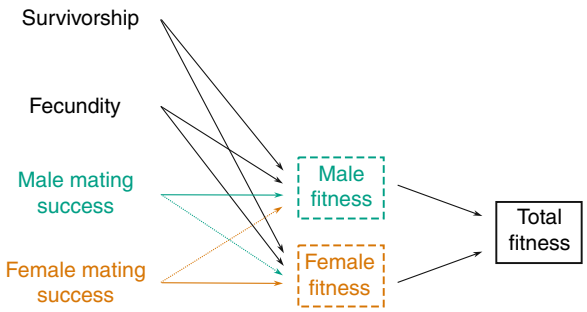


Figure 1 The two routes to fitness in hermaphrodites. In hermaphrodites, survivorship, fecundity, and mating success will often contribute to fitness separately via the male and female sex functions. Nevertheless, we can expect important feedback effects between these different fitness components, only some of which are illustrated here. For example, mating success in one sex function may impact upon reproductive success in the other sex function (so-called cross-sex effects; stippled arrows), as can occur if mating is reciprocal such that additional matings in the male role automatically correspond to more matings in the female role, with potentially negative consequences (see Anthes *et al.*, 2010 for further discussion). Note also that for simplicity only a single fecundity component has been plotted, but this could again be split into male and female components and these may often trade off against each other due to a sex allocation trade-off. Modified from Schärer, L., Janicke, T., Ramm, S. A., 2014. Sexual conflict in hermaphrodites. Cold Spring Harbor Perspectives in Biology doi: 10.1101/cshperspect.a017673, based on an original figure for gonochorists in Arnqvist and Rowe (2005).

have differing optimal body sizes, such that an individual may maximize its total fitness by first exhibiting one sex and later changing to the other (the 'size-advantage model'; Ghiselin, 1969; **Figure 2**). Stated more broadly, the size-advantage model predicts that an individual should want to change sex whenever it can increase its 'reproductive value' by doing so, emphasizing that social and ecological factors come into play in determining the optimal sex-change strategy (Warner, 1975, 1988; Charnov, 1982; Munday *et al.*, 2006). We consider the rationale of the size-advantage model in more detail in Section 'Local Competition and Sex Allocation,' and then provide examples in Section 'Sex in Sequential Hermaphrodites' below.

For simultaneous hermaphrodites, the benefits of dual sexuality may stem from reproductive assurance when the rate of encountering potential mates is low, for example under low population density (Ghiselin, 1969; Schärer, 2009). In contrast to gonochorists, for a simultaneous hermaphrodite each encountered conspecific is a potential mating partner (Tomlinson, 1966). Moreover, even in the complete absence of access to mating partners, exhibiting both sexes simultaneously – or in short sequence, such as in some *Caenorhabditis* nematodes – has the additional benefit of permitting self-fertilization (Charlesworth and Morgan, 1991; Jarne and Charlesworth, 1993; Jarne and Auld, 2006). However, simultaneous hermaphroditism is clearly not restricted to only organisms occurring at low density, and more generally, this sexual system is expected to be stable whenever there are strong diminishing fitness returns on investment into one of the sex functions (Charnov, 1982; Schärer, 2009), an argument that we develop more fully in Section 'Local Competition and Sex Allocation.'

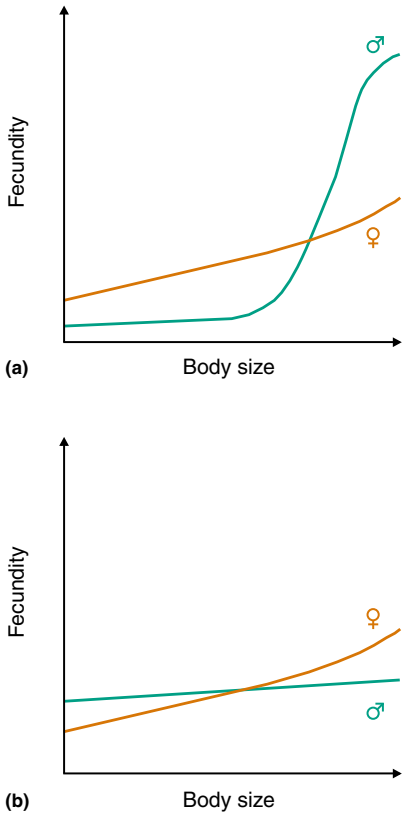


Figure 2 The size-advantage model for sequential hermaphroditism. If the expected fitness returns on operating as either a male or a female change predictably with size (or age), this may – provided that it is physiologically possible and the costs of doing so are not too high (see Kazancıoğlu and Alonzo, 2009) – favor reproductive strategies that involve sex change. (a) Protogyny (female-to-male sex change) is favored when the relationship between size and fecundity is shallower for females than males, for example, because large males are more successful at holding a territory in which they can mate with multiple females. (b) By contrast, if the size–fecundity relationship is steeper for females, for example, because larger females are more fecund, while a male's size is relatively unimportant for its fecundity, this may favor protandry (male-to-female sex change). Modified from Munday, P. L., Buston, P. M., Warner, R. R., 2006. Diversity and flexibility of sex-change strategies in animals. Trends in Ecology & Evolution 21, 89–95.

Whilst these adaptive explanations for hermaphroditism are undoubtedly important in understanding how it can evolve, the current taxonomic distribution of the different sexual systems among animals also reveals a strong degree of phylogenetic inertia (see Renner and Ricklefs, 1995 for data on plants). While some groups are (almost) entirely hermaphroditic (e.g., flatworms, arrow worms, and gastrotrichs) there are other groups that are (almost) entirely gonochoristic (e.g., insects, nematodes, and acanthocephalans), while yet other groups show more variable sexual systems (e.g., coelenterates, polychaetes, and molluscs) (see also Ghiselin, 1969; Schärer, 2009; Weeks, 2012; Collin, 2013).

Irrespective of the exact conditions under which hermaphroditism is favored or maintained, once two routes to fitness are present in the same individual, this leaves open the possibility of strategically varying the amount of resources invested into the two sex functions, both in terms of overall quantity and with respect to the timing during an individual's life history (i.e., simultaneously vs. sequentially). These are questions about 'sex allocation,' which we discuss next.

Local Competition and Sex Allocation

In anisogamous species, every zygote results from the fusion of one male and one female gamete, and so every individual in the population has exactly one father and one mother. This means that at the level of the population, the amount of fitness that can be obtained via the male and the female reproductive strategy is necessarily equal (despite the male gamete's usually minimal material contribution to the zygote). Under frequently made simplifying assumptions of random mating (i.e., any male gamete in the population has the same probability of fusing with any female gamete) and large populations size (i.e., mates and competitors are always unrelated) this then predicts equal resource investment in male and female reproduction (Düsing, 1884; Fisher, 1930; Charnov, 1982). However, in nature local competition between related entities often results from imperfect mixing of gametes, clustering of gametes into ejaculates and clutches, and limited dispersal of offspring leading to mate competition between relatives, meaning that these simplifying assumptions are probably broken more often than not. As a result the optimal resource allocation can differ drastically from equality, leading

to strongly biased individual and population sex allocation patterns (Hamilton, 1967; Charnov, 1982; Frank, 1987). Local competition can also affect the optimal distribution of the two sexual strategies over individuals in the population, so understanding the consequences of the evolution of anisogamy requires an understanding of the logic of sex allocation theory. While we cannot give a detailed account of sex allocation theory here (see Charnov, 1982; Schärer, 2009; West, 2009), we will highlight those aspects most relevant to the study of sex in hermaphrodites.

Local competition between related gametes means that investment toward making more gametes shows diminishing returns. If local competition is sex-specific, say affecting male more than female gametes, then there comes a point at which additional resources would be better invested in the alternative sex function: such individuals benefit from investing less than half of their resources into the production of male gametes, resulting in simultaneous hermaphroditism with a female-biased sex allocation (**Figure 3(a)**). Such 'local sperm competition' may regularly occur under conditions such as low population density, monogamy or in small groups (Charnov, 1982; Schärer, 2009), but it can also result from different processes of sexual selection, such as strong first or last male sperm precedence, cryptic female choice, or random paternity skews (Charnov, 1996; Greeff *et al.*, 2001; Schärer, 2009; Van Velzen *et al.*, 2009; Schärer and Pen, 2013). Exclusive selfing is an extreme example, involving maximal local sperm competition and thus strongly diminishing returns on male reproductive investment.

In a similar way, local competition between socially interacting individuals may curtail the reproductive opportunities more strongly for one than the other sex function. If this effect depends on, for example, body size, then reproducing in one sex early in life and later switching to the other sex, i.e., being a sequential hermaphrodite, might be the optimal sex allocation strategy (**Figure 2**; Charnov, 1982; Munday *et al.*, 2006). Conversely, if investment toward one sex function shows accelerating returns (say because a higher fighting ability or more attractive ornament makes winning in mating competition much more likely), then permanently specializing on one sex role may be the best strategy, thus favoring gonochorism (**Figure 3(b)**; Charnov, 1982). Here individuals can still optimize their sex allocation by producing offspring with biased sex ratios (although under a fairly broad range of conditions the optimal sex ratio may remain close to equality). Note, however, that a gonochorist's sex ratio adjustment is less direct than the sex allocation adjustment within an individual hermaphrodite's lifetime, as the former is played out only in the next generation.

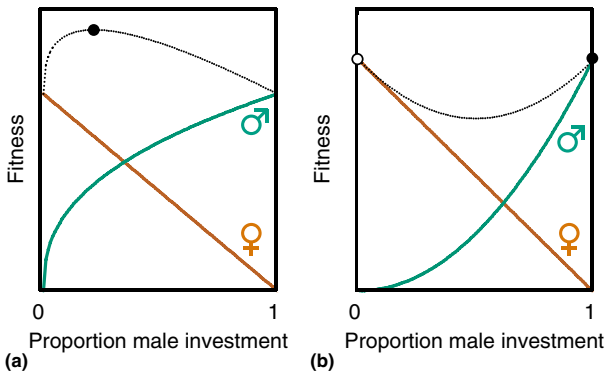


Figure 3 Fitness gain curves from basic sex allocation theory applied to simultaneous hermaphrodites. The graphs depict hypothetical fitness gain curves that represent the total fitness (stippled black line) that an individual can obtain when incrementally shifting the investment of a fixed resource budget from the female (solid orange line) to the male sex function (solid green line) (note that the fitness returns to the female function are here assumed to be linear). (a) If the marginal gains from increasing male investment show diminishing returns (here depicted as a power function with fitness $\propto$ resources$^{0.4}$) then total fitness is maximized (as indicated by the solid black circle) at an intermediate proportion of resources being allocated to the male function, in this case resulting in a somewhat female-biased sex allocation (a likely common scenario). (b) By contrast, if the marginal gains from increasing male investment show accelerating returns (here depicted as fitness $\propto$ resources^2) this does not result in a single fitness optimum, selecting instead for maximal investment in the male function in some individuals (which are then males, solid black circle), and – due to the frequency-dependent nature of sex allocation – an equivalent investment into pure females (open circle), i.e., for gonochorism. Based on Charnov (1979, 1982); see also Schärer (2009) and Kazancıoğlu (2009).

Sex in Sequential Hermaphrodites

The Role of Ecology and Social Interactions

Recall that the size-advantage model predicts that an individual should want to change sex whenever it can increase its fecundity (or reproductive value) by doing so (Section 'The Adaptive Significance of Having Two Routes to Fitness'). Here we emphasize that ecological and social factors combine in interesting ways to generate predictable sex differences in the size–fecundity relationship favoring sex change, but also that they can constrain individual sex-change decisions.

'Protandry' (male-to-female sex change) is often observed if the habitat leads to very small and stable groups of locally interacting individuals, such as is observed in slipper shells that form stable stacks of individuals (**Figure 4(a)**; Collin, 2006) or in anemone fishes that inhabit individual sea anemones (**Figure 4(b)**; Fricke and Fricke, 1977). In both cases, female fecundity is highly body size-dependent, whereas male fecundity is not, because the particular ecological conditions constrain the opportunity for male–male competition (**Figure 2(b)**). In the case of slipper shells, the number of mates that a male can obtain is inherently limited to only the neighbors in its stack (Collin, 1995) and paternity is biased toward the closer neighbors (Proestou *et al.*, 2008). In anemone fishes, there is a stable dominance hierarchy among the group members in which the highest ranking individual, a large female, uses aggression to constrain the reproductive decisions of the lower-ranking individuals, which comprise a smaller male (her only sexual partner) and a variable number of even smaller reproductively suppressed immature individuals (Fricke and Fricke, 1977). It is important to acknowledge, however, that such ecological constraints may also favor bidirectional sex change, as observed in some coral gobies

Figure 4 Sequential hermaphrodites. (a) In the protandrous common slipper shell, *Crepidula fornicata*, individuals form stacks in which the smaller (and younger) individuals closer to the top of the stack are males (the arrowheads indicate very small individuals), while the larger (and older) individuals toward the bottom of the stack are females (note that the individual in the bottom-most shell on the right has already died). Intermediate-size individuals that are in the process of changing from male to female often occur in the middle of the stack (see also Collin, 2006; image 'Stack of Crepidula' by Paul Morris from https://www.flickr.com/photos/aa3sd/5044549409/ licensed under CC BY-SA 2.0). (b) In the protandrous twoband anemonefish, *Amphiprion bicinctus*, many individuals live in stable pairs that inhabit and jointly defend a sea anemone (here the bubble-tip anemone, *Entacmaea quadricolor*). Pairs usually consist of two sexually mature individuals, a smaller male individual and a larger female individual, the latter has previously functioned as a male. Moreover, in some cases there are additional immature individuals that are cueing to take the place of the resident male and female (see also Fricke and Fricke, 1977; image by Lukas Schärer). ((c) and (d)) The protogynous sea goldie, *Pseudanthias squamipinnis*, shows considerable sexual dimorphism, with females (c) being much smaller and largely orange in coloration and males (d) being larger, more variably colored, and carrying a long dorsal fin ray. Males are territorial and can pair spawn with multiple individual females per day; all males start their life as females (see also Shapiro, 1979; images by Lukas Schärer). (e) Like most grouper species, the Red grouper, *Epinephelus morio*, is protogynous and mates in pairs where males experience low sperm competition, while (f) its sibling species the Nassau grouper, *Epinephelus striatus*, is gonochoristic and mates in groups where males experience high sperm competition, supporting the idea that strong sperm competition can make protogyny unstable (see also Erisman *et al.*, 2009; the public domain images are, respectively, from http://www.photolib.noaa.gov/bigs/fish3131.jpg and …/fish3129.jpg).

(Kuwamura *et al.*, 1994; Munday, 2002), or simultaneous hermaphroditism with strongly female-biased sex allocation (Baeza, 2010).

Conversely, in less ecologically restricted conditions that permit the formation of larger groups, male fecundity may strongly depend on an individual's ability to hold a territory, in which it can gain exclusive access to multiple females. Even if female fecundity is still strongly dependent on body size (as is often the case), a large body size may benefit males more than females, thus favoring 'protogyny' (**Figure 2(a)**). This sexual system is frequently encountered in a diversity of different fish families (Munday *et al.*, 2006; Avise and Mank, 2009; Erisman *et al.*, 2013), including some serranid reef fishes (**Figures 4(c)** and **4(d)**), and many labrid and scarid reef fishes (Robertson and Warner, 1978; Warner and Robertson, 1978; Warner, 1991; Schärer and Vizoso, 2003; Kazancıoğlu and Alonzo, 2010). Note that in such species only relatively few individuals may actually reach a size where they change sex and obtain significant reproductive success as male, leading to highly biased population sex ratios (Warner and Hoffman, 1980).

From a mating systems perspective, protandry is expected under conditions where sexual selection is weak, as individuals are often essentially living under monogamy. In contrast, there is ample scope for the evolution of sexual ornaments under protogyny, with multiple males vying for the attention of females. Indeed, males and females often look drastically different in protogynous species (**Figures 4(c)** and **4(d)**), while they often look very similar in protandrous species (**Figure 4(b)**).

Extensions to the Basic Theory

More recently the theoretical foundations of the size-advantage model have been extended in an attempt to explain some apparently counterintuitive empirical observations (see also Munday *et al.*, 2006), one of which is that in some protogynous species large females sometimes do not change sex when the opportunity arises. The Expected Reproductive Success Threshold model (Muñoz and Warner, 2003) incorporates variation in (1) sperm competition intensity (which reduces the payoffs that can be expected from changing to the male sex) and (2) female fecundity (which considers how much fecundity a female gives up when changing sex compared to the aggregate fecundity of the remaining females). In support of the sperm competition scenario, which may result from group spawning or small males that interfere in pair spawns, a recent comparative phylogenetic study showed that protogyny has shifted to gonochorism at least four times independently among groupers, and that these shifts were always associated with the evolution of group spawning (high sperm competition) from the ancestral pair spawning (low sperm competition) character state (Erisman *et al.*, 2009) (**Figures 4(e)** and **4(f)**). Moreover, the same study showed that relative testis size was much larger in gonochoristic than protogynous species, which also matches this scenario very well. Support for the female fecundity scenario stems from a study on the bucktooth parrotfish, *Sparisoma radians*, that initially motivated the extended theory (Muñoz and Warner, 2004; but see critique by Clifton and Rogers (2008) and response by Warner and Muñoz (2008)), and arguably also from a study that shows that in the bluehead wrasse, *Thalassoma bifasciatum*, females that become infected with an ovarian parasite – and thus have a lower female fecundity – change sex earlier and at a smaller size (Schärer and Vizoso, 2003).

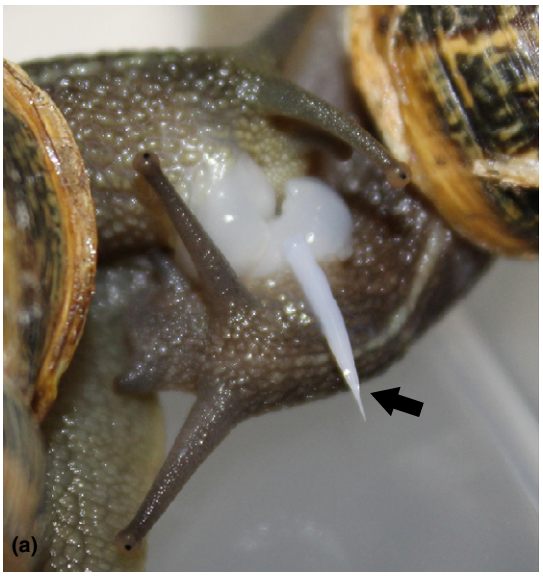
Finally, a recent study in the protogynous sandperch, *Parapercis cylindrica*, suggested that there might be a role for intralocus sexual conflict in sequential hermaphrodites (Sprenger *et al.*, 2012), in that individuals were shown to exhibit a similar aggression level before and after sex change. Given the species' mating system, the authors argued that high aggression may be good when male, but potentially deleterious when female, which would make any allele affecting aggression sexually antagonistic (for a more detailed discussion of both intra- and interlocus sexual conflicts in sequential hermaphrodites, see Abbott (2011) and Schärer *et al.* (2014)). If such antagonistic alleles are widespread in sex changers, this could also affect the stability, expression, and timing of sex-change strategies, a field that would greatly benefit from further theoretical work.

Sex in Simultaneous Hermaphrodites

Pre- versus Post-Mating Sexual Selection

As originally conceived, sexual selection sought to account for conspicuous, sexually dimorphic traits that were difficult to attribute to natural selection for survival (Darwin, 1859, 1871). Given the absence of male and female individuals, sexually dimorphic traits cannot really exist in simultaneous hermaphrodites. Nevertheless, there are clearly many traits that might differentially influence reproductive fitness through one or the other sex function. An interesting question is whether the fact that all individuals in a simultaneously hermaphroditic population would have to express a (presumably costly) sexually selected trait and preference fundamentally alters the operation of sexual selection, or whether it just means that such traits are harder to identify (Morgan, 1994; Schärer and Pen, 2013). The conditions that were originally thought to favor hermaphroditism – low mate availability etc. – might tend to favor relatively indiscriminate mating, obviating the need for elaborate pre-mating mate choice mechanisms. Accordingly, most evidence of mate choice in hermaphrodites to date appears to relate instead to indicators of female fecundity or likely fertilization returns on male investment (Schärer and Pen, 2013).

The importance of post-mating sexual selection (Parker, 1970; Eberhard, 1996) in hermaphrodites is much more clearly established. Indeed, some of the earliest insights about how sperm competition, cryptic female choice, and sexual conflict would play out were those in the hermaphrodite-specific treatment of Charnov (1979). Many of the familiar post-mating sexually selected traits found in gonochorists have also been well described in various hermaphroditic taxa. Some of these adaptations even appear to be especially common in hermaphrodites, as is for example the case for devices that inject manipulative substances into the mating partner, for example, the love darts of various land snails (**Figure 5(a)**)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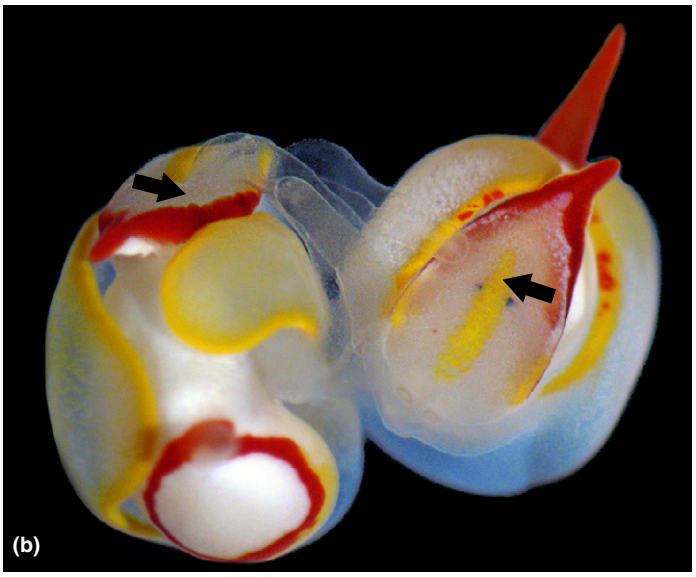
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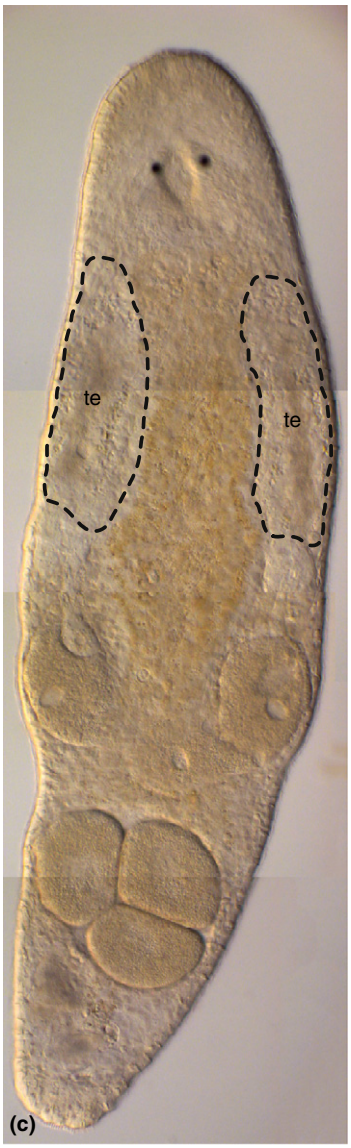
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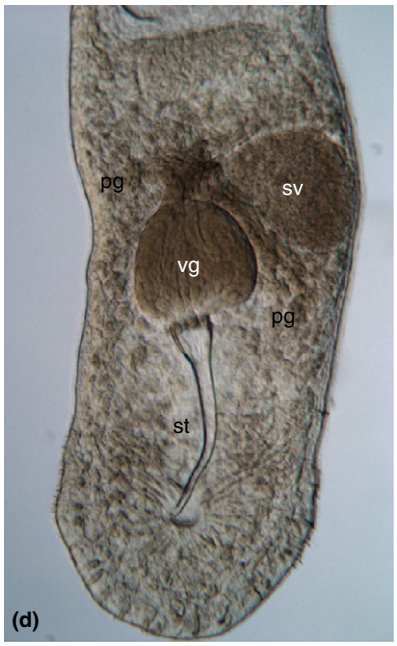
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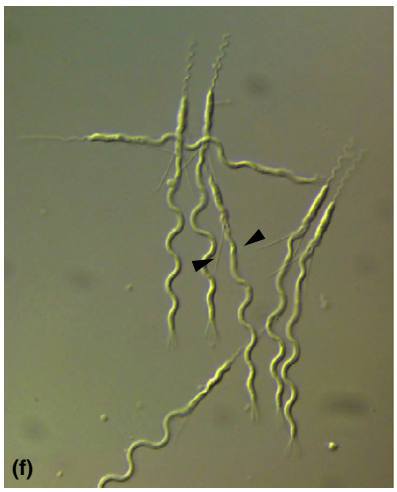
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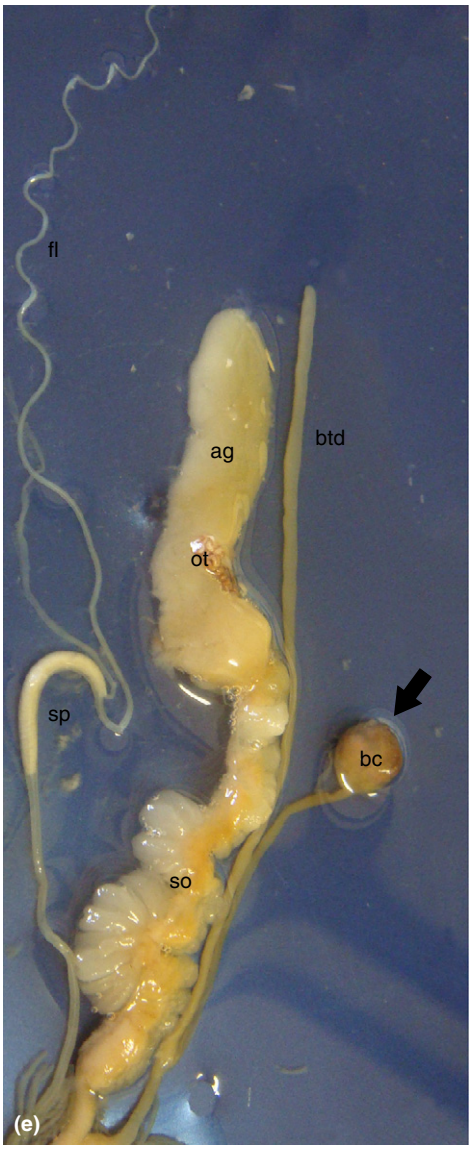
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(Koene and Schulenburg, 2005; Nakadera and Koene, 2013; Kimura and Chiba, 2015), the copulatory setae of earthworms (Koene *et al.*, 2002) and the stylet-like penis appendages of some sea slugs (**Figure 5(b)**) (reviewed in Lange *et al.*, 2013; Schärer *et al.*, 2014). Moreover, large testes (e.g., **Figure 5(c)**) and prominent prostate glands (**Figure 5(d)**), sperm digesting organs (**Figure 5(e)**), and complex sperm (**Figure 5(f)**) are also frequently encountered. In fact, owing to the apparent scarcity of pre-mating sexual selection and the fact that two simultaneous hermaphrodites will often agree on (or due to reciprocity be unable to avoid) mating, there may even be an enhanced role for post-mating sexual selection compared to gonochorists (Schärer *et al.*, 2014). In Section 'Sex Allocation Manipulation as a Unique Post-Mating Arena of Conflict' we focus on one unique aspect of post-mating sexual selection in simultaneous hermaphrodites, the potential ability of an individual to manipulate the sex allocation of its mating partner (see also Abbott (2011) and Schärer *et al.* (2014) for a broader discussion of sexual conflict in hermaphrodites), but first we return to a unique pre-mating aspect of sex, namely the fact that two potential mating partners must first resolve which sex role or roles each of them will play in any mating interaction.

Sex Role Preference as a Unique Pre-Mating Arena of Conflict

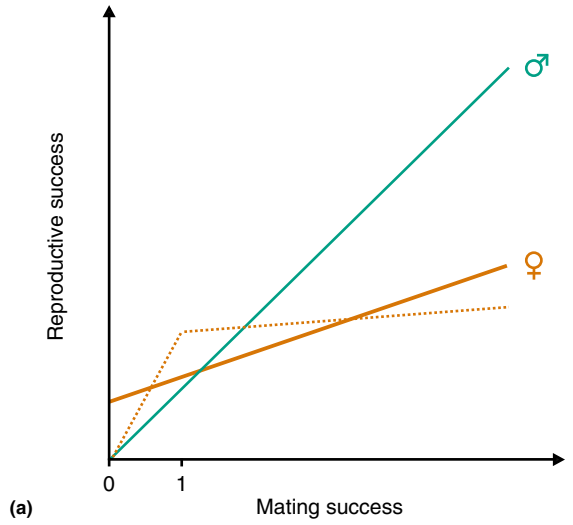
Applying Bateman's principle (Bateman, 1948) to simultaneous hermaphrodites, Charnov (1979) realized that hermaphroditic individuals may often wish to 'copulate not so much to gain sperm to fertilize [the own] eggs as to give sperm away,' basically because the female sex function is typically more limited by access to resources and the male sex function by access to eggs (**Figure 6**) (for a more thorough review see Anthes *et al.*, 2010). If two potential mating partners are then both male and female, this may often create a conflict over which of them adopts the male and female sex role in any particular mating interaction, i.e., a 'mating conflict' (Charnov, 1979; Michiels, 1998; Schärer *et al.*, 2014) (**Figure 7**). This need not always be the case, however, since the expected male preference refers to the average situation, not to a specific interaction in which the individuals may or may not have compatible mating interests; for example, for a virgin individual there may be a considerable fitness gain to be had from mating in the female role (**Figure 6**), so a virgin and a non-virgin individual might readily agree over their different, preferred sex roles.

Whenever there are incompatible mating interests, these could be resolved in various ways, either resulting in realized conflict or not (Charnov, 1979; Michiels, 1998; Schärer *et al.*, 2014). In the former category would clearly be behaviors such as penis fencing exhibited by some polyclad flatworms, in which each individual attempts to stab and hypodermically inject sperm into the partner whilst simultaneously avoiding the same fate itself (Michiels and Newman, 1998; **Figure 8(a)**). More peaceful resolutions occur in those species that agree to reciprocally exchange gametes, meaning they consent to adopt both the preferred and non-preferred roles through either conditional sperm receipt or conditional sperm donation. This could involve interactions during which two individuals may exchange sex roles, but each mating itself is unilateral (e.g., **Figure 8(b)**). For example, empirical evidence suggests that such a resolution of mating conflict manifests as egg trading in serranid reef fishes (**Figure 8(c)**). In one case of proposed sperm trading – in the sea slug *Chelidonura hirundinina* – the predicted conditionality of gamete trading has also been experimentally demonstrated: individuals are more reluctant to mate with non-reciprocating partners (Anthes *et al.*, 2005). In other cases, reciprocal exchange of gametes is achieved by both individuals acting simultaneously as sperm donor and sperm recipient, as in some land snails (**Figure 8(d)**) and flatworms (**Figures 8(e)** and **8(f)**).

Sex Allocation Manipulation as a Unique Post-Mating Arena of Conflict

It was again Charnov (1979) who first suggested that a common target of manipulation by a simultaneously hermaphroditic sperm donor might be the sex allocation of the corresponding sperm recipient. This could occur in two distinct ways, either by boosting the female function of the mating partner, or else by attacking its male function. The former mechanism should normally be straightforwardly

Figure 5 Adaptations to post-mating sexual selection in simultaneous hermaphrodites. (a) Mating in the garden snail *Cornu aspersum* (formerly *Helix aspersa*) involves the attempt to shoot a mucus-covered calcareous 'love dart' (arrow) into the body of the mating partner (here the shooter has missed). The transferred substances cause a conformational change in the female genital system that reduces the digestion of received sperm (see also panel e) and thereby increases the successful shooter's paternity share (see also Chase and Blanchard, 2006; Koene, 2006) (image courtesy of Alexandra Staikou). (b) Mating in the cephalaspid sea slug *Siphopteron* sp. 1 involves the reciprocal traumatic insertion of an accessory copulatory stylet into a precise position on the forehead of the partner (arrows), followed by the injection of a prostate secretion close to the partner's neural ganglia (see also Lange *et al.*, 2014; image courtesy of Nils Anthes). (c) The free-living flatworm *Macrostomum hystrix* has very large testes (te), which represent approximately 15% of the body volume, suggesting that this species experiences high levels of sperm competition (stitched image by Lukas Schärer). (d) The free-living flatworm *Macrostomum evelinae* has a copulatory stylet (st) that is associated, not just with a seminal vesicle (sv), but also with a very prominent prostate-like vesicula granulorum (vg), which is fed by numerous prostate gland (pg) cells that occupy a large portion of the animal's tail plate (image by Lukas Schärer). (e) The garden snail has a complex spermatophore receiving organ system that includes a bursa tract diverticulum (btd) involved in receiving the spermatophore (sp) and a bursa copulatrix (bc) involved in the digestion of most of the received sperm that emerges from the spermatophore (ag, albumen gland; fl, flagellum; ot, ovotestis; so, spermoviduct) (see also Koene, 2006; Garefalaki *et al.*, 2010; image courtesy of Alexandra Staikou). Whether such sperm digestion occurs at a net resource benefit or if it just serves to remove excess sperm is currently unclear (Schärer *et al.*, 2014). (f) The sperm of the free-living flatworm *Macrostomum lignano* are highly complex and carry stiff lateral bristles (arrowheads) that are considered to be a male persistence traits involved in a sexual conflict over the fate of received sperm (see also Vizoso *et al.*, 2010; Schärer *et al.*, 2011; still image extracted from a video taken by Lukas Schärer).



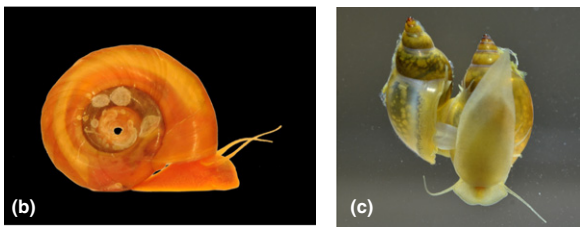


Figure 6 Bateman gradients in hermaphrodites. (a) Hypothetical plot showing the expected fitness returns (measured as reproductive success) on obtaining mating success in the male or the female sex function in hermaphrodites. As in gonochorists, it is often expected that the 'Bateman gradient' (i.e., the slope of the regression of reproductive success on mating success; Bateman, 1948; Arnold, 1994a) is steeper for males (solid green line) than for females (solid orange line). The dotted line illustrates a scenario whereby for the female function there is a large increase in fitness from an initial mating (where the individual's state switches from virgin to mated) but further matings then have little impact on fitness (e.g., because once mated the individual has access to stored sperm) (see also Anthes *et al.*, 2010; Schärer *et al.*, 2014). Recent empirical studies in two simultaneously hermaphroditic snails, (b) *Biomphalaria glabrata* and (c) *Physa acuta* (here engaged in a unilateral mating), confirm that the male sex function indeed exhibits a steeper Bateman gradient than the female sex function (see Anthes *et al.*, 2010; Pélissié *et al.*, 2012). (Images of *B. glabrata* and *P. acuta* courtesy of Nils Anthes and Tim Janicke, respectively.)

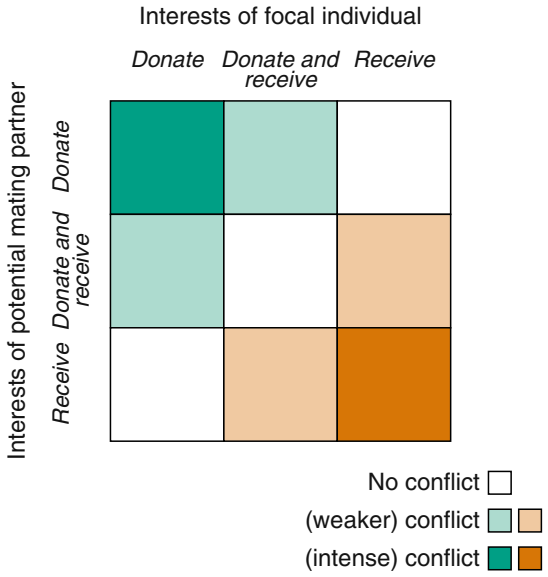


Figure 7 Potential mating conflicts over sex roles in simultaneous hermaphrodites. Two simultaneously hermaphroditic individuals that meet must each decide, not just whether they wish to mate, but also which sex role or roles they wish to play: donate sperm, receive sperm, or both. Mating conflicts arise whenever the two individuals have incompatible mating interests and they are strongest when both individuals either want to only donate (green-shaded square) or to only receive sperm (orange-shaded square), but they also occur wherever there is partial disagreement (lighter-shaded squares). Modified from Schärer, L., Janicke, T., Ramm, S. A., 2014. Sexual conflict in hermaphrodites. Cold Spring Harbor Perspectives in Biology doi: 10.1101/cshperspect.a017673, based on an original figure in Michiels (1998).

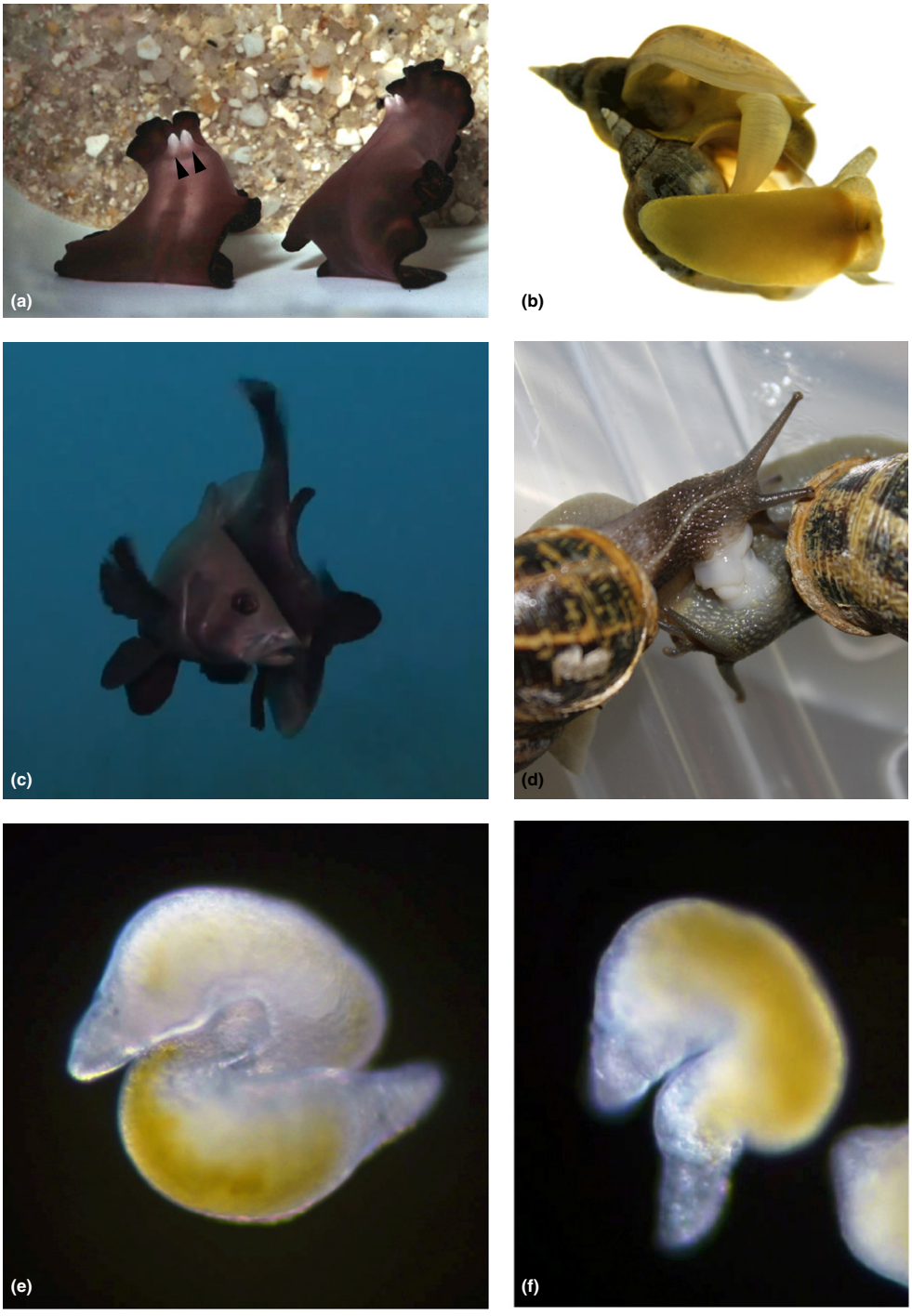
adaptive in the same way that gonochoristic males might attempt to boost the reproductive output of females with whom they mate (cf. Avila *et al.*, 2011), and the latter could be adaptive for a number of reasons (reviewed in Schärer, 2014; Schärer *et al.*, 2014) (**Figure 9**). Recent experimental work in the pond snail *Lymnaea stagnalis* has identified at least two seminal fluid proteins that indeed appear to manipulate the male sex function of the mating partner, reducing the number of sperm that an individual transfers during its next mating (Nakadera *et al.*, 2014). However, the exact adaptive significance of this effect remains to be determined (see also Schärer, 2014).

Outcrossing versus Self-Fertilization

As we already mentioned above, another unique aspect of sex in simultaneous hermaphrodites (and indeed certain sequential hermaphrodites, namely if the gamete type produced first can be stored prior to use) is the phenomenon of self-fertilization. Despite potential negative effects resulting from inbreeding depression, this may still offer a better alternative than being unable to reproduce at all, and current evidence of so-called delayed selfing bears this out: upon reaching sexual maturity, in the absence of outcrossing opportunities many simultaneous hermaphrodites at first wait for such an opportunity to arise (especially if inbreeding costs are high; Tsitrone *et al.*, 2003a; Escobar *et al.*, 2011), and if an opportunity does not materialize they later switch to selfing (e.g., Tsitrone *et al.*, 2003b; Escobar *et al.*, 2011; Ramm *et al.*, 2012). Note, however, that by far not all simultaneous hermaphrodites are capable of selfing, as there may be morphological constraints that prevent ready selfing (cf. Ramm *et al.*, 2012; Ramm *et al.*, 2015) or self-incompatibility systems that normally guard against it (Bishop, 1996; Bishop *et al.*, 1996) that would need to be overcome, and the precise costs and benefits of outcrossing versus selfing will depend strongly on the previous history of selfing within a population (Jarne and Auld, 2006; Escobar *et al.*, 2009; Escobar *et al.*, 2011).

Outlook

Even from this brief account, it should be clear that questions about the sexual system, sex allocation, and sexual selection are inextricably linked, and that we therefore need to learn more about how these processes operate in hermaphrodites compared to the much better investigated gonochorists. In a similar vein, it should be evident that there are important parallels between sex changers, simultaneous hermaphrodites, and gonochorists, and it is worth pointing out that these broad categories gloss over a much richer diversity of sexual systems, including for example mixtures of males and hermaphrodites (such as androdioecious clam shrimp; Weeks *et al.*, 2006), dwarf males with both gonochorists and hermaphrodites (as in barnacles; Yusa *et al.*, 2013), cyclical parthenogenesis in which one clone can make both male and female offspring (as in cladocerans; Innes and Singleton, 2000), and asexual propagation and fissioning in hermaphrodites (as in many



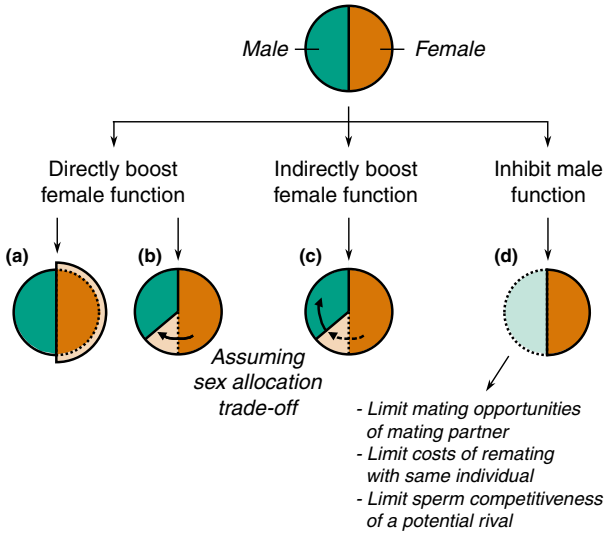


Figure 9 Hypotheses for sex allocation manipulation in simultaneous hermaphrodites. For a sperm donor, manipulating the reproductive allocation of the sperm recipient could be adaptive for a number of reasons. Most straightforwardly, the donor could divert resources toward the recipient's female function, and thereby gain from fertilizing a greater number of eggs ((a) and (b)). Depending on from where these resources are diverted, this may (b) or may not (a) result in a corresponding reduction in the recipient's male allocation (i.e., it may or may not affect the individual's sex allocation in the strict sense). Alternatively, it may pay the donor to directly target the recipient's male sex function, either because doing so results in a reallocation of resources toward the female function due to a sex allocation trade-off (c), or because reducing the recipient's male allocation is by itself beneficial (d). Possible adaptive scenarios for the latter include that it may constrain the ability of the recipient to re-mate (thus reducing the likelihood that the donors faces sperm competition to fertilize the recipient's eggs); that it may limit the costs when a donor re-mates as a recipient with that same mating partner (e.g., if fewer costly substances are transferred, or costly mating duration is reduced); or that the sperm competitive ability of a potential rival donor in other recipients in the population would be reduced. For further discussion, see also Schärer *et al.* (2014) and Schärer (2014).

cnidarians, flatworms, and different colonial marine invertebrates; Hughes, 2005; Janssen *et al.*, 2015; for plants see also Haig and Wilczek, 2006), to name just a few (see also Weeks, 2012). Studying the evolutionary biology of sex in hermaphrodites should therefore be seen as an important part of the wider goal of understanding the biochemical, morphological, and behavioral diversity resulting from the male–female phenomenon, i.e., the evolution of anisogamy and its consequences. The fact that there are multiple origins of anisogamy (Kirk, 2006) necessarily means that there is a mix of common, unifying themes (e.g., the usually greater variance in male reproductive success) coupled with a plethora of divergent solutions that involve considerable phylogenetic idiosyncrasies (e.g., the prominent role of the male gametophyte in plant reproduction vs. the greatly suppressed role of haploid sperm in animals due to their being largely transcriptionally silenced). Thus it is clear that more work to compare plant and animal sexual systems would be insightful (see also Arnold, 1994b; Bernasconi *et al.*, 2004; Bedhomme *et al.*, 2009; Vega-Frutis *et al.*, 2014), as would the better incorporation of insights gained from other anisogamous sexual systems (Coleman, 2012; Nieuwenhuis and Aanen, 2012). Sexual reproduction did not start, nor does it necessarily end, with separate males and females; as they evolved multiple times, the male and female sexual strategies instead explored a far greater parameter space, often settling on one of the different forms of hermaphroditism that we see today.

Acknowledgments

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Figure 8 Types of mating in simultaneous hermaphrodites. Mating conflicts can be resolved in a number of ways, which may or may not result in realized conflict. (a) Two polyclad flatworms of the species *Pseudobiceros bedfordi* engage in penis fencing, which involves the attempt to traumatically inseminate sperm into the mating partner using paired copulatory stylets (double arrowheads), while trying to avoid being stabbed oneself (see also Michiels and Newman, 1998 for data on *Pseudoceros bifurcus*; image 'Two Individuals of *Pseudobiceros bedfordi* About to Have a Sperm Battle' by Nico Michiels from doi:10.1371/journal.pbio.0020183.g001 licensed under CC BY). (b) Unilateral mating in the freshwater snail *Lymnaea stagnalis*, with the male role individual at the top inseminating the female role individual below using its penis-carrying organ, the preputium (see also Koene *et al.*, 2010; image courtesy of Joris Koene and Cathy Levesque). (c) Two black hamlets, *Hypoplectrus nigricans*, engaged in an egg trading exchange in which the male role individual (the egg releaser) is facing downwards and the female role individual (the sperm releaser) has clenched jaws and is curled around the former. Roles are then usually exchanged multiple times in an iterated way (see also Fischer, 1980; Henshaw *et al.*, 2015; still image from a video by the BlennyWatcher.com website hosted at http://www.youtube.com/watch?v=5d2dXRKNCbg and courtesy of Ned and Anna DeLoach). (d) Two garden snails *Cornu aspersum* (formerly *Helix aspersa*) involved in a reciprocal mating, during which they assume a head to head position and reciprocally insert their male copulatory organs (see also Koene, 2006; Garefalaki *et al.*, 2010; image courtesy of Alexandra Staikou). (e) Two free-living flatworms of the species *Macrostomum lignano* engaged in a reciprocal mating, during which they curl around each other in the shape of two interlocking 'G's (see also Schärer *et al.*, 2004; Vizoso *et al.*, 2010; still image from a video by Lukas Schärer). (f) An *M. lignano* individual engaged in the postcopulatory 'suck' behavior, during which it places its pharynx over its own female genital opening and appears to be sucking, presumably to remove ejaculate components that it received during the preceding reciprocal mating (see also Schärer *et al.*, 2004; Vizoso *et al.*, 2010; still image from a video by Lukas Schärer).

See also: Mating Systems, A Brief History of. Mating Systems in Flowering Plants

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Homo, Diversification of

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Glossary

Acheulian Type of stone tools characterized by large handaxes first produced by *Homo erectus* in Africa after about 1.7 Ma, but later also widespread in Eurasia.
Arborealism The behavior of an organism living in, or spending the majority of time in, the trees.
Australopithecine Species in the genera *Australopithecus* and *Paranthropus*; a type of hominin largely preceding (with some overlap) the genus *Homo*.
Basicranial flexion Angle of the cranial base, which differs between hominin species.
Cranium The skull not including the mandible (lower jaw).
Derived Characteristics which are present in a daughter taxon, but not its parent taxon.
Edentulous An individual who has lost their teeth.
Encephalization Relative (taking body size into account) increase in brain volume.
Endocranium The inside of the cranial vault, which houses the brain.
Genetic drift Variation in relative frequency of different genetic make-ups resulting from chance, such as the failure of some individuals to reproduce.
Homoplasy Characteristics present in two taxa, but not in their last common ancestor and thus not shared due to shared ancestry. This is also called convergent evolution.
Hypodigm The fossil or group of fossils from which a taxon is defined.
Introgression Input of genetic material from one species to another.
ka Kiloannus, or thousand years ago.
Ma Megaannus, or million years ago.
Maxillae The paired bones forming the lower part of the face.
Mitchondrial DNA The genetic material in the energy-producing organelles found in cell nuclei, the mitochondria. Unlike the nuclear genome, the mitochondrial genome (the mtDNA) consists of a single ring of DNA, in mammals is inherited only through the female line and is not recombined between generations.
Nuclear genome/DNA The genetic material in the nucleus inherited from both parents and consisting of 23 paired chromosomes in living humans. The material from each parent is recombined in every new generation.
Nutritional cannibalism Cannibalism treating the consumption of human flesh as no different from any other food source, as opposed to ritual cannibalism.
Occipital Bone at the back and rear of the cranium.
Phalanx A finger bone, in the case of the Denisovan fossil, from the last knuckle to the finger tip of the fifth finger.
Pleistocene The geological epoch before present (the Holocene).
Postcrania The skeleton below the skull.
Primitive Characteristics present in a daughter taxon that are also present in its parent taxon.
Prognathism Facial projection.
Sexual dimorphism Difference in size and morphology between males and females of the same species.
Speciation The process by which a new species is born.
Supraorbital tori Browridges or bony protrusions above the eyes.
Taxon (plural: taxa) A taxonomic group (e.g., species, genus, or order).
Temporal Pair of bones forming the lower sides of the skull and housing the ear region.

Homo Habilis and *Homo Rudolfensis*

Homo habilis was named in 1964 by Louis Leakey and colleagues based on fossils from Olduvai Gorge (Tanzania) (Leakey *et al.*, 1964) now dated to 1.9–1.6 Ma (Antón, 2004). *H. habilis* was regarded as intermediate between australopithecines and *Homo sapiens*, due to its larger brain and smaller posterior dentition (Leakey *et al.*, 1964). This 'handy man' purportedly had manual adaptations that facilitated tool-making, although now the earliest currently known stone tools are more than 3 Ma, considerably predating this species (Harmand *et al.*, 2015). Tool use is argued to coincide with changes in cognition and diet, including increased meat eating (Wood, 2012), another supposed distinction between *Homo* species and australopithecines (Lockwood, 2013). Postcranially, body proportions in *H. habilis* are very different to later *Homo*, with long arms probably demonstrating continued reliance on arborealism (Lockwood, 2013). *H. habilis* has always been controversial, initially because it was considered insufficiently distinct from the australopithecines and then, as more material was included in the hypodigm, because some considered that it subsumed too great a morphological diversity (Antón *et al.*, 2014). In particular, the fossils included a larger morph with a flatter face and perhaps less apelike body proportions and a smaller morph with less encephalization and a more apelike postcrania, but a more derived upper face (Wood, 2012; **Figure 1**). Opinions differ as to whether this level of dissimilarity is evidence of sexual dimorphism or of two different species at this time period in East Africa (Wood, 2012). Those who argue for two species have used the name *H. rudolfensis* to distinguish the larger material (Wood, 2012). In 2012, new fossils from Koobi Fora (1.8–2 Ma), similar in

Figure 1 *Homo rudolfensis* (left, shown by a replica of KNM-ER 1470) material has a relatively large, flat face, relatively small anterior teeth and moderately sized posterior teeth, a squared off tooth row and a short palate. The *H. habilis* (right: a replica of KNM-ER 1813, as a possible example) tooth row is more curved and the face flatter (Leakey *et al.*, 2012). Copyright: The trustees of the Natural History Museum, London (NHM).

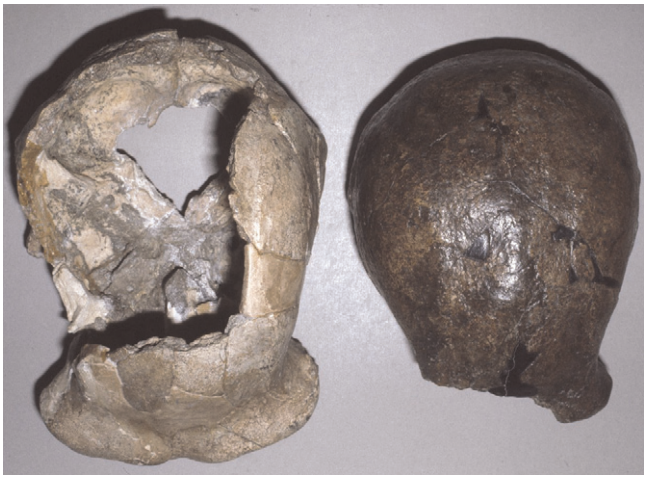

Figure 2 African (left, OH9, Tanzania) and Asian *Homo erectus* (right, Sangiran, Java) skull caps. Copyright: C. Stringer.

shape, but not size, to the larger morph were published, supporting the existence of two taxa (Leakey *et al.*, 2012). The association of facial and mandibular material from one individual in the new material also precludes the coexistence in one species of some of the material previously grouped together in *H. rudolfensis*, further demonstrating considerable complexity in this phase of human evolution (Leakey *et al.*, 2012; Spoor *et al.*, 2015).

Homo Erectus

Homo erectus fossils show larger mean endocranial volumes than *H. habilis*/*H. rudolfensis* combined with very large supraorbital tori (**Figure 2**), thickened bone in the occipital region (Hublin, 2014), and body proportions similar to later *Homo* (Stringer, 2012a). The earliest known *H. erectus* material may come from the Lake Turkana region (Kenya) (**Figure 3**) and the species is traditionally thought to have evolved ~2 Ma, before becoming the first hominin to leave Africa (Stringer, 2012a). Some researchers, however, suggest a Eurasian origin and into-Africa dispersal, due to the early (~1.8 Ma), material from Dmanisi, Georgia (Wood, 2011a, see below). *H. erectus* dispersal may be associated with increased meat eating, as carnivore species are usually wide-ranging (Lockwood, 2013). *H. erectus* might also have been the first hominin to control fire and both cooking and increased meat consumption may be linked to its encephalization and gut reduction (Aiello and Wheeler, 1995; Wrangham *et al.*, 1999). There is a concomitant change in technology; early African and Asian *H. erectus* used tools similar to those of *H. habilis*/*H. rudolfensis*, but from ~1.7 Ma, African *H. erectus* produced larger, multipurpose tools, such as handaxes. These were also used in western Asia and possibly Europe from ~1 Ma (Lepre *et al.*, 2011).

The Dmanisi fossils are as primitive as early African *H. erectus* and it has even been argued that some of the material might demonstrate a new species, '*H. georgicus*' (Gabounia *et al.*, 2002). The Dmanisi material now includes several very complete crania, associated mandibles, and postcrania (Lordkipanidze *et al.*, 2013). There seems to have been a (relatively) rapid accumulation of fossils at the site, representing a short time period and leading the authors to propose a single

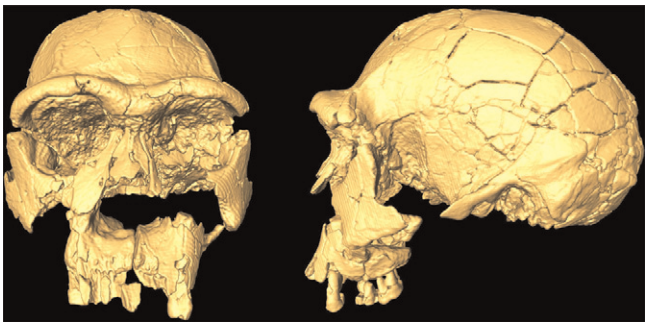

Figure 3 KNM-ER 3733 (Kenya). *Homo erectus* is characterized by larger average brain sizes than *H. habilis*, but the braincase is relatively longer, flatter, and more angular. The browridges are prominent and the face is less projecting than in typical *H. habilis* fossils, with a more prominent, human-like nose. Figure shows virtual reconstructions from CT data. Copyright, L.T. Buck, CT data from National Museums of Kenya.

species, despite considerable variation between individuals (Lordkipanidze *et al.*, 2013). They argue that the range of morphology at Dmanisi, which they propose is comparable to that in recent *H. sapiens* or chimpanzees, may well encompass material otherwise diagnosed as *H. habilis* and *H. rudolfensis*, suggesting that these species should be subsumed in *H. erectus* (Lordkipanidze *et al.*, 2013). Critics argue that the Dmanisi team's measures of variability are flawed and fail to capture characteristic differences between species (Hublin, 2014; Spoor, 2013). There is also criticism of the inclusion of an elderly, endentulous, and juvenile individuals in the analyses and the failure to consider the postcranial material, suggesting that *H. habilis* and *H. rudolfensis* are still best described as separate species from *H. erectus* (Hublin, 2014; Spoor, 2013).

Asian *H. erectus* is best known from 'Java Man' discovered in the 1890s and 'Peking Man' in the 1920–1930s, although many additional finds have since been made in both Indonesia and China, the former being generally older. The Asian finds show some differences from the African material; the skulls are more strongly reinforced with ridges of bone, and the walls of the skulls are generally thicker. Asian *H. erectus* was a very successful species, surviving perhaps into the last 250 ka (Antón, 2013).

Homo naledi

To date, the newest member of the genus *Homo* is *H. naledi*, discovered in the Rising Star cave system near Johanesberg, South Africa and published in September 2015 (Berger *et al.*, 2015; Dirks *et al.*, 2015). The assemblage from this remarkable site comprises over 1500 fossils from at least 15 individuals. A new species has been created by the discovers, due to the fossils' combination of primitive characteristics, such as the small brain, curved fingers, shoulder, hip and trunk morphology, and derived characteristics such as the morphology of the wrist, legs, hands, and feet, which most closely resemble those of Neanderthals and *H. sapiens* (Berger *et al.*, 2015). It is unclear as yet how *H. naledi* fits into the genus *Homo*, in part because of the important issue of the unknown age of the fossils, but they are perhaps morphologically most similar to early, small-bodied *H. erectus*, such as those from Dmanisi (Stringer, 2015, see above).

Homo Floresiensis

In 2003 a tiny skeleton (LB1) was discovered in Liang Bua cave on Flores, Indonesia. Despite its size, the skull was mature and its primitive characteristics, such as the lack of chin and distinct supraorbital tori, led the authors to declare it a new species: *H. floresiensis* (Brown *et al.*, 2004). LB1 is currently dated to <20 ka and was suggested by the discoverers to be the result of insular dwarfism, a process whereby island-living, large-bodied mammals reduce in size due to reduced resources and threat from predators. The potential large-bodied ancestor for *H. floresiensis* was given as Asian *H. erectus* (Morwood and van Oosterzee, 2009). LB1's status as a distinct species has been extremely controversial and many recent *H. sapiens* pathologies that could lead to such a morphology have been suggested (e.g., Henneberg *et al.*, 2014; Martin *et al.*, 2006; Hershkovitz *et al.*, 2007). This controversy reached a nadir when the remains were appropriated by a rival team who, in trying to replicate them, damaged some of the fossils to the detriment of future research (Morwood and van Oosterzee, 2009; Brown, 2012). However, subsequent analyses and the recovery of further individuals from Liang Bua have uncovered a combination of traits inexplicable by any single pathology and, arguably, more similarities with australopithecines than later *Homo* (Morwood and Jungers, 2009; Orr *et al.*, 2013; Brown, 2012). Currently, the interpretation of *H. floresiensis* as a valid species is the best explanation of the data, but the ultimate ancestral species is unsure, as derivation from either *H. erectus*, or an earlier hominin (**Figure 4**), would still require substantial evolutionary reversals or homoplasies. This debate is only likely to be settled with the discovery of more fossil material from Flores or surrounding regions (Stringer, 2014).

Homo Antecessor

The enigmatic remains attributed to *H. antecessor* date to around 850 ka (Parés *et al.*, 2013). The species is known from a single site – Gran Dolina (Atapuerca, Spain), where more than 100 fossils from at least 10 individuals have so far been recovered (Wood, 2011b). The fossils were considered to show craniodental features more derived than *H. erectus*, but more primitive than either Neanderthals or *H. heidelbergensis* and so a new species was created: *H. antecessor* (Bermúdez De Castro *et al.*, 1997;

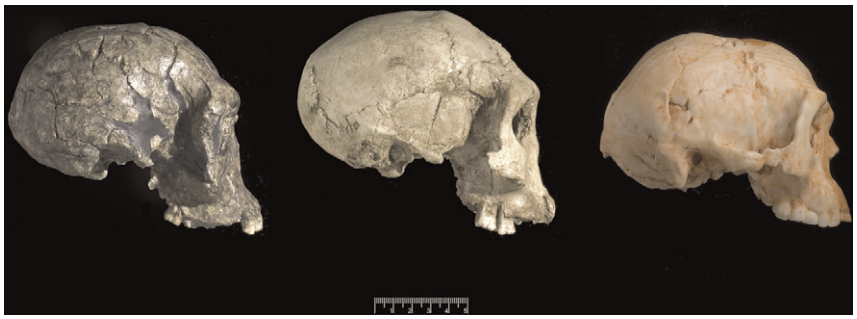

Figure 4 (From left to right) replicas of KNM-ER 1813 (*'Homo habilis'*), D2700 (*H. erectus*) and LB1 (*H. floresiensis*). LB1 is female of about 1 m in height, with a brain volume of around 400 ml (about the same as a chimpanzee) (Stringer, 2014). LB1 has robust limbs with relatively long arms, short legs, and large feet, which lack the longitudinal arch characteristic of *Homo*. The clavicle is short, the scapula protracted, there is little humeral torsion, and the carpal morphology is primitive; these all link *H. floresiensis* with pre-*Homo* hominins, such as the australopithecines (Stringer, 2014). Copyright: NHM.

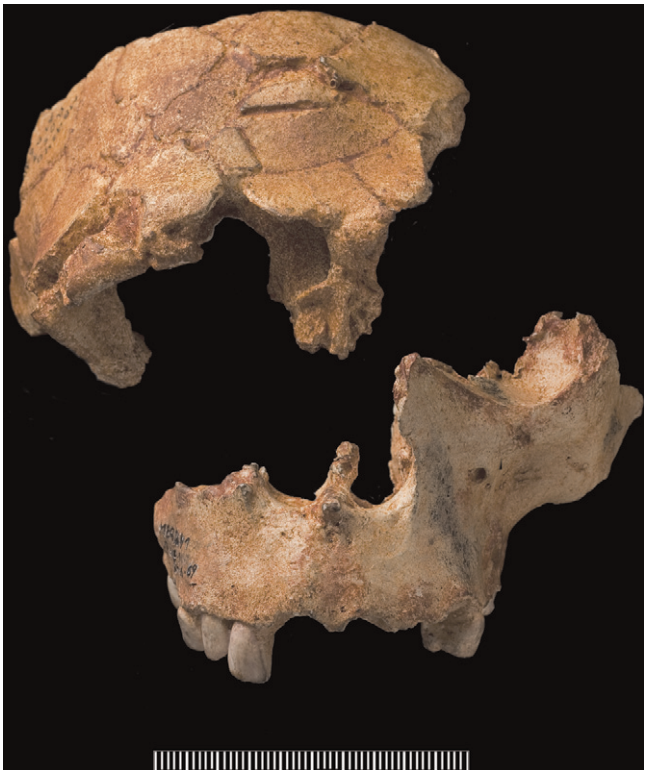

Figure 5 A cast of the most complete *Homo antecessor* fossil, a juvenile partial face, ATD6-69. Copyright: NHM.

Arsuaga *et al.*, 1999). Suggested Neanderthal and *H. sapiens* traits in the fossils have led their discoverers to propose that *H. antecessor* is a common ancestor of both (Arsuaga *et al.*, 1999). Critics, however, suggest that the *H. sapiens*-like facial morphology seen in ATD6-69 facial fragment (**Figure 5**) might be due to its immaturity (Wood, 2011b), whilst the Atapuerca team have themselves noted similarity to the *H. erectus* fossils from Zhoukoudian (China). Most of the hominin remains from the site show human modification and are treated in the same way as fauna in terms of damage and distribution. This suggests that the assemblage is anthropogenic and the result of nutritional cannibalism (Fernández-Jalvo *et al.*, 1999; Saladié *et al.*, 2014). In May 2013, investigations at Happisburgh, UK, revealed the oldest known hominin footprints outside Africa (~1.0–0.78 Ma) in the estuary sediments of an extinct river. Since it is the only known hominin species in western Europe of a similar age, and foot sizes and estimated stature from Happisburgh fall within its estimated range, these footprints are tentatively assigned to *H. antecessor* (Ashton *et al.*, 2014).

Homo Heidelbergensis

Homo heidelbergensis is named for a mandible (~600 ka) found at Mauer, near Heidelberg (Germany) in 1907. The hypodigm is disputed, but the most wide-ranging, and currently

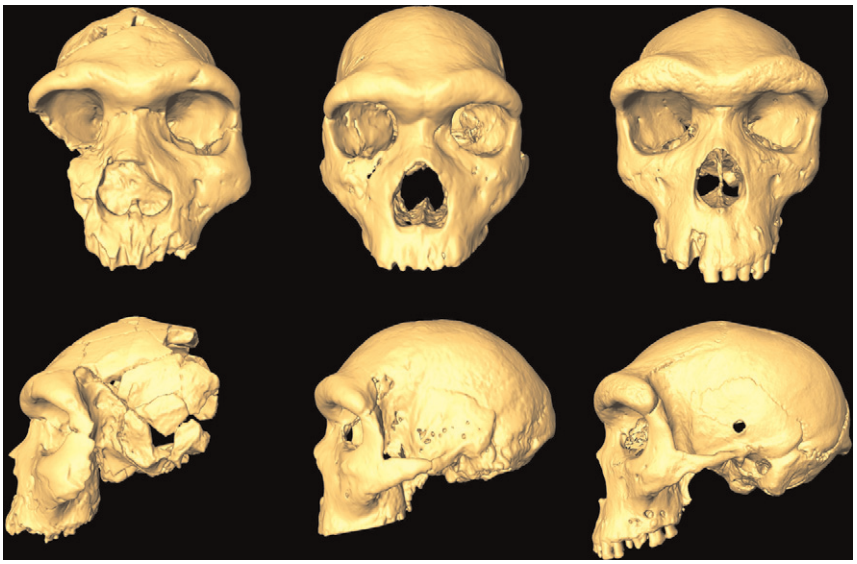

Figure 6 Top and bottom, left to right: Bodo (Ethiopia), Petralona (Greece), and Broken Hill (Zambia). *Homo heidelbergensis* exhibits *H. erectus*-like large browridges (albeit often with distinctively large frontal sinuses), occipital morphology, wide interorbital breadth and pelvic morphology alongside more derived (*H. sapiens*/*H. neanderthalensis*-like) traits including an expanded braincase, reduced overall facial projection, temporal bone morphology, and in some cases, inflated, swept-back maxillae like those seen in *H. neanderthalensis*. *H. heidelbergensis* endocranial volumes overlap the higher end of variation for *H. erectus* and lower end of *H. sapiens/H. neanderthalensis* (Stringer, 2013). Figure shows virtual reconstructions from CT data, fossils scaled to approximately equal size. Copyright: L.T. Buck, CT data from University of Vienna/University of Thessaloniki/NHM.

Figure 7 *Homo heidelbergensis*. Artist's reconstruction of the Boxgrove individual. Copyright: John Sibbick/NHM.

best supported, interpretation of the data includes African, European, and possibly Asian Mid-Pleistocene material and is thought to be the most probable last common ancestor between *H. sapiens* and Neanderthals (Buck and Stringer, 2014; Stringer, 2012b). Similarities between Mauer and remains from Arago (France), which are allied with comparable fossils from Europe and Africa, enable the whole group to be called *H. heidelbergensis*. This Mid-Pleistocene Euro-African (and possibly Asian) group shares a distinctive mosaic cranial morphology (**Figure 6**). The little postcranial evidence that exists shows that *H. heidelbergensis* adults were tall and robust with possible climatic variation in their body shapes (Stringer, 2013). Archeological finds show *H. heidelbergensis* produced sophisticated tools, such as the beautiful Acheulian handaxes from Boxgrove, UK (Roberts *et al.*, 1994) (**Figure 7**). In contrast to the Euro-African hypodigm, some researchers maintain that *H. heidelbergensis* is restricted to Europe and is ancestral solely to Neanderthals. However, this viewpoint is largely dependent on Neanderthal traits in remains from Sima de los Huesos (Atapuerca, see **Figure 8**), which have recently been redated to a younger ~400 ka and are thus probably best viewed as early Neanderthals, rather than *H. heidelbergensis* (Buck and Stringer, 2014; Stringer, 2012b). In this scenario, shared traits linking the African and European material are largely primitive retentions from *H. erectus* (Stringer, 2012b). By comparing mitochondrial DNA (mtDNA) from *H. neanderthalensis* and *H. sapiens*, a divergence date (~410–440 ka) for the two species has been estimated, providing calibration for the potential split of *H. heidelbergensis* into its descendent species (Endicott *et al.*, 2010).

Homo Neanderthalensis

Neanderthals (**Figure 9**) were a successful and long-lived species with a geographical spread from Gibraltar (**Figure 10**) to Siberia and chronologically from ~400–40 ka, if we assume fossils from Swanscombe and the Sima represent very early members of the lineage (Higham *et al.*, 2014; Stringer and Hublin, 1999). The traditional image of Neanderthals as brutes is gradually eroding; over the last few years, evidence of the supposed hallmarks of 'modern humans' has been attributed to Neanderthal agency. This includes pigment use (Zilhão *et al.*, 2010); apparent symbolic use of feathers

Figure 8 Replicas of Sima de los Huesos 5 (Top, Spain, ~400 ka) and Swanscombe (bottom, UK, ~400 ka), which we argue are two early Neanderthals from different sites showing the mosaic accumulation of Neanderthal traits in different populations across Europe (Stringer and Buck, 2014). Copyright: NHM.

Figure 9 Artist's reconstruction of *Homo neanderthalensis*. Copyright: John Sibbick/NHM.

(Finlayson *et al.*, 2012; Peresani *et al.*, 2011); dietary breadth (Stringer *et al.*, 2008); burial of the dead (e.g., Rendu *et al.*, 2014); and the Châtelperronian industry (Bailey *et al.*, 2009; Bar-Yosef and Bordes, 2010; Higham *et al.*, 2010; Zilhão *et al.*, 2006). Although all these findings are controversial individually, as a whole, they point to the reduction in the perceived cognitive demarcation between *H. sapiens* and *H. neanderthalensis*. Improvements in genetic analyses have also enabled exciting new discoveries; the sequencing of the Neanderthal nuclear genome in 2010 has shown that most extant *H. sapiens* populations from outside sub-Saharan African have ~2% Neanderthal introgression (Green *et al.*, 2010). This varies between populations, with a greater extent observed in east Asian populations (Wall *et al.*, 2013), which may be due to the differential effect of genetic drift on separate populations across Eurasia (Prufer *et al.*, 2014) or to multiple interbreeding events (Vernot and Akey, 2014).

The Denisovans

In 2010 a fossil phalanx from the Siberian site of Denisova Cave yielded mtDNA and revealed an unexpected additional hominin in late Pleistocene Eurasia (Krause *et al.*, 2010). As the fossil remains (dated to ~50 ka) attributed to the Denisovans are still limited to the phalanx and three very large molars (from other individuals), their overall morphology is still unknown and their relationships to other Pleistocene hominins are incompletely understood. The ancient DNA (aDNA) preservation in this cold, dry cave was such however, that it could be determined that, although Denisovan mtDNA differs from both Neanderthals and *H. sapiens*, their nuclear DNA suggests they are a sister group to Neanderthals (Reich *et al.*, 2010). Evidence of interbreeding with recent *H. sapiens* in Southeast Asia and Melanesia shows that the Denisovans were once widespread in the region (Reich *et al.*, 2010). As there are Mid-Pleistocene fossils from Asia (e.g., Maba and Dali from China, **Figure 11**), it is possible they could represent a branch of *H. heidelbergensis* ancestral to the Denisovans (Stringer, 2013). Recent advances in aDNA analyses mean that recovering diagnostic genetic material from Mid-Pleistocene fossils (e.g., Meyer *et al.*, 2014) such as the remains from Asia is now a real possibility, which could clarify the evolutionary origins of the Denisovans.

Homo Sapiens

The diagnosis of our own species is controversial in the fossil record. Different diagnoses depend on their underpinning models of speciation and dispersal (Stringer and Buck, 2014). For example, multiregionalists propose a single, diverse species from >1 Ma onwards, with significant gene flow between regions preventing speciation (e.g., Wolpoff *et al.*, 1994).

Figure 10 The Forbes' Quarry (Gibraltar I) female Neanderthal. The species is diagnosed cranially by a long, low cranium, distinctive occipital morphology (Stringer, 1985; Vandersmeersch, 1985; Balzeau and Rougier, 2010), mid-facial prognathism with large nose and swept-back cheek bones (Stringer, 1985), and large, double-arched browridges (Stringer, 1985; Tattersall and Schwartz, 2006). Copyright: NHM.

Figure 11 The Maba fossil from China. Could this Middle Pleistocene hominin and other similarly enigmatic remains from sites such as Dali and Jinnuishan be related to the Denisovans (Stringer, 2013)? Copyright: C. Stringer.

In contrast, the better-supported (by the fossil and genetic data) Recent African Origin model suggests *H. sapiens* originated in Africa relatively recently, and dispersed globally to replace regional archaic populations (Stringer, 2002; Stringer and Buck, 2014). Recent genetic analyses (see above) have shown that living humans retain small contributions from extinct species, thus the currently most plausible explanation of *H. sapiens* origins is one of African beginnings and 'leaky replacement' of regional, preexisting non-*sapiens* populations (Stringer and Buck, 2014). Variation in early *H. sapiens* fossils in Africa and the Near East shows there was no simple, linear change from *H. heidelbergensis* to *H. sapiens*. There was instead

a gradual coalescence of *H. sapiens* characteristics (Stringer and Buck, 2014). This could be the result of climatic variation on population size and expansion/contraction (Stringer, 2012a; Blome *et al.*, 2012; Stewart and Stringer, 2012; Ziegler *et al.*, 2013). 'Anatomically modern humans' (fossils closer to living humans in morphology than to any other species) in the African fossil record (**Figures 12** and **13**) date to ~200 ka, while the origin of the *H. sapiens* lineage is likely to be ~400 ka (Stringer, 2012c). The oldest complete *H. sapiens* genome sequenced to date, from the Ust'-Ishim femur from Siberia (~45 ka), shows evidence of a similar amount of Neanderthal interbreeding to recent non-African humans (Fu *et al.*, 2014). Longer lengths of Neanderthal DNA in this individual suggest that time since introgression was ~55 ka (Fu *et al.*, 2014; Seguin-Orlando *et al.*, 2014). Since this Neanderthal contribution seems to be present in all recent non-Africans, Ust'-Ishim suggests a relatively late date for the global dispersals of *H. sapiens*. However, the possibility of earlier dispersal (as has been suggested by material from middle eastern sites such as Skhul and Qafzeh) is still

Figure 12 *Homo sapiens*. Artist's reconstruction of Cro-Magnon 1. Copyright: John Sibbick/NHM.

Figure 13 Early *H. sapiens*, replicas of Jebel Irhoud 1 (left) and Qafzeh 6 (right) from Morocco and Israel respectively. Copyright: NHM.

plausible (Reyes-Centeno *et al.*, 2014; Reyes-Centeno *et al.*, 2015), and larger amounts of Neanderthal introgression in the ~40 ka Oase mandible from Romania suggest that limited interbreeding continued until this time (Gibbons, 2015). *H. sapiens* in the fossil record are recognized by their cranial globularity, facial diminution and retraction, basicranial flexion, dental microstructure, inner ear morphology and pelvic shape (Stringer and Buck, 2014; **Figure 13**). It has been asserted that *H. sapiens* cannot be diagnosed purely morphologically because of our unique reliance on culture (Wolpoff and Caspari, 1997). '*H. sapiens* behavior,' however, appears in the fossil record in different places at different times (McBrearty and Brooks, 2000) and increasingly there is also potential evidence for such behavior in Neanderthals (see above). Furthermore, where 'anatomically modern' *H. sapiens* are found, they are not always associated with the whole *H. sapiens* behavioral package (Brumm and Moore, 2005). In our view, these problems preclude the utility of cultural evidence for diagnosing *H. sapiens* (Stringer and Buck, 2014).

See also: Biogeography, Human. Life-History Evolution, Human

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Human Life Histories, Evolution and

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Glossary

Adrenarche When the adrenal glands mature and the zona reticularis starts to produce increased levels of androgens such as DHEA and DHEAS. In humans this is associated with the appearance of pubic hair and body odor in children around the ages of 6 to 8.
Allometry The study of the relationship between various measures of body size and shape.
Antagonistic pleiotropy When one gene or allele affects more than one trait, and where at least one of the traits is beneficial to the organism's survival and reproduction and at least one is detrimental to the organism's survival and reproduction.
Encephalization Increasing brain size relative to body size.
Gestation Pregnancy, or carrying an embryo or fetus inside the female body.
Hominin Refers to modern humans and extinct human-like bipedal species (including members of the genera *Homo*, *Australopithecus*, and *Ardipithecus*).
Interbirth interval The length of time between births.
Lactational amenorrhea The temporary postnatal cessation of menstruation, associated with infertility.
Life history theory How a species allocates energy across the competing physiological demands of growth, reproduction, and somatic maintenance.
Menarche The first menstrual period.
Menopause The last menstrual period, recognized in retrospect after 12 months without menstruation.
Metabolic load The amount of energy required, for example, to carry a pregnancy to term, or to breastfeed.
Neonatal period From birth through the first 4 weeks of an infant's life.
Obligate bipedalism The form of bipedalism (walking on two legs) that is carried out as the habitual means of locomotion.
Secular trend Generally refers to increases in height and decreases in ages at menarche over a long period of time, i.e., 100 years.
Senescence Age-related changes, both structural and functional, that decrease the ability of an individual to withstand stress, reduce the probability of reproduction, and increase the likelihood that an individual will die.
Subfecundity Having a low probability of conceiving during a month of exposure to unprotected intercourse.

Introduction

Over the course of the past 6 or 7 million years, humans have evolved some unique life history characteristics. Compared to other primates, humans have the slowest-growing offspring, the latest age at first birth, and the longest life span. Human females experience a universal menopause and prolonged post-reproductive life. Although human offspring are slow-growing, they are weaned at earlier ages than chimpanzees and gorillas, and interbirth intervals are shorter, resulting in more offspring during the reproductive period (Bogin and Smith, 2012; Robson *et al.*, 2006).

Life history theory, a branch of evolutionary biology that focuses on the timing of life cycle events, has been applied to explain the uniqueness of the human life span. Life history theory is concerned with the optimal allocation of energy across energetically expensive physiological demands: somatic maintenance, immune function, physical activity, growth, reproduction, and energy stores. Everything has a cost (Jasienska, 2013), and life history theory presumes that when the body's energy is directed for one purpose (e.g., growth), that same energy cannot be used for something else. Through most of human evolution, energy was not consistently available, and trade-offs occurred (Trevathan, 2010). Life history theory is, therefore, about trade-offs, as a species balances limited energy across competing physiological demands (Stearns, 1992).

This article will use life history theory to provide a coherent framework to explain why humans evolved unique life history characteristics. Human life history will be compared with our closest living relatives (chimpanzees, gorillas) and sometimes with the more distantly related macaque. Evidence will also be drawn from fossil hominins. Contemporary foraging populations will be referred to, albeit with caution, as their ways of life may be somewhat like that of our hominin ancestors. Human life history cannot be understood apart from the development of human culture and sociality (Sears and Gibson, 2009).

This article is organized around the life events of gestation, infancy, childhood and adolescence, adulthood and reproduction, and menopause and post-reproductive life. Although these life cycle events are universal across all humans, there is variability in the timing of life events both within and between populations. The conditions for natural selection include heritability and variation in the traits of interest (Langdon, 2005), thus the variability observed in the timing of life events in contemporary populations suggests how natural selection was able to shape the unique trajectory of the human life span. Of course, the third condition for natural selection is an association with reproductive success, and that is the central premise of life history theory (Ellison, 2003). Ultimately, the species-specific pattern of energy allocation across the human life span results in reproductive success.

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Gestation

One perplexing life history question associated with human gestation pertains to why humans are not more fully developed at birth given the amount of time they spend *in utero*. Humans have a longer length of gestation (38–40 weeks) than chimpanzees (32 weeks) and gorillas (37 weeks); however, human infants are behaviorally more altricial at birth compared to other primates. Whereas chimpanzees are born with brains that are about 40% of their adult brain size, humans are born with brains less than 30% of their adult brain size (DeSilva and Lesnik, 2006). Human infants are born helpless, highly dependent, and require prolonged care from mothers and others (Hrdy, 2000, 2009).

Birth cuts short the period of prenatal development. Some have argued that the timing of birth is an evolutionary trade-off made necessary by pelvic changes associated with obligate bipedalism and an enlarged brain (Krogman, 1951; Lovejoy, 1981; Rosenberg and Trevathan, 2002; Trevathan, 2010). The changes to the pelvis and cranial capacity relevant to birthing date to about 2 million years ago (Langdon, 2005); however, the true conflict between pelvic morphology and encephalization occurred much more recently, probably during the last few hundred thousand years (Weaver and Hublin, 2009). Natural selection favored a shorter gestation and less developed offspring in order to accommodate the passage of a big-brained neonate through a pelvis modified for locomotion and heat dissipation (Weaver and Hublin, 2009).

Other investigators have addressed the uniqueness of long gestation coupled with slow intrauterine development with a focus on the trade-offs associated with fetal demands and maternal metabolic load (Ellison, 2001). Life history theory suggests that energetic considerations constrain how large a fetus can grow before its energetic needs cannot be met within the mother's body (Martin, 1996; Peacock, 1991). Dunsworth *et al.* (2012) note that, although human brains are small at birth compared to their adult brain size, relative to other primates the human neonatal brain is larger than expected after controlling for the relationship between neonatal brain size and mothers' body size. In other words, there is more maternal investment than expected up to the point at which maternal metabolic constraints limit maternal support. By 9 months, the metabolic demands of the fetus threaten to push maternal energy requirements beyond the mother's metabolic capacity.

During her lifetime, a woman has a finite quantity of energetic resources that she can allocate to reproduction. By limiting the length of gestation, she can allocate resources to more offspring, increasing her lifetime reproductive success (Peacock, 1991). Of course, the developing fetus might wish for a little more time *in utero*.

Infancy

The parent–offspring conflict that starts in gestation continues in infancy. 'Parent–offspring conflict' describes the trade-off between parental investment that benefits a particular offspring and the cost of that investment to the parent and the parent's ability to invest in other offspring (Trivers, 1974). The length of gestation is a species-level compromise between the human mother and her fetus. Life history theory points to the duration of breast-feeding as another point of potential conflict between mother and child. Related to breast-feeding is the question of when mothers should get pregnant again (i.e., what is the human interbirth interval?)

The determination of how long women should breast-feed is complicated by human culture. For example, the Quran instructs women to breast-feed for 2 years (Al-Jassir *et al.*, 2006). The World Health Organization advocates exclusive breast-feeding for up to 6 months, and supplemented breast-feeding for up to 2 years or beyond. Dettwyler (1995) addressed the question of how long humans should breast-feed with a comparative approach across species and across cultures. She found that humans in traditional cultures breast-feed for 2 to 5 years, and chimpanzees breast-feed for 4 to 5 years. After examining a variety of allometric relationships across primate species (e.g., age at weaning in relation to gestational length, birth weight, and eruption of the first permanent molar), Dettwyler argued that the hominin 'blueprint' for breast-feeding is between 2.5 years and 6 years of age. She suggests that the wide range of variation is due to the variety of ecological conditions to which early hominins adapted, the influence of cultural norms, the ability to process food that infants can eat, and the hominin capacity for social support.

High maternal metabolic investment in breast-feeding constrains the energy available to support a new pregnancy (Ellison, 2003). This results in a period of lactational amenorrhea, during which ovarian function is suppressed. The length of time spent in lactational amenorrhea varies across populations and is associated with breast-feeding intensity, energy availability, and maternal workload (Valeggia and Ellison, 2001). The resumption of ovulation has a direct impact on the interbirth interval and lifetime reproductive success.

It is in the interest of the infant to gain as much nutritional and immunological benefit from breast-feeding as possible, for as long as possible. There is, however, a point at which it is in the interest of the mother to allocate energy to subsequent offspring. The interbirth interval is about 4 years in gorillas and about 5 years in chimpanzees (Harvey and Clutton-Brock, 1985; Knott, 2001). Humans have a faster fertility rate than apes and can conceive every 2 years. Walker *et al.* (2008) suggest that more energy is available for rapid reproduction in humans due to slow offspring growth and social assistance in provisioning.

Childhood and Adolescence

Childhood is defined as a period of postweaning dependency, from approximately 3 to about 7 years of age. It is characterized by slow and steady growth, with a high percentage of energy devoted to the growth and development of the brain. Bogin and Smith (2012) argue that human childhood is characterized by immaturity in a suite of features related to growth, dentition, and cognition. They suggest that human children remain dependent on their caretakers to a degree unique among mammals because of their need for a low-volume diet dense in energy, lipids, and proteins. Human children do not have the appropriate dentition, or the motor

and cognitive skills necessary to prepare such food for themselves. By the end of childhood, brain growth is complete and children are able to find, process, and eat adult foods.

Around the ages of 6 to 8 years, there is a slight acceleration in growth velocity and adrenarche occurs. Adrenarche is associated with a prepubertal increase in circulating adrenal androgens, DHEA and DHEAS. The slight acceleration in growth appears to be unique to humans (Bogin and Smith, 2012), although adrenarche occurs in great apes and may occur in other primates as well (Conley *et al.*, 2012). In humans, increased DHEAS production continues into the 20s and may promote brain maturation, learning, and the acquisition of skills (Campbell, 2006, 2011).

A juvenile stage, characterized by very slow growth, occurs between childhood and adolescence. In human females, this occurs between about 7 and 10 years of age and lasts for another 2 years in males (Bogin and Smith, 2012). This period may be especially important for social learning.

Adolescence begins with the hormonal changes associated with puberty. Adolescence in girls is characterized by breast development, increased pubic hair, development of the external genitalia, sexual dimorphism in body shape and composition, and the adolescent growth spurt. The first menstrual period (menarche) occurs on the downside (end) of the growth velocity curve. Following menarche, gorilla, chimpanzee, and human females have a period of adolescent subfecundity that lasts for about 2 years (Knott, 2001; Wood, 1994). In boys, adolescence is characterized by an increase in pubic hair, facial hair, development of the external genitalia, sexual dimorphism in body shape and composition, and a deepening of the voice. These changes occur on the upside (at the beginning) of the adolescent growth spurt. The pubertal growth spurt is unique to humans (Bogin, 1999; Bogin and Smith, 2012).

Relative to other primates, humans spend a long period of time between infancy and the onset of puberty, and another long period of time between puberty and first birth. The delay between puberty and parenthood is less than 3 years in monkeys and apes, but about 10 years in humans (Bogin and Smith, 2012). Life history theory provides a commonly accepted explanation for the timing of the adolescent growth spurt: energy is directed to the development of the brain during infancy and childhood, to somatic growth during adolescence (Campbell, 2006; Gluckman and Hanson, 2006), and finally to reproduction during adulthood (Trevathan, 2010).

Trade-offs in the allocation of energy are particularly visible in the relationship between female stature and reproductive success. Growth and reproduction are both energetically costly, and a woman's final height is partly determined by when the body stops directing energy to growth and starts to allocate energy to reproduction (Sear, 2010). In environments where energy is not consistently available, children have the option of growing faster during periods of plenty, or growing for a longer period of time. As Sear (2010) points out, both 'growing fast' and 'growing longer' are associated with reproductive costs. Growing for a longer period of time delays the start of reproduction which may mean fewer offspring overall. On the other hand, it is disadvantageous for a woman to enter the reproductive stage too early. The pelvic dimensions do not reach their maximal size until several years after menarche (LaVelle, 1995), and, when energy is shifted from growth to reproduction, shorter height is associated with lower reproductive success (Nettle, 2002).

Much attention has been given to variation in age at menarche, and human biologists are in agreement that there has been a secular trend toward an earlier age at menarche since the turn of the twentieth century (see Eveleth and Tanner (1990) for a classic summary). A more provocative idea has been put forward by Gluckman and Hanson (2006). They suggest that menarche occurred between the ages of 7 and 13 in Paleolithic times. They base this on the earlier age of puberty experienced by chimpanzees (6 to 9 years), and on estimates of life expectancy at birth for Neolithic humans corrected for the likelihood of maternal mortality, childhood mortality, probable interbirth interval, and a 1% population growth per generation. They propose that human females evolved to enter puberty at a relatively young age, but with the transition to agriculture about 10 000 years ago the average age of menarche was delayed due to childhood disease and postnatal undernutrition. Delayed menarche was a good match for the need to learn how to function socially in an increasingly complex society; however, with modern hygiene and improved nutrition, the constraints on puberty were removed and age at menarche now occurs at earlier ages. This has resulted in a mismatch between physiological and psychosocial maturation in contemporary adolescents (Gluckman and Hanson, 2006).

Adulthood and Reproduction

In life history theory, the function of adulthood is the same in all mammals. Adulthood is a time of reproduction: finding mates, defending mates, pregnancy, lactation, provisioning, and protecting offspring. Although humans are rapid reproducers relative to chimpanzees and gorillas, humans are similar to monkeys and apes in their focus on the quality of their offspring.

During adulthood, the trade-off between reproduction and somatic maintenance becomes a salient feature of life history as females outlive males. Human biologists who focus on the reproductive ecology of males have been particularly interested in how androgens (e.g., testosterone) influence somatic, reproductive, and immune metabolisms. The same hormone that contributes to male success in competition for mates (e.g., bigger muscles) comes with a cost of compromised immune function. There is a trade-off between reproductive effort and survivorship (Bribiescas, 2001; Muehlenbein and Bribiescas, 2005). Although females regularly outlive males, they do not complement their longer lives with continued reproduction.

Menopause and Post-Reproductive Life

Human females are not unique in their capacity to outlive their ability to reproduce (Cohen, 2004; Ward *et al.*, 2009). Among primates, a longitudinal study of Japanese macaques found that reproduction ceased after the age of 25; however, only 3% of the monkeys lived to the age of 26 (Pavelka and

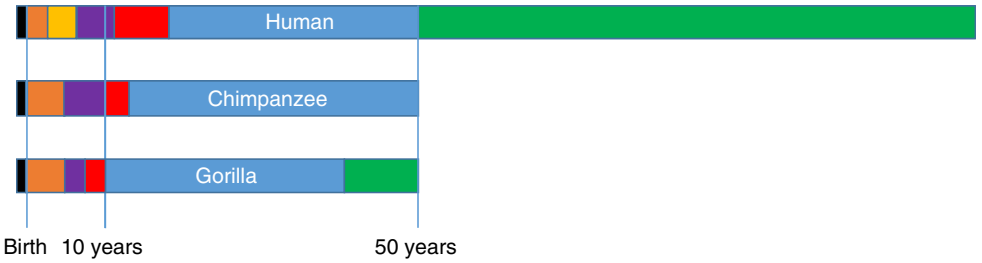

Figure 1 Gestation, infancy, childhood, juvenile period, adolescence, adulthood, post-reproductive period to maximum potential life span. Human: duration of stages, gestation through adulthood, based on Bogin, B., Smith, B.H., 2012. Evolution of the human life cycle. In: Stinson, S., Bogin, B., O'Rourke, D. (Eds.), Human Biology: An Evolutionary and Biocultural Perspective, second ed. New York, NY: John Wiley & Sons, Inc., pp. 515–586. Maximum potential lifespan drawn out to 122 years based on age of Jeanne Calment at her death. Chimpanzee: duration of infancy and adolescent subfertility (in red) based on Thompson, M.E., 2013. Reproductive ecology of female chimpanzees. American Journal of Primatology 75, 222–237. Estimated age at last birth based on Alberts, S.C., Altmann, J., Brockman, D.K., *et al.*, 2013. Reproductive aging patterns in primates reveal that humans are distinct. Proceedings of the National Academy of Sciences of the United States of America 110, 13440–13445. Gorilla: duration of adolescent subfertility (in red) based on Knott, C., 2001. Female reproductive ecology of the apes: Implications for human evolution. In: Ellison, P.T. (Ed.), Reproductive Ecology and Human Evolution. New York, NY: Aldine de Gruyter, pp. 429–463. Estimated ages at first and last birth based on Alberts, S.C., Altmann, J., Brockman, D.K., *et al.*, 2013. Reproductive aging patterns in primates reveal that humans are distinct. Proceedings of the National Academy of Sciences of the United States of America 110, 13440–13445. Maximum longevity based on Atsalis, S., Margulis, S.W., 2008. Perimenopause and menopause: Documenting life changes in aging female gorillas. Interdisciplinary Topics in Gerontology 36, 119–146.

Fedigan, 1999). Among gorillas and chimpanzees, the completion of fertility is not universal before death (Alberts *et al.*, 2013; Atsalis and Margulis, 2008; Emery Thompson *et al.*, 2007). In contrast, women in hunting/foraging groups without access to modern healthcare universally demonstrate a capacity for menopause and long post-reproductive life (Alberts *et al.*, 2013; Hawkes, 2004; Hawkes and Blurton Jones, 2005). It is post-reproductive longevity, rather than menopause, that sets humans apart from other primates in the latter part of the life span (see **Figure 1**). Hominin longevity most likely exceeded 50 years of age more than 1 million years ago (Smith, 1991), and fossil remains from Paleolithic Neanderthals and early *Homo sapiens* suggest that 10% to 24% of the population survived beyond the age of 40 years (Kennedy, 2003; Trinkaus, 1995, 2011).

Anthropologists have argued that menopause may have been selected for to ensure that old eggs are not fertilized (Hrdy, 2000), or to ensure that mothers are young enough to survive pregnancy, delivery, and the infancy of their offspring (Hill and Hurtado, 1991; Peccei, 2001; Pavard *et al.*, 2008). The 'grandmother hypothesis' is probably the best known argument for the evolution of menopause and post-reproductive longevity. This hypothesis asserts that post-reproductive grandmothers provide care and food for their grandchildren (Hawkes, 2004; Scelza, 2009; Sear *et al.*, 2000), and increase their own inclusive fitness by investing in their daughters' fertility and their grandchildren's survival rather than continuing to produce children of their own.

There is ethnographic and historical support for the idea that menopause and post-reproductive aging were selected for by the evolutionary benefits gained through grandmothering (Lahdenperä *et al.*, 2004). However, not all studies have shown positive effects of maternal grandmothers, or of paternal grandmothers, or grandfathers on the survival of grandchildren (Gibson and Mace, 2005; Jamison *et al.*, 2002; Madrigal and Meléndez-Obando, 2008; Voland and Beise, 2002).

Some anthropologists argue that menopause is a type of antagonistic pleiotropy. Waves of developing follicles are needed to produce hormones during pre-reproductive life and the reproductive period. However, this pattern of follicular development and degeneration results in the exhaustion of ovarian reserves because we are a long-lived species (Wood *et al.*, 2001). Menopause can also be understood as the by-product of the highly conserved mammalian pattern of oogenesis and follicular atresia coupled with a lengthened life span (Sievert, 2011).

The lengthened life span, for both males and females, is associated with senescence. From an evolutionary perspective, human senescence has been explained by three (not mutually exclusive) theories. The disposable soma theory follows the tenets of life history theory by proposing that energetic resources are limited and must be allocated for growth, maintenance, and/or reproduction (Kirkwood, 1977). Once growth has finished, natural selection favors investment in reproductive success; however, with increasing age there is a loss of reproductive potential. As reproductive potential declines, natural selection loses its strength. For example, there is less pressure to repair degeneration in somatic tissues. Natural selection allocates energy to reproduction at the expense of somatic maintenance and, with increasing age, senescent changes begin to emerge (Rose, 1991).

The second theory of senescence suggests that deleterious mutations that act late in life are not selected against because most of our ancestors who carried the mutations died at an earlier age from environmental hazards (Medawar, 1946). As a result, deleterious alleles accumulate within populations, and when life spans lengthen the accumulated late-acting deleterious mutations are expressed. The visible effect of late-acting deleterious mutations is senescence.

Finally, Williams (1957) proposed that some genes could have beneficial effects early in life, but detrimental effects late in life. Because the effects are counteracting, the theory is called 'antagonistic pleiotropy.' Testosterone activity in men is

one example of antagonistic pleiotropy because testosterone contributes to secondary sex characteristics, the regulation of puberty, and reproductive success early in life. These are positive effects. Unfortunately, this same hormone may contribute to the risk of prostate cancer and cardiovascular disease later in life (Bribiescas, 2006). As a theory for aging, antagonistic pleiotropy has gained traction recently due to the increasing popularity of evolutionary medicine (Trevathan *et al.*, 2008; Williams and Nesse, 1991). Antagonistic pleiotropy has been used to explain many aspects of human physiology that may be beneficial early in the life span, but deleterious later on.

In Conclusion

Evolutionary theory contributes an understanding of how natural selection shaped species-level patterns of life history within particular environmental contexts. Life history theory focuses on energy appropriation. Every question about the human life span, from length of gestation to reproductive senescence, is approached from the perspective of the kinds of trade-offs that occur when energy is finite (Trevathan, 2010). Humans are developmentally immature at birth, in spite of a long gestation, because of maternal physical and metabolic constraints. Although humans have evolved to breast-feed offspring for as long as 6 years, there is a trade-off between continuing to nourish individual offspring and producing more offspring quickly. Human brains grow during infancy and childhood, and then energy is directed toward an adolescent growth spurt and growth of the body. Energy is then allocated to reproduction during adulthood, at times at the expense of immune function and somatic maintenance.

This article of human life history has been done with very broad brush strokes. There are certainly finer details to be examined in the application of life history theory to ovarian sensitivity in response to specific ecological and energetic constraints (Ellison *et al.*, 1993), the early biological responses of offspring to variation in maternal conditions (Gluckman and Hanson, 2006; Hales and Barker, 2001; Kuzawak, 2008), and population-level variation in ages at menarche and menopause (Eveleth and Tanner, 1990; Sievert, 2006).

See also: Evolutionary Medicine I. An Overview and Applications to Cancer. Evolutionary Medicine III. Mismatch. Life-History Evolution, Human

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Hybrid Speciation

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Glossary

Allopolyploid speciation Hybridization of two different species that results in the origin of a new hybrid species that has double the chromosome complement of the progenitor species.

Cytotypes It is a characteristic of the cell, referring to variations in chromosome number and genome structure.

Dosage compensation It is equalization of gene expression in males and females, which differ in the number of sex chromosome copies.

Gametic imbalance An imbalance of the nuclear and cytoplasmic sizes (nuclearcytoplasmic ratio) that can interfere with proper cell division during meiosis.

Genetic incompatibilities A failure of the interaction between genes from different parents required for proper function.

Homoploid hybrid speciation (HHS) Hybridization that results in the origin of new hybrid species without a change in chromosome number (ploidy).

Hybrid swarm It refers to a population of hybrids generated by multigenerational intercrossing between hybrids and parental types or species.

Incomplete lineage sorting (ILS) When same allelic variants at a locus are inherited by two or more lineages (e.g., species) from a common ancestor it is called incomplete lineage sorting.

Magic trait Traits under divergent selection that also influence nonrandom mating.

Minority cytotype exclusion It refers to the loss of low frequency cytotypes, such as polyploids, due to swamping out effects of the more common cytotype.

The concept of hybridization being involved in the origin of new species has a long history that began with Linnaeus in 1744 (Rieseberg, 1997). Over the past couple hundred years, the importance of hybridization in adaptive evolution has become clear and evident, as seen in numerous hybrid, domesticated plants and animal strains. The potential for hybridization to produce offspring that have a higher fitness than their progenitors and has been championed as a major force in adaptive radiations (Rieseberg *et al.*, 1999; Seehausen, 2004). If hybridization can have such a major influence adaptive divergence, then surely hybridization could also influence the evolution of reproductive isolation between the hybridizing groups. More importantly, can hybridization result in the origin of a new species, and if so, how often does this happen in nature?

What Is Hybrid Speciation?

Hybrid speciation can be broadly defined as the hybridization between two or more distinct lineages that contributes to the origin of a new species. More specifically, hybridization must result in a hybrid population that is at least partially reproductively isolated from the parental species. Recently, three criteria were established to demonstrate hybrid speciation: (1) there must be evidence of hybridization between species, (2) the hybrids must be reproductively isolated from the parental species, and (3) there should be evidence that hybridization is the cause of the isolation (Schumer *et al.*, 2014). Studies of potential examples of hybrid speciation in nature and the lab over the past 100 years have shown that there are several ways that hybridization can lead to origin of a new hybrid species.

The most well-known route to hybrid speciation is through the doubling of chromosome numbers in hybrids (allopolyploidy), so hybrids have twice the number of chromosomes as their parents. These allopolyploid hybrids can be reproductively isolated from the progenitor species that have a different ploidy, due to improper chromosome pairing during meiosis (Grant, 1981) and genetic incompatibilities between the hybridizing genomes (Abbott *et al.*, 2013). Therefore, the doubling of chromosomes offers a rapid route to hybrid speciation. Examples of allopolyploidy provide some of the clearest evidence of hybrid speciation, since the increased ploidy of hybrids resulting from hybridization directly causes reproductive isolation.

Hybrid speciation can also occur with no change in chromosome number, which is referred to as homoploid hybrid speciation (HHS) (also known as recombinational speciation, see Grant, 1981). In HHS, viable, true-breeding hybrids evolve that are reproductively isolated from the parental species. Homoploid hybrids do not have the advantage of being immediately isolated from the parent species, like allopolyploids do. Therefore, HHS requires the evolution of reproduction isolation while gene flow is ongoing with the parental species, which may explain why HHS is unlikely or more difficult to demonstrate than allopolyploid hybrid speciation (Barton, 2001; Coyne and Orr, 2004).

It is also important to understand what hybrid speciation is not. Hybrid speciation is not simply the production of F1 or backcross offspring between distinct species, as it requires the establishment of a third, distinct species. Therefore, interspecific hybrids such as ligers and geeps, which are sterile like mules and cannot persist beyond a single generation, are not examples of hybrid species. Similarly, hybridogenesis, a reproductive strategy that involves backcrossing between hybrids and a parental species with different ploidy (as seen in edible European frog *Rana esculenta* (Tunner and Nopp, 1979)), does not qualify as hybrid speciation since the hybrids are

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obviously not reproductively isolated from the parental species. Additionally, hybrid speciation should also be considered distinct from reinforcement where natural selection against deleterious hybridization drives the evolution of increased reproductive isolation. Although reinforcement involves both hybridization and speciation, it does not necessarily involve the origin of a new, hybrid species.

The concept of hybrid speciation may seem counter-intuitive to some. If species are defined as reproductively isolated groups, then by definition, hybridization should not occur. Hybrid speciation requires that the reproductive barrier between the parental species is either incomplete or has been lost, so that hybridization can occur. When considering hybrid speciation, it may be more useful to define species as distinguishable groups of individuals with clusters of shared genotypes that remain distinct in the face of gene flow (Mallet, 1995). Importantly, this definition, referred to as the Genotypic Cluster Species Concept, acknowledges that divergence and speciation can occur even with hybridization and gene flow.

Theory and Background

Hybridization has long been considered an opposing force to the speciation process. Hybridization and the subsequent mixing of genetic variation among sexually reproducing organisms was thought to most often result in 'despeciation,' the collapse of the two species into a single lineage. Even worse, the hybrids themselves often suffered developmental, physiological, and behavioral abnormalities that made them less fit than the parental species, suggesting at best they may be mere 'hopeful monsters' (Dittrich-Reed and Fitzpatrick, 2013). These preconceptions made it difficult for many to envision that hybridization could have a positive impact on the speciation process. However, it was the rediscovery of Mendel's 'Experiments in Plant Hybridization' by Hugo de Vries, Carl Correns, William Jasper Spillman, Erich von Tschermak that rekindled interest in the question: can hybridization lead to the origin of a true-breeding hybrid lineage? Tschermak was particularly intrigued by the 'hybrid vigor' phenomenon, where many of his experimental crosses that involved 'foreign pollen' yielded offspring with greater fitness than either parent (Tschermak, 1902). Would it not be possible for these vigorous hybrids to persist as their own distinct species? In 1914, John Gerould formally hypothesized that species may be 'built' through hybridization, based on a review of breeding experiments in several insects (Gerould, 1914). Over the past 100 years, we have established theoretically and empirically that under certain conditions hybrid species may frequently evolve in both plants and animals.

Allopolyploid Speciation

The major advantage of alloplyploids, and perhaps why it is the more common form of hybrid speciation, particularly in plants, is the instantaneous postzygotic isolation from the parental species due to the differences in ploidy. However, allopolyploid hybrids still have major hurdles to overcome in order to persist. When the hybrids and parent species continue to hybridize, the rare cytotypes will rapidly be lost, a well-known phenomenon called "minority cytotype exclusion" (Levin, 1975). Therefore, the allopolyploids will often rapidly go extinct, if there is no isolation from the parental species.

There are two factors that can enable an allopolyploid species to persist: pre-zygotic isolation and ecological divergence. Pre-zygotic isolation can alleviate the risk of extinction, and allow the hybrids to breed true. Polyploid plants and insects are often larger and more robust than their diploid parents, which could result from larger cells and slower development in polyploids (Levin, 2002, 1983; Otto and Whitton, 2000). It is possible that increased ploidy can confer rapid pre-zygotic isolation and ecological divergence, due to the physical change in cell sizes (the 'gigas effect'). Interestingly in plants, flowering time asynchrony and pollinator shifts are often associated with changes in ploidy, which may reflect the slower development associated with larger cells (Ramsey and Schemske, 2002; Levin, 1983, 2002). Similarly, the larger allopolyploid cells of the hybrid gray tree frog, *Hyla verisicolor*, appears to directly impact their mating call, thereby isolating the hybrids from the progenitors (Keller and Carl Gerhardt, 2001). Ecological divergence can also provide the isolation necessary for a hybrid species to persist in the presence of its progenitors. Hybridization can often generate hybrid genotypes that can persist in certain environments that their progenitors are disadvantaged, which could be an effective ecological barrier that facilitates hybrid speciation (Barton, 2001; Rieseberg, 1997).

Homoploid Hybrid Speciation

The traditional model of HHS, formalized by Stebbins (Stebbins, 1950) and Grant (Grant, 1981) (referred to as 'recombinational speciation'), involves chromosomal rearrangements between progenitor species that typically cause low fertility in F1 hybrids. Hybridization results in chromosomal deficiencies and duplications that confer low F1 fertility. However, through hybridization and recombination, novel 'genetically balanced' genotypes with recovered fertility can form. These viable hybrids can mate with each other, but are intrinsically isolated from the parental species, since there is gametic imbalance between the hybrids and progenitors. Simulations of this model reveal that HHS is definitely possible if F1 hybrids are at least partially fertile, and that the chances of HHS are much higher when there are extended periods of hybridization and/or the organisms are self-reproducing (Mccarthy *et al.*, 1995). In addition, simulations that include extrinsic barriers, such as ecological barriers, showed that strong ecological selection greatly increased the chance of HHS (20% of simulations resulted in HHS) (Buerkle *et al.*, 2000), and that factors that contribute to ecological or intrinsic isolation can become quickly stabilized in a hybrid genome (Buerkle and Rieseberg, 2008), demonstrating that ecological barriers can be a major factor in the establishment of a hybrid species.

There are two models of HHS that have been described, which involve quite different evolutionary processes and genomic signatures: *mosaic genome hybrid speciation* and *hybrid trait speciation* (Jiggins *et al.*, 2008; **Figures 1(a)**, **1(b)** and

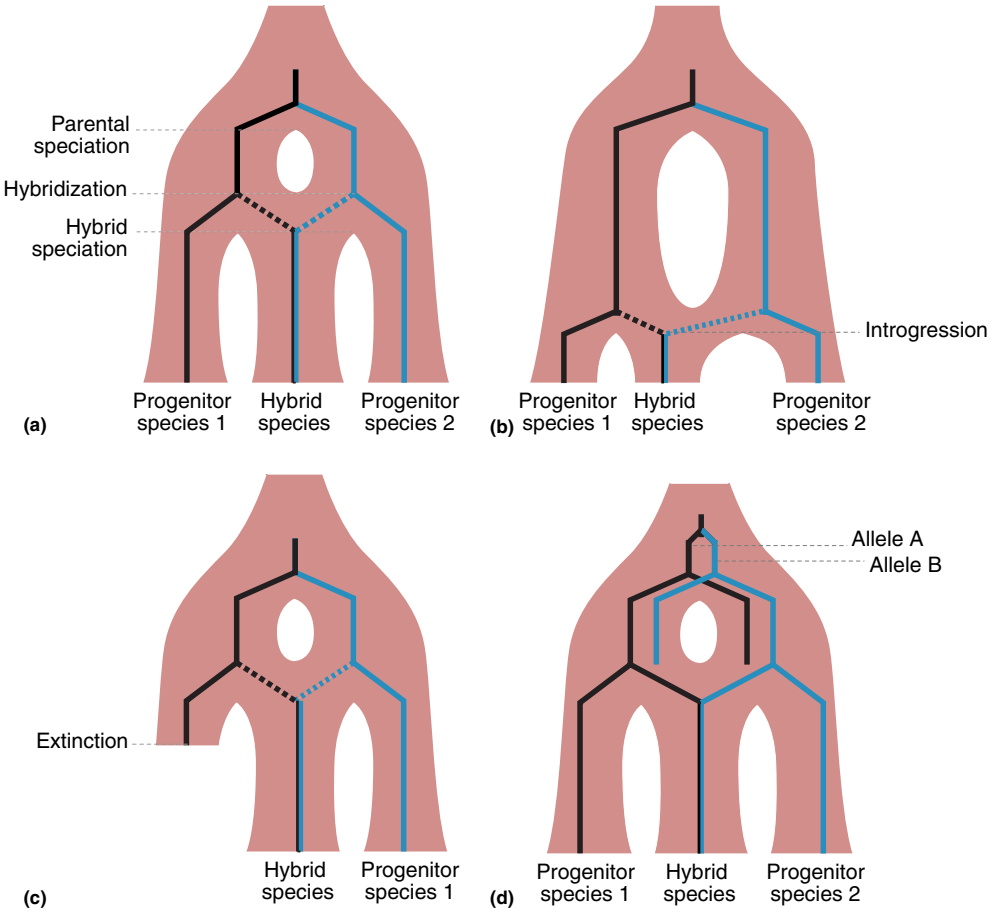

Figure 1 Paths to hybrid speciation. For each panel, shaded regions reflect the species tree, the solid lines reflect gene trees and the dotted lines reflect hybridization. (a) Classic form of hybrid speciation that can be generated through allopolyploidy or homoploid hybrid speciation (HHS) (e.g., mosaic genome hybrid speciation). (b) Hybrid trait speciation model with introgression from species 2 into a genomic background that is predominantly from species 1 (e.g., through repeated backcrosses) drives the origin of new hybrid species. (c) Demonstrates how extinction can prevent the inference of a hybrid origin. (d) Example of how incomplete lineage sorting (ILS) of A and B alleles from a common ancestor can generate similar patterns predicted from hybrid speciation.

Figure 2(a)). The mosaic genome hybrid speciation follows the traditional model discussed above, where a 'genetically balanced' hybrid species differs from its progenitor species at several loci that confer intrinsic reproductive isolation (e.g., hybrid sterility or inviability) and/or ecological divergence. The genomes of these hybrid species would be true mosaics of the parental genomes, although not necessarily with equal contributions from both parents. Hybrid trait speciation may be considered a more extreme case of HHS where introgression drives rapid ecological divergence *and* reproductive isolation. The key difference in this model is that introgression at only a single or few loci may be sufficient for hybrid speciation (Figures 1 and 2). The model posits that introgression of a so-called evolutionary 'magic trait' that influences both adaptive divergence and reproductive isolation can rapidly, if not instantaneously, lead to hybrid speciation. Importantly, hybrid trait speciation does not require intrinsic barriers and provides a model for ecological divergence to drive hybrid speciation that needs further exploration. These two models of HHS are not mutually exclusive; rather these models represent different ends of the spectrum when considering the patterns of hybridization that can drive HHS.

Evidence of Hybrid Speciation

Allopolyploid speciation is the most commonly known route to hybrid speciation. This is not surprising with 40–70% plant species being polyploidy. A review of ploidy in plants suggests that polyploidy was involved in speciation events of ~2–4% of flowering plants and 7% of ferns (Otto and Whitton, 2000). Perhaps the most familiar examples are the numerous allopolyploid crops that humans have domesticated (including wheat, cotton, tobacco, strawberry, and rapeseed). There are also multiple examples of recently formed allopolyploid species (<200 years old; for example, *Cardamina* (Mandáková *et al.*, 2013), *Mimulus* (Vallejo-Marin *et al.*, 2015), *Senecio* (Abbott and Lowe, 2004), *Spartina* (Ainouche *et al.*, 2004b), *Salsola* (Ayres *et al.*, 2009), and *Tragopogon* (Soltis *et al.*, 2004)), that have provided insights into the types of genetic changes

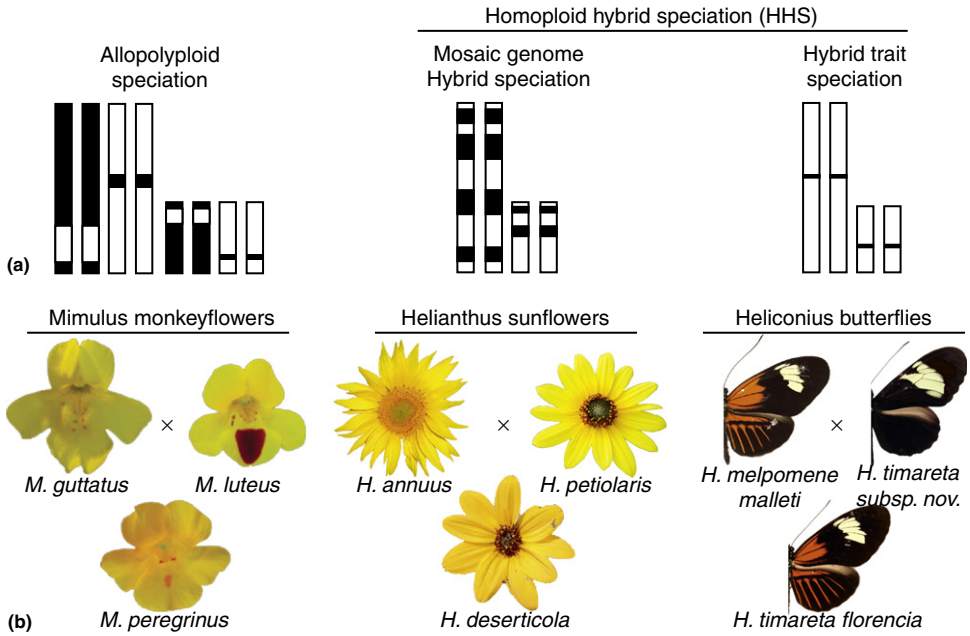

Figure 2 Examples of hybrid speciation. A genomic representation (a) and well-documented examples (b) of the three models for hybrid speciation.

involved in origin of hybrid species (Matyášek *et al.*, 2007; Lim *et al.*, 2008; Tate *et al.*, 2009; Ainouche *et al.*, 2004a; Salmon *et al.*, 2005, also see Soltis and Soltis, 2009 for a review).

Allopolyploid speciation appears to often involve hybridization of recently introduced, potentially invasive species, as seen in the *Mimulus* monkey flowers introduced to Scotland only 140 years ago (Vallejo-Marin, 2012; **Figure 2(a)**). Hybridization between *Mimulus guttatus* and *Mimulus luteus* in their native North American range results in sterile, vegetatively vigorous, triploid offspring. *Mimulus peregrinus* is found in Scotland where the two introduced species co-occur and is vegatatively vigorous like the triploid hybrids, but has twice as many chromosomes (polyploid) and is highly fertile (Vallejo-Marin and Lye, 2013; Vallejo-Marin, 2012). Population genomic sequencing of *M. peregrinus* confirmed the genome is mosaic from the two progenitors, and that allopolyploidy has independently evolved at least twice since the species introductions (Vallejo-Marin *et al.*, 2015).

In animals, allopolyploid speciation appears to be rare, since there is no overrepresentation of species with even-numbered chromosome counts (Otto and Whitton, 2000). In general, ploidy is uncommon in sexually reproducing animals, likely due to the presence of sex chromosomes and improper *dosage compensation*, the mechanisms that balances the gene products between the sexes, which can become unbalanced in polyploids (Orr, 1990). However, in the fishes polyploidy is very common and appears to have evolved repeatedly (Leggatt and Iwama, 2003). The repeated origin of polyploidy and frequent hybridization among polyploid fish in nature suggests allopolyploidy may be more common in fish than other animals. As expected, most allopolyploid examples in animals are often parthenogenetic or self-reproducing such as stick insects (*Bacillus*) and freshwater snails (*Bulinus truncatus*) (Otto and Whitton, 2000). The allopolyploid gray tree frog with the distinct mating calls mentioned previously is also a clear example of a sexually reproducing allopolyploid, where the evolution of female preference for the hybrid call could quickly result in speciation. Perhaps the best evidence of allopolyploidy speciation in animals comes from laboratory strains of tetraploid hybrid silk moths (*Bombyx mori* × *Bombyx mandarina*) (Astaurov, 1969). These tetraploid hybrids were laboratory engineered using a 'triploid bridge' strategy that involves the fusion of rare, unreduced (diploid) gametes of one species with a normal haploid gamete of the other species to form a fertile tetraploid hybrid offspring. Although these hybrids are synthetically engineered and do not necessarily constitute a distinct species, this example demonstrates a possible route to allopolyploidy in animals.

Similar to allopolyploidy, HHS has been more commonly reported in plants than animals. However, the number or putative hybrid species has dramatically increased across a broad range of organisms that demonstrate the many routes to hybrid speciation, such as: chromosomal rearrangements in hybrid yeast species (Greig *et al.*, 2002), host-choice shift in hybrid honeysuckle-maggot flies (*Rhagoletis*) (Schwarz *et al.*, 2005), the outperformance of intermediate hybrid cyprinid fish (*Gila seminude*) in the Virgin River (Demarais *et al.*, 1992), the invasion of hybrid sculpin fish (*Cottus gobio* group) in the murky waters of the Rhine River (Nolte *et al.*, 2005), host plant divergence and altitudinal differences in intermediate hybrid alpine butterflies (*Lycaeides*) (Gompert *et al.*, 2006; Nice *et al.*, 2013), the allopatric establishment of hybrid daisies on the British Isles (*Senecio squalidus*) (James and Abbott, 2005), the hybridization-derived 'swordtail' mating signals in *Xiphophorus* (Schumer *et al.*, 2013; Meyer *et al.*, 2006), the introgression of wing color mating cues in hybrid *Heliconius* butterflies (Sanchez *et al.*, 2015; Salazar *et al.*, 2010), the sorting of genetic incompatibilities and plumage-based mate choice in the

hybrid Italian sparrows (Hermansen *et al.*, 2014; Bailey *et al.*, 2015), and the establishment of rock-edge adapted, genotypic hybrid *Penstemon clevelandii* in Southern California (Straw, 1955; Wolfe *et al.*, 1998).

In plants, there are over 20 well-documented examples of HHS (Rieseberg, 1997; Gross and Rieseberg, 2005), with the best evidence coming from multiple hybrid species of desert sunflowers (*Helianthus*). Hybridization between the common sunflower (*Helianthus annuus*) and the prairie sunflower (*Helianthus petiolaris*) lead to the establishment of at least three desert-adapted homoploid hybrid species: *Helianthus anomalus* (Ungerer *et al.*, 1998), *Helianthus deserticola* (Gross *et al.*, 2003), and *Helianthus paradoxus* (Welch and Rieseberg, 2002; Rieseberg *et al.*, 2003a; **Figure 2 (b)**). The *transgressive segregation* of variation from the two parental species resulted in individuals with a mosaic genomic makeup and 'hybrid vigor' that allowed them to persist in extreme environments where the progenitors could not, which appears to be a common result of hybridization (Rieseberg *et al.*, 1999, 2003b). Laboratory crosses were able to reconstruct the chromosomal arrangements and transgressive phenotypes found in the desert species (e.g., small leaf size, seed dormancy, and high drought and salt tolerance), which supports the predictions of the *mosaic genome hybrid speciation* model (Rieseberg *et al.*, 2003a; **Figure 2**). For these desert sunflower species, chromosomal rearrangements and ecological isolation appears to have been the key to their establishment (Gross *et al.*, 2007; Gross *et al.*, 2003; Gross and Rieseberg, 2005, 2004).

Heliconius butterflies, particularly the *Heliconius melpomene/cydno* species complex, provide one of the most thoroughly examined cases of HHS. *H. melpomene* and *H. cydno* are closely related, divergently colored species that geographically overlap in Central America and the northern Andes. In nature, they are isolated ecologically (i.e., divergence in larval host plant and adult food plant preferences) and altitudinally, as well as through hybrid F1 female sterility (reviewed in Jiggins, 2008). However, hybrid males are fertile and backcrossing facilitates introgression between the species (Salazar *et al.*, 2008). *Heliconius hueripa* is a putative hybrid species of *H. cydno* and *H. melpomene* that has an intermediate color pattern, comes into geographic contact with *H. melpomene* (Salazar *et al.*, 2005) and clusters phylogenetically in the *H. cydno* species complex (based on nuclear gene sequences and genome-wide markers) (Quek *et al.*, 2010; Flanagan *et al.*, 2004; Beltrán *et al.*, 2007). In the wild, *H. hueripa* is isolated from its progenitors by color pattern-based mate choice (Mavarez *et al.*, 2006). Surprisingly, the first-generation backcrosses that resemble *H. hueripa* also preferred mates with their own color pattern, suggesting at least partial reproductive isolation may be able to rapidly evolve in *Heliconius* hybrids (Melo *et al.*, 2009). A custom spatial, individual-based, multilocus evolutionary model was built to explicitly examine the likelihood of the HHS scenario for *H. hueripa* (Duenez-Guzman *et al.*, 2009). The model showed clear support for the possibility of a hybrid origin of *H. heuripa* when the initial hybridization is followed by an extended period of geographic separation from the progenitor species.

Genomic evidence suggests *H. huerripa* may be a hybrid trait species. Genetic mapping has identified the color pattern loci responsible for color pattern differences between *H. heurripa* and its progenitors. Population genomic analyses across these color pattern loci and neutral regions, uninvolved in color pattern variation, reveal a hybrid genomic makeup, with relatively few *H. melpomene*-derived alleles in a predominantly *H. cydno* background (Salazar *et al.*, 2010). At the color pattern loci, *H. hueurippa* is homozygous for the same *H. melpomene* and *H. cydno* allelic combinations that produced the *H. hueripa*-like color pattern in the laboratory backcrosses. Although the data thus far support HHS of *H.* hueripa (however, see Brower, 2012 for a critique), the observed mosaic genome makeup in *H. heurripa* could also result from the incomplete sorting ancestral variation (see **Figure 1(d)**). However, unlike most *Heliconius* color patterns, *H. heuripa* has a unique non-mimetic color pattern not found in any other *Heliconius* species, which makes it unlikely to have been an ancestral form. Further evidence comes from another member of the *H. cydno* species complex, *Heliconius timareta*, where genomic evidence suggests introgression of a novel wing color pattern, from the sympatric species *H. melpomene*, has resulted in a new reproductively isolated hybrid lineage of *H. timareta* that has melpomene-like color pattern (Dasmahapatra *et al.*, 2012). In the laboratory, backcross individuals with the reconstructed *H. timerata florencia* color pattern showed clear males clearly preferred to approach and court females with the same hybrid color pattern, providing an excellent case of hybrid trait speciation (Sanchez *et al.*, 2015; **Figure 2(b)**). Again, it appears that with only a few generations of crosses, hybridization and introgression of warning color patterns that also act as mating cues can result in the establishment of a homozygous, true-breeding hybrid lineage. These examples demonstrate the efficacy of 'magic traits' in evolutionary diversification and highlight the importance of hybridization during adaptive radiation.

Is Hybrid Speciation Important?

A corollary question to whether hybrid speciation is important is: how common is hybrid speciation? It is estimated that 25% plant and 10% of animal species hybridize (Mallet, 2005), providing ample opportunity for hybrid speciation. Polyploid speciation is rare in animals and explains about 2–7% of vascular plant species (Otto and Whitton, 2000). Potential cases of HHS are fewer, but reports have dramatically increased and there are now several likely examples in a broad range of plants and animals (Schumer *et al.*, 2014). However, a critical review of 'How common is HHS' suggests there are few examples with sufficient evidence to satisfy the three criteria for hybrid speciation, particularly the evidence of reproductive isolation required for criteria 1 and 3 (**Table 1**). Many factors likely contribute to there being so few examples, which has

Table 1 Proposed cases of HHS that meet the three criteria of Schumer *et al.*, 2014

	Criterion 1	*Criterion 2*	*Criterion 3*
No. of proposed HHS cases (fungal/animal/plant)	8 (2/4/2)	43 (8/22/13)	4 (0/1/3)
%	19%	100%	9%

likely resulted in a gross underestimation of the importance of hybrid speciation.

The greatest difficulty with HHS is that theoretically it seems unlikely to occur. The concern is that the hybrid lineage will be swamped out by backcrosses with the progenitor species, if isolation is incomplete. However, genomic studies have revealed that ecological divergence can be extremely effective at maintaining the species boundary, even in the face of gene flow (reviewed in Seehausen *et al.*, 2014). The role of ecological divergence in the establishment of hybrid species needs to be further explored, as this is a key step in most models of hybrid speciation and there are novel genotypes continuously being generated by hybridization to explore. Both theoretical developments and empirical examples demonstrate that hybrid speciation does occur, but also that there are conditions that greatly increase the chance of hybrid speciation occurring.

Evidence for the hybrid origin of a species will always be difficult, if not impossible, to attain. Perhaps the most obvious difficulty is extinction. If either progenitor species becomes extinct after the hybrid species becomes established and leaves no little or no fossil record, then it is very unlikely that anyone would hypothesize hybridization was involved in the divergence of the extant species (**Figure 1(c)**). Even with whole genome sequencing of the extant species it would be unlikely to be able to infer a hybrid origin, since the extinct progenitor would not have been sampled. The other major difficulty is distinguishing between genomic evidence of hybrid speciation versus the incomplete sorting of ancestral variation (referred to as incomplete lineage sorting, ILS; **Figure 1(d)**), since both could result in highly mosaic genomic compositions. Both of these processes will result in shared alleles between the putative hybrid and the parental species, however the species are expected to share a certain number of alleles by chance alone, simply because they share a recent common ancestor. One approach to this problem has been the development of phylogenetic models that can account for ILS when testing genomic evidence of hybridization (Yu *et al.*, 2013; Meng and Kubatko, 2009). Another difficulty is that there is only a limited window of evolutionary time that hybrid speciation is likely to be detectable. If there are too few differences between the progenitors and hybrids, then they may appear to be nothing more than a hybrid swarm. If there are too many differences and too few shared phenotypes, then a hybrid origin may never be hypothesized or tested. For these and many more reasons, the frequency of hybrid speciation in nature will always be underestimated, but the theoretical and empirical evidence suggests that the importance of hybrid speciation should never be underappreciated (Abbott *et al.*, 2013).

See also: Reinforcement

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Inbreeding and Nonrandom Mating

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Glossary

Autozygosity The proportion of the genome that is homozygous because it is identical by descent.
Identical by descent Probability that the two homologous alleles in a diploid individual are derived from one particular allele possessed by a common ancestor of the parents.
Inbreeding Mating between relatives that results in an increased level of identity by descent in the progeny of the mating.
Inbreeding depression The detrimental effect on fitness due to increased inbreeding.
Lethal equivalents Group of genes that, when made homozygous, on average would cause $2B$ deaths.
Negative-assortative mating An increase in the frequency of mating between individuals with different phenotypes compared to random mating.
Positive-assortative mating An increase in the frequency of mating between individuals with the same or similar phenotypes compared to random mating.
Run of homozygosity Segment of genome that is autozygous based on homozygosity of a number of adjacent SNPs.

In much of population genetics, it is assumed that the individuals in the population choose mates by chance, that is, there is 'random mating' among the potential mates in the population including relatives. Here we will discuss two exceptions to this pattern, inbreeding and assortative mating, where there are deviations from this assumption. 'Inbreeding,' which is the result of mating between relatives greater than expected by chance, results in an increase of homozygotes and a decrease in heterozygotes throughout the genome. On the other hand, assortative mating, which is based on the tendency of particular phenotypes to mate, only influences the genes determining those phenotypes. We will discuss both 'positive-assortative mating,' an increase in the frequency of matings between individuals with the same or similar phenotypes compared to random mating, and 'negative-assortative mating,' an increase in the frequency of matings between individuals with different phenotypes compared to random mating.

Inbreeding

A major exception to random mating is when relatives mate and consequently their resulting progeny are inbred. For example, inbreeding in some plants is high when there is a high proportion of self-fertilization (both the female and male gametes come from the same parental individual). Small captive populations of endangered species often have high inbreeding because of past and present mating between related individuals. Inbreeding levels are generally low in human populations, where only a small proportion of matings occur between relatives, but in some societies first-cousin matings are the norm.

Inbreeding by itself does not cause a change in allele frequency, but it causes a reorganization of the alleles into genotypes. In a population that is inbred, the frequency of homozygotes is increased and the frequency of heterozygotes is reduced relative to random-mating (Hardy–Weinberg) proportions. The genotype changes caused by inbreeding are expected to equally influence all loci in the genome. However, the effect on genotype frequencies may be quite ephemeral if the mating system changes. For example, the excess frequency of homozygotes resulting from self-fertilization can be eliminated completely in one generation of random mating.

Inbreeding and genetic drift appear to have similar overall effects on heterozygosity when averaged over loci or over populations, but when examining a given locus within a population, the predicted effect is different. Inbreeding (such as self-fertilization in a large plant population) can result in a deficiency of heterozygotes with no change in allele frequency for a given locus within a population. On the other hand, genetic drift may cause a chance change in allele frequency at a locus but generally causes little or no deficiency of heterozygotes compared to Hardy–Weinberg proportions within a population.

The level of inbreeding is generally quantified by the inbreeding coefficient, f. The inbreeding coefficient is the

Table 1 The three different parent genotypes when there are two alleles and self-fertilization, the frequency of these genotypes in the population, and the frequency of the three types of progeny

Parent	*Frequency*	*Progeny*		
		A_1A_1	A_1A_2	A_2A_2
A_1A_1	P_0	P_0	–	–
A_1A_2	H_0	$H_0/4$	$H_0/2$	$H_0/4$
A_2A_2	Q_0	–	–	Q_0
Total	1	$P_0 + H_0/4$	$H_0/2$	$Q_0 + H_0/4$

Table 2 Several types of matings between relatives and the inbreeding coefficient in the offspring from the different matings

Mating	*Inbreeding coefficient (f)*
Self-fertilization	0.5
Full-sibling	0.25
Parent–offspring	0.25
Half-sibling	0.125
Uncle–niece or Aunt–nephew	0.125
First cousin	0.0625
Second cousin	0.0156

probability that the two homologous alleles in a diploid individual are 'identical by descent (IBD)' – that is, that they are derived from one particular allele possessed by a common ancestor. The proportion of the genome that is homozygous, because it is IBD, is called the level of 'autozygosity.' This concept can be illustrated with self-fertilization, the most extreme type of inbreeding generally found.

As shown in Table 1 with complete self-fertilization and two alleles, there are just three parental genotypes, A_1A_1, A_1A_2, and A_2A_2, and they occur in the relative proportions of the genotypes in the population before inbreeding, P_0, H_0, and Q_0, respectively. Allowing for segregation in the heterozygote, the proportions of the genotypes in the progeny generation are

$$P_1 = P_0 + H_0/4 \qquad H_1 = H_0/2 \qquad Q_1 = Q_0 + H_0/4$$

In the progeny from the heterozygous parent A_1A_2, half the progeny are IBD, either A_1A_1 or A_2A_2, and therefore the inbreeding coefficient after one generation of self-fertilization is $f=0.5$. Notice that due to the inbreeding, the frequency of heterozygotes is half what it was in the parents and the frequency of both the homozygotes are increased by $H_0/4$.

To illustrate that inbreeding does not change allele frequency, the frequency of allele A_1 in the next generation p_1 is the frequency of genotype A_1A_1 plus half the frequency of genotype A_1A_2 or

$$p_1 = P_1 + H_1/2 = (P_0 + H_0/4) + H_0/4 = p_0$$

A general formulation of the proportion of the three genotypes in a population with inbreeding level f is (Wright, 1931)

$$P = p^2 + fpq \qquad H = 2pq - 2fpq \qquad Q = q^2 + fpq$$

where the first term is the Hardy–Weinberg proportion and the second is the deviation due to inbreeding from that value. The size of the coefficient of inbreeding reflects the deviation from Hardy–Weinberg proportions of the genotypes such that when f is 0, the zygotes are in Hardy–Weinberg proportions, and when f is positive, there is a deficiency of heterozygotes. Because f as we have defined it here is a probability, it has a range from 0 to a maximum of 1 when all the individuals are homozygotes. Estimates of f can sometimes be less than 0, either due to chance or to the impact of other evolutionary factors.

Other matings between relatives also result in an increase in the inbreeding coefficient in their progeny (Table 2). For example, a mating between two full siblings and between a parent and an offspring (sometimes called first-degree relatives) result in an inbreeding coefficient of 0.25 in their progeny. A mating between two first cousins results in $f=0.0625$ in their progeny, a much lower level but one that results in an increase in the frequency of rare recessive diseases (see below).

Often, in captive populations of endangered species or in livestock breeds, there might be complicated pedigrees with multiple matings between relatives resulting in inbreeding. There are approaches, such as the chain-counting technique based on the count of the number of links between the two parents through a common ancestor, to calculate inbreeding in these cases. As an example of a more complicated pedigree, Figure 1 gives the pedigree of the wolves on Isle Royale National Park (Hedrick *et al.*, 2014).

In pedigrees, squares indicate males, circles indicate females, and in this wolf pedigree, the numbers in them indicate their studbook or identification numbers. When there are multiple individuals from a mating that cannot be identified, or it is not important to identify them, a diamond is used with a number that indicates the number in this group. In pedigrees, the oldest individuals are generally at the top and their offspring and later individuals are lower in the pedigree.

For example, in the wolf pedigree, male 93 and female 99 (considered founders in this pedigree) had 13 offspring (individuals 58, 70, 62, and 102, plus 9 additional offspring that are not identified individually here). After several years, female 99 died and male 93 subsequently mated with his daughter 58, a consanguineous (between relatives) mating that is indicated by a double line. The 21 offspring of this mating (individuals 135, 147, and 152, plus 18 others), all had inbreeding coefficients of 0.25. Two full sibs from this mating, 135 and 147, mated and the two progeny from this mating had an $f=0.375$ because inbreeding accumulated over two generations. The eight individuals alive in 2014 are indicated by shading and have inbreeding coefficients ranging from 0.0 for 160 (she has no known inbreeding in her ancestry) to 0.312 for 193, who is the offspring from a mother–son (160 and 183) mating, and the son 183 was himself inbred.

This example illustrates that IBD is always defined with respect to an ancestral population but this base population might be arbitrary (Powell *et al.*, 2010; Speed and Balding, 2015). For example, in the analysis of the wolf population, it was assumed that oldest animals born on Isle Royale, females 99, 55, and 67 and male 61 were unrelated (male 93 was an immigrant from Canada). However, if the base population and pedigree information were available more generations in the past, there was probably contemporary IBD from common ancestors in this older reference population as well as from recent matings.

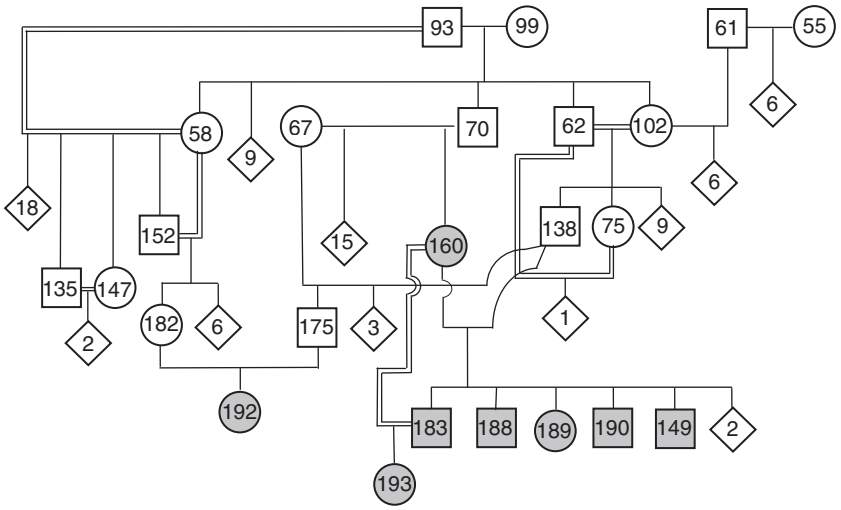

Figure 1 The pedigree of the Isle Royale National Park wolf population (Hedrick *et al.*, 2014).

Inbreeding and Selection

Because inbreeding increases the frequency of homozygotes, the frequency of rare, recessive diseases is increased in inbred individuals. For example, in a sample from Japan, approximately 5% of the marriages were between first cousins, but the proportion of first-cousin marriages among families with offspring having albinism was 10-fold higher, approximately 56%. In a European sample, approximately 2% of the marriages were between first cousins, but 20% of the marriages that had offspring with albinism were among first cousins.

To demonstrate the basis of this, we can compare the proportion of recessive homozygotes for a given inbreeding coefficient (Q_f) to that in a non-inbred population (where the frequency of recessive homozygotes is $Q=q^2$). The ratio of these two quantities is

$$\frac{Q_f}{Q}=\frac{q^2+fpq}{q^2}=1+\frac{fp}{q}$$

This ratio is very large for low allele frequencies (small values of q), as is generally seen for recessive diseases, and increases with the level of inbreeding. For example, when there is a first-cousin mating, $f=0.0625$ and $q=0.01$, the ratio above is 7.2. That is, there are more than seven times as many affected individuals in the inbred group as in the random-mating group, an excess similar to the differences discussed above for albinism.

Inbreeding also has a general detrimental effect on fitness and results in the decline of fitness due to increased inbreeding, called 'inbreeding depression' (Charlesworth and Willis, 2009). Although inbreeding depression seems to be a nearly universal phenomenon, the extent of inbreeding depression varies for different species and even for different populations of the same species, depending upon the evolutionary history of the population.

An example of inbreeding depression is in the Australian tree *Eucalyptus grandis*, which is used for timber production throughout the world. For a group of 28 progeny produced by self-fertilization from a single parental tree, the whole genome was sequenced (Hedrick *et al.*, 2016a). Overall, 9560 genes were heterozygous in the parent and were examined in the progeny group. As we have discussed above, it would be expected that 50% of these progeny would be homozygous (IBD) for these heterozygous loci, given that there was no selection. However, only 34% of these genes were homozygous in this progeny group, a deficiency that was present on all 11 chromosomes. **Figure 2** gives the observed proportion of the three genotypes for 1019 genes along chromosome 1 and except for a short region on the far right end of the chromosome, the proportion of heterozygotes is much greater than 0.5 and averages 70%. This effect appears to be the result of very strong selection at many genes, or genes associated with them, that cause high mortality when made homozygous by this one generation of self-fertilization.

To quantify the effects of inbreeding on survival, Morton *et al.* (1956) developed a model that assumes that the loci affecting survival act independently and multiplicatively. In this case, the fitness of individuals with inbreeding f becomes approximately

$$w_f=w_0e^{-Bf}$$

where w_0 and w_f are the mean fitnesses when there is no inbreeding or inbreeding to the level f. B is the regression coefficient that measures how fast fitness decreases with inbreeding; it is equal to 0 when there is no inbreeding depression. This expression can be solved to give the number of lethal equivalents as

$$2B=-\frac{2}{f}\ln\left(\frac{w_f}{w_0}\right)$$

The number of 'lethal equivalents' in a diploid zygote is defined as a group of genes that, when made homozygous, would on average cause $2B$ deaths. For example, if there is one lethal allele present as a heterozygote, there would be one lethal equivalent. Using data documenting the survival proportion for different inbreeding categories, investigators have used this relationship extensively to determine how much inbreeding depression is present in a number of different

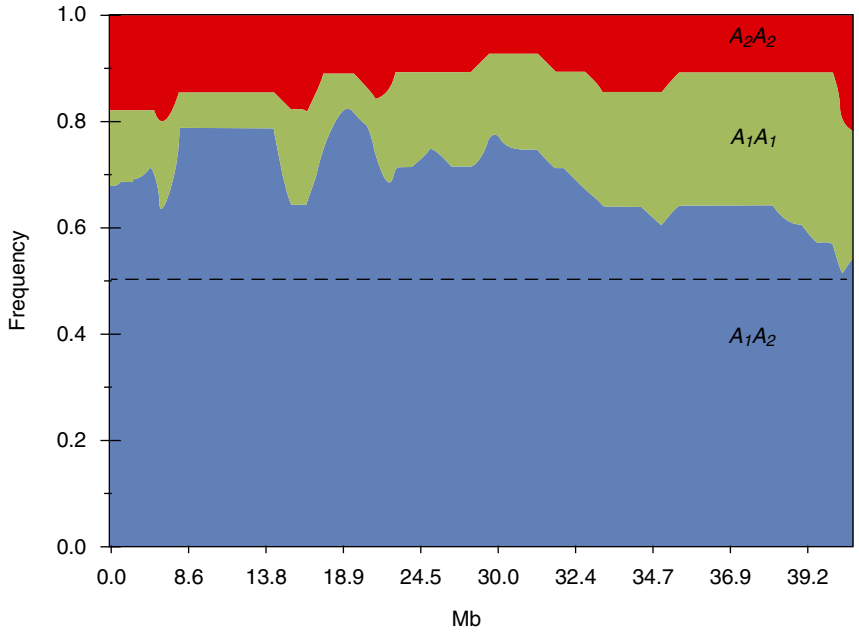

Figure 2 The frequency of heterozygotes A_1A_2 and homozygotes A_1A_1 and A_2A_2 in 28 progeny from a plant heterozygous for 1019 genes along chromosome 1 (location of genes given here are in Mb) in *Eucalyptus grandis* Hedrick *et al.*, 2016a). The broken horizontal line indicates the 50% heterozygosity expected.

species. In particular, this approach has been used to examine the amount of inbreeding depression for survival in a number of captive endangered species.

Arkush *et al.* (2002) determined the survival of outbred ($f=0$) and inbred (from brother–sister matings, $f=0.25$) endangered winter-run chinook salmon exposed to the whirling disease parasite. In this case, survival of the outbred salmon was 82.2% and survival of salmon from full-sib matings was only 59.4%. Using the expression above, then

$$2B = -\frac{2}{0.25}\ln\left(\frac{0.594}{0.822}\right) = 2.6$$

That is, the equivalent of two to three genes were segregating in the winter-run chinook salmon population that if IBD would result in mortality from whirling disease.

Assortative Mating

Assortative mating is nonrandom mating based on phenotypes rather than between relatives. Positive-assortative mating or negative-assortative mating occurs if the mated pairs in a population are composed of individuals with the same phenotype more often, or less often, than expected by random mating, respectively. Positive-assortative mating is in some ways analogous to inbreeding in that similar phenotypes, which might have similar genotypes, are more likely to mate than random individuals from the population. Some types of assortative mating are also similar to inbreeding in that they do not change allele frequencies but do affect genotype frequencies. On the other hand, negative-assortative mating may result in balancing selection and the maintenance of genetic variation. Many assortative mating models do change allele frequencies because the proportion of individuals in the matings differs from the proportion in the population. An important point to remember is that assortative mating affects the genotype frequencies of only those loci involved in determining the phenotypes for mate selection (and genotypes at loci nonrandomly associated with those loci), whereas inbreeding affects all loci in the genome.

In a survey of assortative mating studies, Jiang *et al.* (2013) found that most of the examples of assortative mating were for positive-assortative mating. There appears to be positive-assortative mating for a number of traits in humans, such as height, skin color, and intelligence, although the consequent phenotypic correlation is often not very large. In addition, there also appears to be positive correlations among mates in humans that have particular phenotypes, such as deafness, blindness, or small stature. Of course, there are many different genetic (and nongenetic) causes for deafness, blindness, or small stature so that such a phenotypic correlation may not result in a genetic correlation (for deafness, see Nance and Kearsey, 2004). Rather strong positive-assortative mating may occur in plants when a pollinator forages at a given height or is attracted to a given flower color and, as a result, tends to pollinate plants similar to the ones where the pollen was collected. Similar effects may also occur when flowering time is variable, and only plants that flower simultaneously pollinate each other.

Jiang *et al.* (2013) found few examples of negative-assortative mating in their review. Some examples are, however, in some plants where successful fertilization occurs only between individuals with different flower types. Although less generally accepted, another example is in populations where rare males (or females) have a mating advantage over more common types. Some reports suggest that negative-assortative

Table 3 The number of matings observed between gray and black wolves in Yellowstone National Park from 1995 to 2014 (Hedrick *et al.*, 2016b)

Mating		*Observed*	*Expected*	*Observed – Expected*
Male	*Female*			
Gray	Gray	54	71.0	−17.0
Gray	Black	67	50.1	16.9
Black	Gray	99	82.0	17.0
Black	Black	41	57.9	−16.9
Total		261	261	

mating in mammals and other vertebrates may be based on major histocompatibility complex (MHC) differences.

The overall support for MHC-based, negative-assortative mate choice in humans is mixed and contentious. The most widely known example is the 't-shirt study' in which female Swiss university students ranked the smell of t-shirts worn by male students on characteristics such as pleasantness (Wedekind *et al.*, 1995). The findings of this study suggested that females preferred the odor of males that differed at MHC genes, except when they were on birth control pills, in which case they preferred males that were similar at MHC genes! Further, a follow-up study examining some of the same pairs found no correlation between the rankings for the two different studies.

Several recent studies have examined the correlation of mates for MHC in humans compared to the correlation of genes in the rest of the genome. The first such study found a small but significant (partly due to high statistical power) negative correlation in 30 couples at the MHC region of −0.043 compared to the average in the rest of genome of −0.00 016 (Chaix *et al.*, 2008). However, this level of assortment appears small in a biological sense and nine other genomic regions had higher levels. Subsequently, Derti *et al.* (2010) concluded that the findings of Chaix *et al.* (2008) were not statistically robust and they found nonsignificant results in another small-sized, independent sample. These sample sizes appear much too small to draw inference about genomic assortative mating but a study underway of assortative mating in about 10 000 pairs using genome-wide SNP data (P. Visscher, personal communication) should give definitive estimates.

A striking example of negative-assortative mating is in wolves from Yellowstone National Park for gray and black coat color (Hedrick *et al.*, 2016b). Black coat color in wolves is caused by a dominant allele at a beta-defensin gene. In the surveys of mating pairs at Yellowstone from 1995 to 2014, 166 out of 261 (64%) of the matings were between different color wolves, either gray males × black females or black males × gray females (**Table 3**) with a significant negative correlation of −0.27.

Future for Inbreeding

The major change in population genetics in recent years is the advent of genomic data (around tens of thousands of SNPs or more) in many organisms to accurately document factors important in population genetics. In particular, genomic estimates of the level of inbreeding have enabled a higher degree of resolution, a topic that we will discuss here. In addition, genomic estimates have begun to identify genes that result in inbreeding depression and genes involved with assortative mating, developments that will bring new detailed information for those areas. For example, estimates of genomic inbreeding appear to predict inbreeding depression for a number of traits in humans (Joshi *et al.*, 2015).

Traditionally, the use of pedigree data to estimate inbreeding (f_{PED}) has been thought to be the best approach. However, relatedness and inbreeding of pedigree founders and the limited depth of most pedigrees can result in an underestimation of actual inbreeding levels when pedigrees are used. The estimation of inbreeding using genomic data can potentially overcome these problems and provide more information in many situations (Keller *et al.*, 2011; Kardos *et al.*, 2015). For example, if the homozygosity of many SNPs are determined, then an estimate of inbreeding is

$$f_H = \frac{O(\text{Hom}) - E(\text{Hom})}{N - E(\text{Hom})}$$

where O(Hom) and E(Hom) are the observed and expected numbers of homozygotes in an individual and N is the number of loci examined. This is actually the same as the fixation index

$$f_H = \frac{H_E - H_O}{H_E}$$

where H_E and H_O are the expected and observed frequencies of heterozygotes. This expression can also be derived from the expression for heterozygosity that we gave earlier for deviations from Hardy–Weinberg due to inbreeding, $H = 2pq - 2fpq$, assuming that $H_E = 2pq$.

Given genomically mapped data in a diploid individual, genetic regions in which there are 'runs of homozygosity (ROH)' can be identified. To indicate autozygosity, these ROH consist of a given number of homozygous, adjacent SNPs over a given number of Mb (Keller *et al.*, 2011). The inbreeding coefficient f_{ROH} estimated from these data is the total length of these ROH for a given individual divided by the total length of the examined genome, or

$$f_{ROH} = \frac{\sum L_{ROH}}{\sum L_{AUT}}$$

where the numerator is the sum of the lengths of all the ROH of a given size or larger and the denominator is the sum of the length of autosomal genome covered by the SNPs utilized.

Keller *et al.* (2011) and Kardos *et al.* (2015) provided the following reasons why such genomic estimates of inbreeding might be superior to estimates from pedigrees:

1. Genomic estimates, which directly measure the homozygosity in given individuals, can be more accurate than pedigree estimates. The pedigree estimate of inbreeding (f_{PED}) is the expectation of the inbreeding level for a given individual and there is a substantial variation among progeny from a given mating. For example, the percentage of the genome that is expected to be autozygous from a first-cousin mating is 6.25% but the standard deviation of this is 2.4% (Hill and Weir, 2011).

2. Genomic estimates incorporate autozygosity from distant common ancestors well beyond that given in most pedigrees which generally only go back a few generations. For example, Woods *et al.* (2006) examined 38 individuals that were the result of a first-cousin mating. Instead of having an average of 6.25% of their genomes IBD, they observed 11.0% IBD, a difference probably the result of other more distant common ancestors contributing to IBD. Further, because longer ROH are thought to be the result of more recent inbreeding (recombination has had few generations to break them up) and shorter ROH are more likely to the result of more distant common ancestors, the time to common ancestors can potentially be estimated.
3. Genomic estimates of inbreeding can be calculated in many organisms in which pedigree information is not available or the pedigree is only known for a few individuals or generations (e.g., f_H in harbor seals by Hoffman *et al.*, 2014 and ROH in wooly mammoths by Palkopoulou *et al.*, 2015). For estimation of f_H, the SNPs do not need to be mapped while for estimation of f_{ROH}, they do.
4. Genomic estimates of inbreeding when the SNPs are mapped can potentially be used to examine whether the level of estimated inbreeding is specific to particular regions of the genome or particular chromosomes. Comparison of such genomic data over different chromosomes and genomic regions and to pedigree expectations can provide insight into the impact of factors such as different types of selection, recombination, and mutation on genetic variation.
5. Genomic estimates can also avoid the problem of defining a reference population when working with the IBD probabilities. Further, precise genomic estimates of kinship and inbreeding among founders, or another cohort in later generations, can provide a baseline or a reference population from which to predict future kinship and inbreeding using traditional pedigree analysis.

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See also: Coalescent and Models of Identity by Descent. Mating Systems in Flowering Plants

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Industrial Melanism, History of

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Glossary

Evolution Evolution refers to changes in the composition of a population over long periods of time that reflect an underlying change in the frequencies of genes within the population's genetic pool. Some evolutionary changes, such as the phenomenon of industrial melanism, are referred to as 'microevolutionary,' to indicate they are short term reversible changes. Other evolutionary changes, such as the extinction of an entire species, are irreversible, and are often referred to as 'macroevolutionary.'

Lamarckian inheritance A discredited mechanism for evolutionary change popularized by Jean Baptiste de Lamarck (1744–1829) that posits changes that occur over the course of an individual organism's lifetime can be passed on to its offspring. For example, Lamarck suggested that the reason why modern giraffes have such long necks is because their shorter necked ancestors habitually stretched their necks to reach foliage high up in the canopy of trees, and this continued stretching by generations of giraffes had resulted in their offspring being born with longer necks.

Mutation A mutation is a rare change in the DNA (deoxyribonucleic acid) that serves as the hereditary material in nearly all organisms. DNA is a long molecule that contains long segments called genes that code for specific traits and behaviors. Because mutations occurring within a gene may affect the sequence of the gene, they have the potential to alter the code, and therefore how the trait or behaviour is expressed. Mutations occasionally arise due to copying errors when the DNA is duplicated in cells, despite the presence of repair mechanisms. Certain environmental stimuli, such as radiation, may increase the rate at which mutations occur.

Natural selection A well-documented mechanism for evolutionary change that accounts for, among other things, the origin of adaptations in nature. It was first proposed by Charles Darwin (1809–82) and Alfred Wallace (1823–1913) who drew attention to the predictable consequence of three circumstances in nature. First, populations in nature exhibit variation, i.e., the individuals that comprise a population do not all look and behave exactly alike, and at least part of this variation is inherited. Second, some of this variation affects the ability of organisms to survive and find mates. And third, there is a competition in nature, owing to the fact that not all who are born can possibly survive owing to limits on the availability of resources, mates etc. Darwin and Wallace pointed out that when all three of these circumstances take place, a logical and entirely predictable consequence is that those individuals that vary in ways that help them survive and reproduce will contribute more to the next generation. And as a consequence, the frequency of the favored variations will gradually, over many generations, increase.

Saltationism A discredited mechanism for evolutionary change endorsed by Étienne Geoffroy Saint Hilaire (1772–1844) that attempted to account for the origin of new species and new adaptations by means of sudden dramatic changes. In the early twentieth century, saltation was thought to occur by means of large mutations.

Industrial melanism refers to a trend toward darker coloration in many moth species that has been observed in areas affected by large-scale air pollution. It was first noticed in Britain and Continental Europe in the wake of the Industrial Revolution. It has since been observed in hundreds of other moth and insect species throughout the world wherever large-scale air pollution occurs.

The Peppered Moth

The most famous and well-documented example of the phenomenon of industrial melanism is the peppered moth, *Biston betularia*, a common moth in Britain and Continental Europe. Like other moth species, the adult form of this moth is nocturnal (active at night), spending most of the day motionless on trees, rocks, and other resting sites. The peppered moth was first described by Moses Harris (1766), who in a brief description of the moth drew attention to the 'white, freckled' appearance that gives it its common name. Harris was an acute observer of nature, and his failure to mention the adult form as having any other form strongly implies the existence of another dark form was unknown at the time of his writing.

Naturalists initially greeted the chance discovery of a melanic (dark) form of the peppered moth (ca. 1848) in the vicinity of Manchester, a large manufacturing center at the time, as simply an anomalous sport of nature. But over the course of just a few decades, an ever increasing number of subsequent additional sightings by other naturalists led commentators to recognize something dramatic was taking place before their eyes. The dark form had gone from being unknown to being quite common in the vicinity of Manchester in the space of just a few decades. A similar trend was also noticed in other industrial areas in both Britain and Europe, not merely in the peppered moth, but also in many other moth species.

Multiple explanations were proposed to account for this trend among insects in the vicinity of manufacturing centers. Some, such as Nicholas Cooke (1877), suggested that the general darkening of moths in the affected areas was a direct response to changes in the environment. He pointed out that increased humidity or large-scale air pollution had the effect of

darkening the resting sites of the moth, and suggested the moths became darker in order to match their surroundings to avoid predators. In other words, moths have a physiological ability to darken to match their backgrounds over the course of their lives, and they pass this on to their offspring. This is often referred to as Lamarckian inheritance. James Heslop Harrison (1927–28) alternatively proposed that the general darkening in the affected species was due to the ingestion of pollutants by larvae (caterpillars) that feed on leaves covered by soot. In particular, he proposed lead salts had mutagenic properties that led the caterpillars to mutate to the dark form on the basis of a series of experiments he conducted in the 1920s. These investigations were subsequently called into question by others who were unable to reproduce his remarkable results. A third explanation popularized by James W. Tutt (1896) explained the phenomenon in terms of Darwin's theory of natural selection. According to this explanation, the dark form initially arose by means of a chance mutation. In unpolluted environments, the resulting dark form was easily spotted by birds and thus quickly removed from the population. This explains why it is virtually unknown in unpolluted environments. But in polluted environments, such as those visibly darkened by air pollution (owing to the dying off of pale lichen that covered the trees and the slow accumulation of soot), the dark form was no longer at a disadvantage. Indeed, in soot-darkened forests, it is the pale form that is now at a disadvantage, and as such, the pale form that is removed. Comparing the moths as they rest on pale and dark backgrounds associated with the two environments reveals the basic intuition behind this explanation (see **Figure 1**).

E.B. 'Henry' Ford, founder of the Oxford School of Ecological Genetics that championed the systematic study of natural selection in field populations openly questioned whether Tutt's analysis was sufficient to account for the speed of the spread. Ford doubted it could be that simple. His own explanation, in terms of pleiotropy (the possibility a gene might have more than one affect on the phenotype of an organism), was that the spread was the result of two selection pressures. The first, documented by breeders who had established that the gene responsible for dark coloration was invariably dominant in the affected species, was that the dark form was 'hardier' or better able to survive toxins in their environment. This physiological advantage accounted for the rapidity of the spread. To account for why the spread had been limited to the vicinity of manufacturing centers, Ford drew attention to the obvious handicap of dark coloration in unpolluted environments, where the dark form would be readily spotted by predators.

To the modern eye, explaining the phenomenon of industrial melanism in terms of natural selection seems obvious. But it wasn't at the time of Kettlewell's investigations. The discovery of the phenomenon of industrial melanism coincided with what historians refer to as the 'Eclipse of Darwinism,' around the turn of the century when relatively few biologists believed that natural selection was the primary mechanism of evolution. During this time, scientists considered numerous alternative mechanisms, such as the possibility that new forms might arise at once my mutation (Saltationism), or evolution might be driven by the inheritance of changes that occurred during the

Figure 1 *Biston betularia*: one typical and one *carbonaria* resting on a lichen-covered tree in unpolluted country (Dorset); and, one typical and one *carbonaria* resting on blackened and lichen-free bark in an industrial area (the Birmingham district). These photos originally appeared separately as Plates 14 and 15 in Ford (1975).

life of an organism (neo-Lamarckianism). The phenomenon of industrial melanism, as witnessed in the peppered moth, represented a particularly striking example of natural selection in action, both by virtue of the fact that it appeared to be the result of selection operating on a single gene and also the sheer speed of the spread. J.B.S. Haldane (1924) seized upon the example of industrial melanism as illustrated in the peppered moth in an influential mathematical paper to draw attention to just how rapid natural selection could operate and the strength of the selective pressure involved, well in excess of what anyone previously would have believed.

H.B.D. Kettlewell

It is in this context that H.B.D. Kettlewell enters the story. Kettlewell was trained as a medical practitioner, but in midlife decided to change careers to pursue his boyhood love of natural history in E.B. Ford's newly established subunit of Genetics at Oxford University. Kettlewell's work on industrial melanism began with a large-scale survey of moth frequencies throughout Britain. The survey was conducted over many years with the assistance of literally hundreds of amateur entomologists, who collected yearly records of the frequencies of the pale and dark forms of the peppered moth in their local environments. In this way Kettlewell documented that rises in the frequencies of the dark form of the peppered moth were indeed correlated with areas of large-scale air pollution (Kettlewell, 1958).

Kettlewell is most famous for a series of field experiments he conducted in the early 1950s as an attempt to account for why the spread was occurring. It was quite clear at the start of his investigations that he was a proponent of Ford's explanation in terms of two selective forces mentioned above. In his initial report on the subject, Kettlewell states that he regarded Ford's suggestion that the dark form was physiologically superior to the pale form as established (Kettlewell, 1955). His task, in his early papers on the subject, was to document the second part of Ford's explanation, namely that birds preferentially remove moths that rest on non-matching backgrounds. At the time Kettlewell began his work on the subject it was neither obvious that birds were significant predators on moths, nor that they had the same difficulty humans do when we attempt to spot a moth when it rests on a matching background.

Kettlewell pursued his investigation by a mark–release–recapture experiment, a technique Ford and Ronald Fisher had previously used to estimate population size in insects that (unlike moths) persist in the form of colonies. As the name suggests, Kettlewell marked a combination of bred and local adult moths representing the pale and dark forms with a dab of cellulose paint on the underside of the wings. He then released large known quantities of these marked specimens onto tree trunks in a relatively well-confined area in a soot-darkened countryside near Birmingham, another manufacturing center where the phenomenon had been observed. As the final step, Kettlewell subsequently attempted to recapture as many marked moths as possible over the course of several nights by means of a combination of mercury light and assembling traps. The logic behind his investigation was that, all things being equal, the recapture rates for the pale and dark forms should be the same. If, on the other hand, one form was at an advantage in this polluted environment compared to the other, for example, the dark form was better able to hide from predators, one would expect the recapture rates to favor the dark form. And this is what Kettlewell found – in the polluted environment the recapture rate for the dark form was nearly twice that of the recapture rate for the pale form. A companion experiment, conducted a couple of years later in an unpolluted forest in Dorset, documented the reverse – here the recapture rate for the pale form was much greater than for the dark form. During the course of these investigations, Kettlewell also filmed the order in which birds preyed on moths. With the assistance of Niko Tinbergen, he demonstrated that, given a choice, birds would regularly pass over the inconspicuous form that matched its background in favor of the more conspicuous form (Kettlewell, 1955, 1956).

The dramatic nature of Kettlewell's results, namely the fact he was able to document selection pressures well above what previously been considered possible in nature, caused quite a stir among scientists and the lay public. Kettlewell's pictures and films of the relative order of bird predation were particularly influential in this regard (Rudge, 2003). The results came at a particularly auspicious time, when American textbook publishers were searching for simple examples to illustrate natural selection. Kettlewell's work on the subject fit the needs of educators beautifully. The phenomenon of industrial melanism represents the simplest kind of natural selection: directional selection acting in response to one effect of a single gene. The selective environment, soot-darkened and pale lichen-covered trees, was familiar to students. The selective mechanism, namely birds having difficulty finding moths when they rest on matching backgrounds, was intuitively obvious. The experimental design was also simple and the mathematics straightforward. Kettlewell's field investigations, conducted by means of field experiments further demonstrated that evolutionary biology (which was largely regarded at the time as an observational science, could conform to the standards of good science (Hagen, 1999). For all of these reasons, starting in the early 1960s, the phenomenon of industrial melanism became the example of choice to illustrate natural selection among textbook writers in the United States and elsewhere (Rudge and Fulford, 2012).

Kettlewell's research was received differently by the lay public and scientists. The vast majority embraced Kettlewell's work as a particularly well-documented example of natural selection that was perfect for use in science teaching. Fellow scientists who actually worked on the phenomenon were much more skeptical. Some objected to how he released the moths onto trees, a procedure he introduced out of reasonable assumption at the time that moths spend the day at rest on tree trunks. Others objected to the large numbers of moths used for the experiment. Kettlewell used large numbers in order to increase the odds he would be able to detect a difference between the different forms, but doing so raised a fundamental question about whether birds that normally do not eat moths at all might do so if moths were present in large enough numbers. Still others objected to his use of a combination of both bred moths for which he knew the

underlying genetics and moths caught on the site for whom the genetics was unknown, because the dark form could result from the melanic gene being heterozygous or homozygous (Berry, 1990; Grant, 1999).

As one might expect, critics of evolution, such as Jonathan Wells, have seized upon discrepancies between how the phenomenon is depicted in biology textbooks and what scientists say about it in their research publications as an indication that the phenomenon of industrial melanism is no longer regarded as a good example of natural selection (Wells, 2000, but see Rudge, 2002). Judith Hooper, in the first book-length study of Kettlewell, implies Kettlewell committed fraud, an allegation that is completely without merit (Hooper, 2002, but see Rudge, 2005). We should note the standards by which scientific investigations are judged have changed over time, so it should come as no surprise that an investigation conducted in the early 1950s might fall short when it comes to contemporary standards.

Current Status

At least eight follow-up studies, many of which were conducted specifically to address problems in Kettlewell's original investigations have all documented that Kettlewell's basic conclusion, i.e., the phenomenon is due to natural selection and the most important selective factor is differential predation by birds, is still correct. An impressive study conducted on two continents documented a similar rise and predictable fall in the frequency of dark peppered moths in Britain and the United States following the advent of clean air legislation (Grant *et al.*, 1998). More recently, a large-scale six-year predation experiment specifically designed to address perceived problems associated with the conduct of Kettlewell's original investigations conducted by the late Michael Majerus provides the most direct evidence yet for the selective role of bird predation (Cook *et al.*, 2012). The phenomenon of industrial melanism is still regarded as a great example of natural selection and selective predation by birds remains the primary selective force responsible. What this additional research documents is that the situation is far more complicated than textbooks would have us believe. Sulfur dioxide concentrations and differential migration might both play a role in accounting for the spread of the dark form (Cook, 2003). Discussion of the problems associated with the original conduct of Kettlewell's investigations does not undermine this example. These problems actually augment the continued use of the phenomenon of industrial melanism and Kettlewell's work on it for science teaching, because doing so draws attention to a variety of issues associated with the nature of science often lost on students, such as the tentative nature of scientific knowledge (Rudge, 2000).

See also: Directional Selection and Adaptation. Life History, What is?. Population Structure and Gene Flow

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Inheritance: From Quantitative Genetics to Evolutionary Stable Strategies

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Glossary

Adaptive landscape The association between a phenotype and fitness; the phenotype can be univariate or multivariate.
Additive genetic variation Variation in the phenotype that is attributed to the additive effect of genes, usually estimated across parents.
Evolutionarily stable strategy The strategy with the greatest fitness that is expected to increase in frequency until it dominates the population.
Inheritance function A function in an 'integral projection model' that describes the association between a parental character measured at time t and an offspring character measured at time $t+1$ when it recruits to the population.
Integral projection model A structured population model where individuals are classified by at least one continuous character.
Phenotypic variance Variation in the phenotype, usually estimated across parents.
Structured model A class of population model where individuals are classified by discrete or continuous characters.
Trade-off function An association between two characters and fitness where fitness is maximized at some midpoint of the function.

Population Level Means and Variation Around Them

Many descriptors of life history, including life expectancy, age at first reproduction and generation length, describe averages across individuals within a population. For example, generation length can be defined as the average age of mothers when they give birth (Caswell, 2001). However, there can be considerable variation among these quantities both between individuals within a population, and between populations. For example, within populations of iteroparous species, different individuals can reach sexual maturity at different ages, can reproduce at different ages, and can have reproductive lives of different lengths (Clutton-Brock, 1988). This means that the average age at which females give birth can vary substantially among individuals.

How important is this within population variation? Ecologists frequently ignore much of it, as do comparative life history theorists who focus on variation among species. But for those researchers who are interested in within population evolutionary change, this variation is crucial (Stearns, 1992). Evolution requires such variation, and requires differences between individuals to be heritable. Without differences between individuals, populations cannot evolve, and species cannot adapt to changing environments. So how important is understanding this variation for life history research? And how do biologists study it? To a large extent the answer to this question depends upon the question the researcher is asking. But in addition, assumptions about whether, and how, this variation is transmitted between generations are also important.

In this article, the author considers individual variation in life history and how it is inherited. He discusses how it is treated in analyses and models, and some of the insights that the work has yielded.

Individual Variation

Individual variation is ubiquitous. Individuals differ in their rates of development, their propensity to survive and reproduce, and the way they respond to different environments. Some of this variation can be attributed to measurable differences between individuals. For example, Jones *et al.* (2008) describe how age influences both survival and reproduction in mammals, birds, and plants. But traits like personality, ability to fight disease, genotype, and body size can independently influence the propensity of an individual to survive and reproduce. Indeed, there are potentially so many sources of individual-level life history variation that even when measurable characteristics have been corrected for, there is still considerable unexplained variation that varies between individuals (Tuljapurkar *et al.*, 2009). Much of evolutionary ecology has focused on characterizing this variation, and attributing it to measurable characteristics of individuals.

It is preferable to identify sources of individual variation but sometimes this is not possible. For example, many characters cannot be easily measured on individuals living in the wild. Life history researchers have recently championed powerful statistical methodologies, including linear mixed effect models, to partition variation unexplained by measured fixed effects in regression models into that attributable to repeated measures on individuals (Cam *et al.*, 2002). This has revealed that considerable differences between individual variations can exist even when measured characteristics are corrected for. However, the role that this variation plays on life history varies as a function of how it is inherited.

The inclusion of traits like age and body size into models of life history dynamics influences quantities like generation length, the variation around generation length, and life expectancy (Coulson *et al.*, 2011). For example, Plard *et al.* (2015) have shown that the way age is incorporated into integral projection models of roe deer influences macroscopic life history parameters like generation length, but impacts on the population growth rate is substantially less. Vindenes demonstrated that non-normal distributions of unmeasured individual differences can also generate impacts on population dynamics (Vindenes and Langangen, 2015). In contrast, Ellner and Rees (2006) and Coulson (2012) concluded that

normally distributed unmeasured individual differences were unlikely to generate substantial effects on summary statistics of life history and population growth.

In order to understand why different studies may have reached different conclusions it is informative to understand how population structure can impact life history (and population dynamics). Life history, and the dynamics of populations, is determined by survival, recruitment, and dispersal. If the distribution of a character, like body size, fluctuates over time within a population, then the life history and population dynamics will also change depending upon the way population fluctuations influence population structure (Coulson *et al.*, 2001). Those studies that capture fluctuations in the structure of fitness-associated traits will likely conclude that individual differences matter, while those studies that do not capture such fluctuations will likely reach the opposite conclusion. In reality, population structure does fluctuate, and it should be incorporated in models – for both analyses of transient dynamics, as well as for those of longer-term dynamics.

The models of Plard, Coulson, Rees, and Vinderes assume a particular type of inheritance, but there are other ways that inheritance is dealt within models. In the remainder of this article we consider different ways in which inheritance is treated.

Finessing Inheritance in Life History Research

Genetic inheritance is necessary for evolutionary change. It describes how genes are passed from one generation to the next. It might consequently initially seem surprising, then, that genetic inheritance itself is rarely included in life history research. Instead, assumptions are made about what is inherited, and what different patterns of inheritance mean. The reason life history theorists finesse inheritance is that our understanding of the genetic architecture of life histories is usually so rudimentary we have very little idea about the role that genotypes at specific loci play (Falconer, 1960). In this section, the author briefly considers the theoretical underpinnings that have led to life history theorists treating inheritance in the way they do. The theory has arisen from the way that inheritance is treated in population models.

Often the focus of life history research is on life history traits – for example, age-specific body size, or age at first reproduction (Stearns, 1992). Stage structured and age–stage structured models describe the life history of the group of individuals over which they are parameterized – usually the entire population (Caswell, 2001). The models dynamically iterate forwards the distribution of a character from one time step (usually a month, or a year) to the next. In order for the approach to work, it is necessary that phenotypic characters in adults are mapped to phenotypic characters in offspring (Easterling *et al.*, 2000; Coulson, 2012). This map is referred to as the inheritance function. This function requires that traits are measured at different ages in the adult and the offspring; models consequently capture the birth process. In many animal studies, individuals with relatively large adult trait values often produce offspring with relatively large offspring traits, and the inheritance function has a positive slope (Coulson *et al.*, 2010). Models such as these are termed integral projection models (Easterling *et al.*, 2000), and they are agnostic to the inheritance mechanism that the inheritance function captures, and whether it is genetic, environmental, or both. These population level models are usually analyzed at equilibrium, which means that parameter values in the model remain constant with time. A consequence of this is that the mean of the phenotype shows no temporal trend as no evolution occurs (Coulson *et al.*, 2010). Nonetheless, the models can be used to analyze evolutionary change through the use of sensitivity analysis and by asking what happens to the life history if parameters in the inheritance function, or in any other parts of the model, evolve?

As well as using models to study evolution over fixed time steps like the integral projection models described above, population level models are also used to study evolution on a per generation time step. The most widely used such model is the breeders equation (Lande, 1979). Instead of iterating a distribution of a phenotypic character forward one fixed step, it iterates the mean of the character value forward by one generation. The model is static, not dynamic. Because the model assumes a per generation time step, the component of the model that captures inheritance provides a map not from parents to offspring, but from the parental generation to the offspring generation – i.e., from age a in the parental generation to age a in the offspring generation (Falconer, 1960). Models do not capture the birth process. A positive association between parental and offspring character on this per generational scale is interpreted as meaning the trait is (at least partly) determined by genes. The trait iterated forwards can be measured at any point in the life course – for example, at birth, as a juvenile, or as an adult – but it must be measured at the same age in each generation. These models assume blending inheritance. These models can only be used to predict evolution across a single generation; they cannot be used to predict multigenerational evolutionary change. The reason for this, as we discuss below, is that they are population level models, with parameters describing what happens across all individuals within the population. If evolution occurs, then parameters in the model would change. This is why many models can only be used to predict forwards one generation at a time. If model parameters were held constant over multiple generations, the assumption would be that no evolution occurs, as by definition evolution will lead to changes in model parameters. To explicitly study evolution in models it is necessary to have different classes of individuals within a population, with the life history of each class determined by its model's parameter values. But the first framework we will consider that uses this approach assumes clonal life histories.

The above population level approaches assume that the trait itself is inherited. The third approach, evolutionary game theory, assumes something different. It assumes that life-history strategies are inherited (Childs *et al.*, 2003). One way to view this is that a structured model, like the population models described above, describes the dynamics of a single strategy. A mutant strategy – a new model with a slightly different set of parameter values – is released to compete with a resident strategy. If the mutant strategy increases in frequency it is predicted to displace the resident strategy. This approach is known as evolutionary game theory or adaptive dynamics. It

assumes that strategies do not mix – they are pure breeding clones. There is consequently no blending inheritance: strategies are passed with very high fidelity. Over time, the frequency of different strategies changes. In most cases one strategy dominates, but under some circumstances competing strategies can coexist.

The astute reader will be thinking why are these different strategies not genetically determined, and strategies inherited via Mendelian inheritance? This is a good question, and there has been considerable theoretical work where different genotypes at a single locus have different structured models, and consequently different life histories and fitnesses (Charlesworth, 1994; Coulson *et al.*, 2011). These models reveal the processes that erode, and maintain, genetic variation – for example, spatial variation in selection pressures, frequency, or density-dependent selection and selection-mutation balance. However, application of such models to real systems is rare as the number of examples where fitness related traits are under simple genetic control is limited. And for this reason, for most species, and for most life history traits, assumptions about patterns of inheritance are consequently required. This means that when the genetic architecture is not known, evolutionary ecologists and life history theorists have to make a decision on how to treat inheritance, and they usually turn to the structured model (Coulson *et al.*, 2010), quantitative genetic (Falconer, 1960) or game theoretic (Childs *et al.*, 2003) approaches described above.

But when should the approach be used, and what can each tell us?

Structured Models

The strength of the structured modeling, or integral projection modeling, approach is that models are dynamic, and very flexible. They can include multiple traits, environmental stochasticity, demographic stochasticity, frequency dependence, and density-dependence (Childs *et al.*, 2004; Rees and Ellner, 2009; Coulson *et al.*, 2011). They can also be used to simultaneously study how population dynamics, life history, character distributions, and selection change as model parameters are altered. This allows the theoretical study of ecological and evolutionary dynamics. However, the approach can also be applied to real systems. For example, if model parameter values change from one period to the next, as a result of environmental change or evolution, integral projection models can be used to give a broad description of how multiple aspects of the system are expected to change (Ozgul *et al.*, 2010). The weakness of this class of model is that most are not evolutionarily explicit; evolution can only be examined in a phenomenological manner through sensitivity analysis, and these methods (usually) make the assumption that model parameters will change independently of one another. Models have been extended to include simple genetic architectures (see Section Simple Genetic Inheritance) but this has only been done once.

Application of these models has shown that population dynamics, life history, the strength of selection, and character distributions are expected to simultaneously respond to environmental change. Ecology, evolution, and life history should be studied together, not in isolation (Smallegange and Coulson, 2013). Models will only provide accurate predictions if (1) they are appropriately specified and (2) it can be assumed that parameter values will not evolve over the time period over which projections are made. As evidence of rapid evolutionary change in the wild is scarce (see Section Quantitative Genetics) it seems that this assumption is probably appropriately for the few generations over which population, or life history, projections are required.

Quantitative Genetics

The great strength of the quantitative genetic approach is that it has proven extremely successful in artificial settings. The approach was devised to predict the evolutionary consequences of artificial selection on one or a few traits across a single generation. In such circumstances the environment is usually benign and constant, artificial selection is strong, and is very precisely targeted at one or a few traits. This means that most individuals survive (unless they are deliberately culled) while only those with desirable trait values do all the reproduction.

The great limitation of the quantitative genetic approach is that predictions should only be made for a single generation (Lande, 1979), and that when applied to free-living populations predictions rarely match observation (Merila *et al.*, 2001). The reason for this is natural selection on one, or a few, characters is typically weaker than artificial selection (Kingsolver *et al.*, 2001), that the environment is often far from benign, and that selection is not so specifically targeted, but instead operates on the entire life history (Childs *et al.*, 2011). In spite of this, application of the approach to free-living populations has provided useful insight. In order to understand these insights, it is useful to have a brief background on implementation of the quantitative genetic approach to the field.

The approach assumes the phenotype is, in part, determined by a large number of loci of small, additive effect. Each locus contributes to the phenotype independently of the other. Most research has focused on estimating how much variation between individuals in phenotypic characters can be attributed to these numerous, small effects. This means that quantitative genetics, when applied to field systems, is primarily concerned with decomposing phenotypic variation across individuals within the population into a component due to additive genetic contributions and other non-additive genetic components. This is done by comparing (statistically corrected) phenotypic traits among individuals of known relatedness, using either midpoint parent–offspring regression or the animal model (Kruuk, 2004). The heritability describes the proportion of phenotypic variation that is attributable to the additive genetic differences between individuals. A heritability of zero means that all variation in the trait among individuals is due to environmental variation. In contrast, a heritability of unity means all variation is attributable to additive genetic differences.

What has the application of this approach shown? Most life history traits in free-living populations appear to have some additive genetic variation. Most life history traits in the field

have a heritability of 0.1 to 0.2 (Kruuk, 2004). Values substantially higher than this are outliers, potentially suggesting there have been issues in implementing the approach.

The heritability frequently declines, the more strongly a trait is correlated with lifetime reproductive success (Kruuk *et al.*, 2000) as predicted by Fisher (Fisher, 1930). In other words, in traits that are strongly correlated with lifetime reproductive success, most of the variation among individuals cannot be attributable to the additive effect of genes. However, even these traits have some genetic variation – selection has not eroded all of it, as standard theory would predict.

This research, and others like this, suggests that life history traits are subject to directional selection, and that there is sufficient additive genetic variation for evolution to occur. However, convincing evidence of rapid microevolution from the application of the quantitative genetics approach is scarce (Merila *et al.*, 2001), and much of quantitative genetics in the wild is concerned with explaining why we fail to see microevolutionary change.

Clonality and Evolutionarily Stable Strategy

The great strength of the evolutionarily stable strategy (ESS) approach is the expected end point of evolution can be identified. The ESS is the peak of an adaptive landscape and populations are predicted to evolve toward this point (Maynard-Smith, 1982). Whether they can achieve the ESS will depend upon whether genetic constraints allow it. The weakness of the approach is that it is often hard to identify trade-offs within populations (Cam *et al.*, 2002). In addition, the approach cannot be expected to accurately capture transient dynamics, which are likely determined by the details of inheritance mechanisms. This weakness has not stopped people using the approach to study transients, but results on short-term life history evolution should be treated with caution.

As previously mentioned, evolutionary game theory competes strategies against one another. In order to apply the approach, a trade-off between two components of the life history needs to be identified, and strategies at points along this trade-off function are competed against one another. Most applications focus on the trade-off between offspring number and offspring size (Childs *et al.*, 2003, 2004, 2011), although other trade-offs can be used. The fittest strategy is the one that cannot be invaded by any other.

Application of this approach, primarily to monocarpic perennial plants, suggests that most life histories appear to be close to the ESS (Metcalf *et al.*, 2008). This would suggest we would expect to see little evidence of evolutionary change. However, ESSs are environment dependent, with different ESSs in different environments. In those cases where environmental variation is included, populations appear to be close to the ESS for the average environment (Childs *et al.*, 2004).

One very nice insight from the ESS approach is that selection on the life history is very different to selection on specific traits (Childs *et al.*, 2011). In other words, selection on a trait like body size is very different to selection on the entire life history. In artificial settings it is sensible to think of selection operating on individuals traits, as these are what selected. However, in natural settings, it is more sensible to think about selection operating on the entire life history, as natural selection cannot be as targeted as artificial selection. Studies of life history evolution in the wild should ideally focus on selection estimated over the entire life history, and to do that it is necessary to work with the structured models that underpin the ESS approach, and the integral projection approach described above.

Simple Genetic Inheritance

Instead of assuming a particular map between genotype and phenotype it would be preferable to work with cases where the genetic architecture underlying a phenotypic character is known. Unfortunately, however, this is rarely possible because traits that influence life history that biologists are interested in are usually under the control of multiple loci. Two cases where they are not are sickle cell anemia in humans and coat color in gray wolves – both traits that are determined by genotype at a single genetic locus. In both cases inheritance is Mendelian. The great strength of this approach is the life history of each of the three competing genotypes can be calculated, and their fitnesses assessed. The limitation of this approach is few traits are under simple genetic control.

Within North America there is a cline in wolf coat color. To the North, black wolves are rare and often absent. As one moves further Southwest, the frequency of black wolves increases. Wolf coat color is determined by genotype at a single locus – CBD103. The locus is a β-defensin locus with two alleles. The heterozygote is black. Initial hypotheses proposed that the black coat was found at a higher frequency to the Southwest because the Southwest is more forested. Black wolves were hypothesized to have a fitness advantage through improved hunting success in shade (Anderson *et al.*, 2009). There are two problems with this hypothesis. First, wolves are not ambush hunters – they do not rely on cover, or camouflage, when hunting prey. Instead, they are cursorial hunters in that they run their prey down. Camouflage is not important for wolves. Second, the black homozygote and the black heterozygote have the same coat color, but they have very different fitness. Black homozygotes never fare well, while black heterozygotes have higher fitness than either of the homozygotes – in Yellowstone National Park at least (Coulson *et al.*, 2011). Recent genetic work by Robert Wayne and colleagues hints that genotype CBD103 may play a role in immunity. Perhaps the coat color cline found in North America, and possibly aspects of wolf life history, are determined by the frequency of disease outbreaks rather than by the amount of shade?

Sadly until we have a better understanding of the map between genotype and phenotype, being able to measure the life history and fitness of genotypes with known phenotypic consequence will be limited to a few special cases like the North American wolves.

The Challenge of Inheritance

Adaptive evolution requires variation among individuals, selection, and genetic inheritance. There is considerable variation among individuals in terms of their life history strategies

within populations. Occasionally some of this variation is under simple genetic control, and when that is the case, studying life history evolution within a population is (relatively) straightforward. Unfortunately, this is rare and in cases where the genetic architecture is not known, assumptions about the mechanisms of inheritance are required. The additively assumption of quantitative genetics suggests many traits are under partial genetic control, and that additive genetic variation for the traits is frequently present. In spite of this important insight, little can be done with it, as mutigenerational predictions of evolutionary change are not possible with current theory.

The second widely used assumption is of clonality. In sexually reproducing species this assumption is violated. This doesn't preclude the predictions of where evolution is likely to take the population in the long-term, but it does limit the utility of predictions of short-term change.

Until we gain a better understanding of the (likely complex) genetic architecture of life histories and life history traits we will need to continue to make assumptions about the modus operandi of inheritance. The clonal assumptions of game theory, and the blending inheritance of quantitative genetics are very sensible assumptions, but both have their limitations, as does use of the inheritance function. A useful focus while we await better characterization of genotype–phenotype maps would be to explore the consequence of assumptions for both long-term and short-term evolution. Currently, researchers often seem to just champion one approach while being dismissive of the others. This is a shame. All approaches have merit, with perhaps fruitful gains being possible from attempting to integrate the approaches together. Inheritance is certainly key to understanding life history evolution in the field, but it is frustrating, as it is so hard to mechanistically characterize.

See also: Life History Evolution, Plants. Multivariate Quantitative Genetics. Quantitative Genetic Variation

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Insects and Ecdysozoa, Diversification of

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Insecta: Animal's Major Radiation

Comprising around one million described species, insects are by far the most species-rich animal group. Nevertheless, estimates of the actual number of insect species considering the high proportion of undescribed ones, range from 5 to 30 million species (Erwin, 1982; Gaston, 1991). The known diversity of the Class Insecta is classified into 31 recent orders (Grimaldi and Engel, 2005), including some notorious terrestrial invertebrates, such as beetles (Coleoptera), bees, wasps, and ants (Hymenoptera), butterflies and moths (Lepidoptera), and cockroaches (Blattodea). Not only are insect orders species-rich, most are also highly abundant, consequently causing a great impact in their natural systems. Few continental niches have not been occupied by some insect group, which are distributed on every continent, including Antarctica. Insects are intrinsically responsible for nutrient recycling in terrestrial and aquatic habitats and plant reproduction and dispersal, besides from interacting with other biota as population regulators or food resources and sometimes being considered as keystone species of ecosystems.

The vast majority of insects and their related entognathan classes (Collembola, Diplura, and Protura) comprising the subphylum Hexapoda (**Figure 1(a)**), dwell in terrestrial habitats. Extinct marine hexapod lineages were still present until 390 million years ago during the early Devonian (Haas *et al.*, 2003). However, at that point hexapods had already invaded continental habitats where they diversified and flourished, based on the earliest fossil of an extant hexapod order, a typical marshy-habitat collembolan, from 400 to 410 million years ago from the early Devonian of Scotland (Pragian) (Hirst and Maulik, 1926). Molecular dating methods suggest a much earlier diversification of the crown group of continental Hexapoda than diversification dates based on fossil data, estimating it to be between 510 (Cambrian) and 450 (Ordovician) million years ago (Misof *et al.*, 2014). Despite this extraordinary terrestrial radiation, approximately 3% of the known insect diversity inhabit, at least as immatures, aquatic habitats. Of these, approximately several hundred species are marine or intertidal, but individuals of these species are never submerged throughout their whole life (Cheng, 1976a). Proposed reasons why insects did not successfully invade the marine environment include respiratory and osmoregulatory limitations and competition with the already diverse and abundant marine arthropods, the crustaceans (Cheng, 1976b).

Several adaptive features have been named as contributing to insects' remarkable diversity. A chitin-based exoskeleton certainly provided an evolutionary advantage in the provision of protection of internal organs, large area for muscle attachment and development, and evaporation control, the latter being extremely important when taking into account radiation of the Hexapoda in terrestrial environments. Even though many animal lineages share this exoskeleton, a unique arthropod characteristic, articulated limbs, made possible an extreme adaptability of these limbs performing highly dissimilar functions in different insect groups (Ross *et al.*, 1991). For example, the three pairs of head appendages, usually referred to as mouthparts, are strongly modified in different insect groups allowing feeding of the most varied sources, such as the plesiomorphic chewing mouthparts for grinding solid animal or plant matter or piercing–sucking mouthparts, which evolved many times independently in different insect groups, to suck internal fluids of other animals or plants.

Considering that half of the number of insect species are herbivores, the phytophagous habit is also hypothesized to be an important cause of diversification (Mitter *et al.*, 1988; Wiens *et al.*, 2015). Plants, however, are not an ideal food source, because of their low nitrogen composition and unbalanced profile with low concentration of many of the animal's essential amino acids, and because plants have evolved many defense mechanisms against herbivores, such as chemical and mechanical protection. Nevertheless, energy resources for herbivores are greater than those available to higher trophic levels, plants did provide diverse new host niches with different ecologies or chemistry driving insect diversification, and as a result interspecific competition is probably low (Southwood, 1973; Mitter *et al.*, 1988).

Unique to the insects, but not present in all of them, there are two other characteristics, which definitely helped shape the current diversity of the Hexapoda. Insects are the sole invertebrate group able to fly and the first organisms to do so, between 90 and 170 million years before the first flying vertebrates (Engel and Grimaldi, 2004). Their two pairs of thoracic wings enhance their dispersal ability, being advantageous for fleeing threats, finding new resource opportunities, mates, or oviposition sites; besides acting in protection, courtship, and thermoregulation. Two main theories address their origin: either insect wings have originated from lateral expansions of the thoracic dorsal sclerites (Quartau, 1986) or from basal processes (exites and endites) of the thoracic legs (Kukalová-Peck, 1983; Trueman, 1990).

Lastly, the great majority of insect species, comprising the Holometabola, share a synapomorphic complete metamorphosis in their post-embryonic development, which is considered a key innovation in the diversification of insects (Rainford *et al.*, 2014). These insects, after hatching from eggs, have characteristic immature wingless larval stages, which are responsible for most of the feeding and resulting growth of the insect. Larvae usually utilize a completely different food resource, for example, caterpillars are leaf chewers, while butterflies are nectarivorous, and live in a different microhabitat than adults. Adults, when they do, have to feed only to maintain its metabolism and sometimes maturation of sperm and eggs, and adapt to particular roles of dispersal and reproduction. Morphologically and anatomically, larvae and adults are completely different and the transition between the two is through the pupal stage, another immature and mostly

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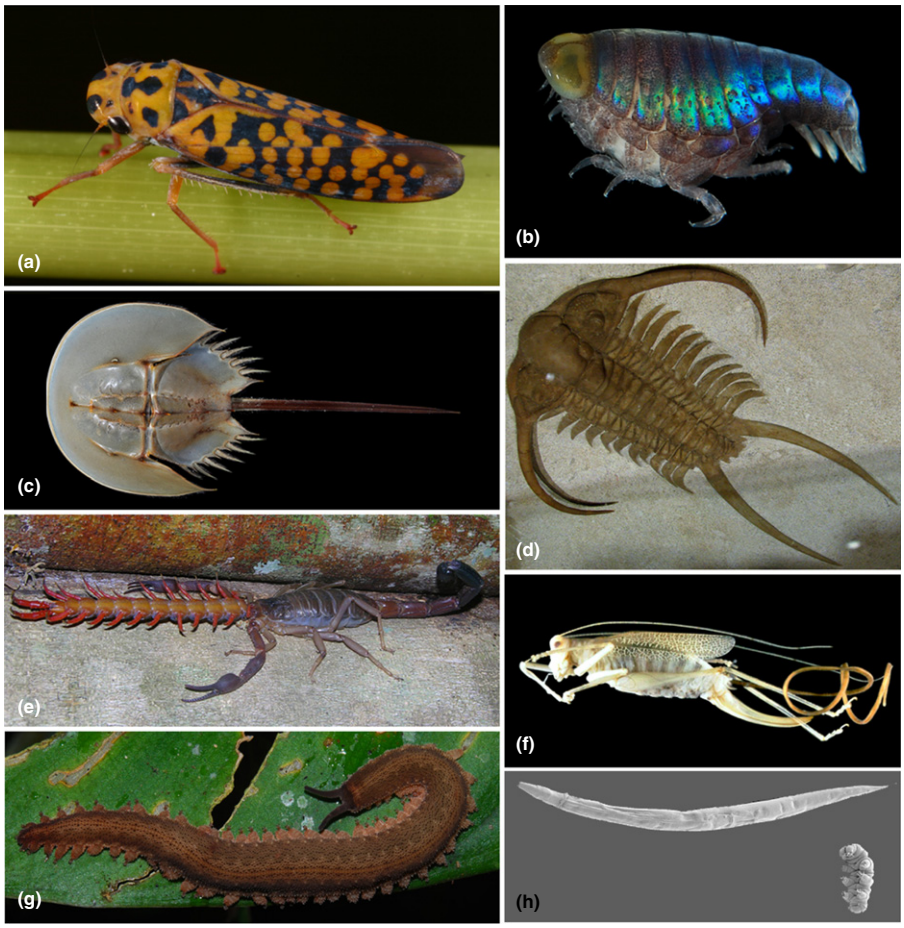

Figure 1 Morphological diversity of major groups of ecdysozoans. (a) Sharpshooter leafhopper *Pawiloma victima* (Hexapoda), by Christopher H. Dietrich. (b) Iridescent hyperiid amphipod crustacean (*Euthamneus* sp.), by Arthur Anker. (c) Coastal horseshoe crab *Tachypleus gigas* (Cheliceriformes), by Arthur Anker. (d) *Paraceraurus* trilobite (Trilobitomorpha) from the Middle Ordovician, by Vassil. (e) *Grosphus* scorpion (Cheliceriformes) feeding on large *Scolopendra* centipede (Myriapoda), by Arthur Anker. (f) *Spinochordodes tellinii* (Nematomorpha) leaving its dead host *Meconema thalassinum* (Hexapoda: Orthoptera), by Andreas Schmidt-Rhaesa. (g) *Peripatus* velvetworm (Onychophora), by Arthur Anker. (h) Scanning electron micrograph of water bear *Hypsibius dujardini* (Tardigrada) and *Caenorhabditis elegans* (Nematoda), by Bob Goldstein and Victoria Madden.

quiescent stage responsible for an extreme internal organs rearrangement and development. Nevertheless, this niche segregation between larvae and adults helped eliminate their competition for resources and open up a wide variety of niches for holometabolans to explore and diversify into 65% of all animals species. Stem lineages of many of the holometabolous orders are estimated to be present in the Late Carboniferous (~310 million years ago), but it was not until the Early Cretaceous (~120 million years ago) that the true diversification of these megadiverse orders occurred, contemporary with the radiation of flowering plants (Misof *et al.*, 2014).

Arthropod Evolutionary Relationships

Arthropods are familiar animals and encompass, besides insects, crustaceans, millipedes, centipedes, spiders, scorpions, mites, ticks, and others. The Phylum Arthropoda is divided into the extinct Trilobitomorpha (**Figure 1(d)**) and four extant subphyla: Cheliceriformes (**Figures 1(c)** and **1(e)**), Myriapoda (**Figure 1(e)**), Crustacea (**Figure 1(b)**), and Hexapoda (**Figures 1(a)** and **1(f)**). Arthropods are represented amongst the oldest fossil invertebrates dating back to the Cambrian explosion (~540 million years ago), and more questionably suggested to be present in the late Precambrian faunas, approximately 60 million years before (Waggoner, 1996). Representatives of crown groups of Crustacea (Anostraca) and Cheliceriformes (Pycnogonida) were already present in the Late Cambrian, between 500 and 490 million years ago (Edgecombe and Legg, 2013). Trilobites also originated in the Cambrian and became one of the most abundant arthropod groups of the Paleozoic seas, but going extinct by the most extreme mass extinction event, the end-Permian (Erwin, 1994). Considerably before the first hexapods and any other animal group, by the mid-Silurian (428 million years ago), scorpions (Cheliceriformes) and millipedes (Myriapoda) had already invaded the terrestrial habitat (Dunlop *et al.*, 2013).

Monophyly of Arthropoda is almost universally accepted, strongly supported by morphological, molecular, and developmental evidence (Edgecombe, 2010). On the other hand,

relationships among major arthropod lineages are still controversial. In the last two decades, addition of new data, especially molecular, has challenged traditional views about phylogenetic relationships among arthropod lineages, and even widely accepted clades were questioned.

Considering the extant arthropod subphyla, most published phylogenies support a traditional clade including Myriapoda, Crustacea, and Hexapoda – the Mandibulata (e.g., Giribet *et al.*, 2001; Regier *et al.*, 2010; Rota-Stabelli *et al.*, 2011). However, the placement of Myriapoda is still controversial, being sometimes recovered as a sister to Cheliceriformes, in a clade named Paradoxopoda or Myriochelata, based on rDNA data (Pisani *et al.*, 2004; Mallatt *et al.*, 2004) and also in more recent studies with large datasets from complete mitochondrial genomes or transcriptomes. Paradoxopoda, however, is seen in many of these more widely sampled studies as an artifact of outgroup choice or saturation of fast evolving sites (Rota-Stabelli and Telford, 2008; Rota-Stabelli *et al.*, 2010; Andrew, 2011).

Within mandibulates, traditionally, hexapods and myriapods were seen as sister taxa, forming a group named Atelocerata (in reference to the loss of the second pair of antennae) or Tracheata (in reference to their tracheal respiratory system), a hypothesis almost undoubted until the end of twentieth century (e.g., Snodgrass, 1938; Hennig, 1981; Bitsch and Bitsch, 2004). However, recent studies based on molecular data and reinterpreted anatomical information, consistently recover Hexapoda nested within Crustacea, a clade named Pancrustacea or Tetraconata (in reference to ommatidia tetrapartite crystalline cones) (e.g., Shultz and Regier, 2000; Cook *et al.*, 2001; Regier *et al.*, 2010; Rota-Stabelli *et al.*, 2011). Thus, hexapods can be viewed as the 'crustacean' lineage, which successfully invaded and diversified on the terrestrial habitat. Consequently, synapomorphic traits of Atelocerata, such as the tracheal system and Malpighian tubules (specialized excretory organs), are more likely adaptive convergences to life on land by hexapods and myriapods. Unfortunately, few studies included a wide taxonomic sampling of Crustacea to address which is the extant sister group of Hexapoda, and results are inconsistent, but often involve Branchiopoda, Remipedia, and Cephalocarida (Regier *et al.*, 2005, 2010; Oakley *et al.*, 2013).

Panarthropoda

Two small animal phyla, Onychophora (velvet worms, **Figure 1(g)**) and Tardigrada (water bears, **Figure 1(h)**), are considered to be closely related to arthropods – the three phyla together being known as Panarthropoda (sometimes also referred as Aiolopoda, see Ortega-Hernández, 2014). Monophyly of Panarthropoda is supported by several morphological synapomorphies (Nielsen, 2012), but of particular importance are the paired segmental ventrolateral claw-bearing appendages operated by intrinsic and extrinsic muscles. These appendages are also found in several extinct lineages of panarthropods, including the lobopodians (Edgecombe, 2010). Lobopodians also originated in the Cambrian explosion and are known from various Paleozoic (541–252 million years old) deposits around the world, thus a major component of early metazoan-dominated ecosystems (Ortega-Hernández, 2015). They were initially thought to be marine ancestors of onychophorans, but are now regarded as a polyphyletic assemblage of stem-groups of Arthropoda, Onychophora, and Arthropoda + Onychophora (Legg *et al.*, 2013; Yang *et al.*, 2015). Neither Onychophora nor Tardigrada have Cambrian fossil representatives.

All possibilities of relationships among these three groups have been proposed and received some support, even recently. However, a closer relationship between Onychophora and Arthropoda is most commonly accepted based on molecular analyses (e.g., Dunn *et al.*, 2008; Rota-Stabelli *et al.*, 2013) and several morphological synapomorphies, including segmental musculature, nephridial derivatives that arise from the walls of coelomic cavities, addition of segments from a posterior growth zone, and arthropod-type hemocyanin (Edgecombe, 2010; Nielsen, 2012). Placement of Tardigrada remains uncertain, since recent studies with broad sampling has recovered it as closely related to the nematode clade (Philippe *et al.*, 2005; Nesnidal *et al.*, 2010).

Ecdysozoans: The Molting Animals

At the same time that molecular data has overturned the phylogenetic understanding among main lineages within Arthropoda, leading to the proposal of the so-called Pancrustracea hypothesis, the position of Panarthropoda in the animal tree of life has also changed dramatically. Until the first papers addressing metazoan relationships based on molecular data, Panarthropoda and Annelida (segmented worms, such as polychaetes, earthworms, and leeches) constituted a robust taxon, named Articulata established in beginning of the nineteenth century (Cuvier, 1817). Morphological features supporting Articulata are mainly related to body segmentation (or metamerism) and segmental substructure (Scholtz, 2002). Rejecting this hypothesis means assuming independent origins of metamerism in panarthropods and annelids or a more ancient origin of this body organization pattern in animal evolution, followed by independent losses in several animal groups. However, despite the strong morphological support, since the first single gene phylogenetic analyses based on 18S rDNA sequences (Giribet *et al.*, 1996; Aguinaldo *et al.*, 1997) to the current phylogenomic era (Nesnidal *et al.*, 2010; Giribet, 2015), Articulata has no support from molecular data. Panarthropoda is commonly recovered within a clade of molting animals, the so-called Ecdysozoa (Philippe and Telford, 2006; Edgecombe *et al.* 2011). Even before the first molecular study proposing Ecdysozoa (Aguinaldo *et al.*, 1997), some morphological studies have questioned the close relationship between arthropods and annelids, recovering annelids most closely related to mollusks instead (Eernisse *et al.*, 1992). Recent alternative hypothesis to Ecdysozoa and Articulata have placed arthropods closer to chordates than other molting animals (specifically Nematoda) based on genomic data calling for a 'Coelomata' group (Blair *et al.*, 2002, Telford, 2004, Wolf *et al.*, 2004; Longhorn *et al.*, 2007). There is little morphological support for this group and molecular support is apparently an artifact of poor and distantly related taxon sampling, while better sampling usually recovers a robust Ecdysozoa.

Besides the three panarthropod phyla, Ecdysozoa includes the five Cycloneuralia phyla: Nematoda (**Figure 1(h)**), Nematomorpha (**Figure 1(f)**), Kinorhyncha, Priapulida, and

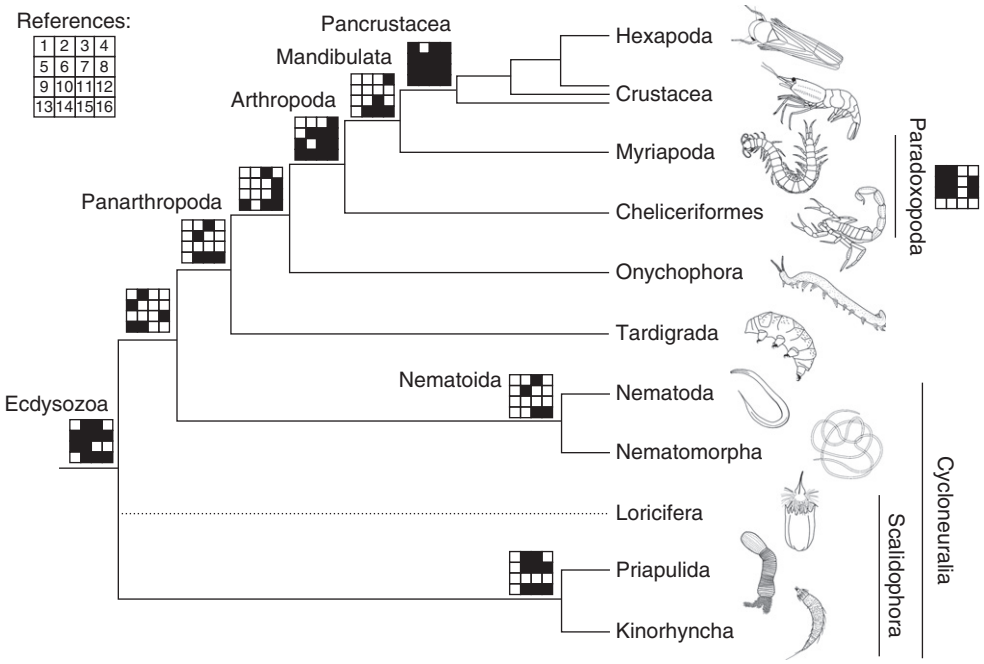

Figure 2 Summary phylogeny of major groups of ecdysozoans based on molecular analyses. Support for respective group is given by black boxes according to the following studies: (1) Friedrich and Tautz (1995), (2) Aguinaldo *et al.* (1997), (3) Garey (2001), (4) Giribet *et al.* (2001), (5) Mallatt *et al.* (2004), (6) Mallatt and Giribet (2006), (7) Park *et al.* (2006), (8) Dunn *et al.* (2008), (9) Sørensen *et al.* (2008), (10) Hejnol *et al.* (2009), (11) Regier *et al.* (2010), (12) Meusemann *et al.* (2010), (13) Andrew (2011), (14) Rota-Stabelli *et al.* (2011), (15) Campbell *et al.* (2011), (16) Rota-Stabelli *et al.* (2013).

Loricifera (see **Figure 2** for a summary of current phylogenetic hypotheses). All cycloneuralians together comprise less than 600 obscure species of marine benthos or arthropod parasites, except of the diverse Nematoda with approximately 30 000 described species. Nematodes are one the most abundant animal groups, occurring in almost all habitats, both aquatic and terrestrial, and can be free-living or parasites of animals and plants, those having great economic importance (Brusca and Brusca, 2003; Nielsen, 2012). Close phylogenetic relationships among cycloneuralians have long been hypothesized based on the shared unique belt-like brain (without paired ganglia) (Nielsen *et al.*, 1996; Brusca and Brusca, 2003), previously thought to be homologous to the one of the non-molting phylum Gastrotricha (Nielsen *et al.*, 1996). Nevertheless, recent molecular analyses do not recover Cycloneuralia monophyly, mostly because of the close relationship between Nematoida (Nematomorpha + Nematoda) and Panarthropoda (Aguinaldo *et al.*, 1997; Mallatt *et al.*, 2004; Rota-Stabelli *et al.*, 2011) and the few data to support the position of Loricifera (Park *et al.*, 2006; Sørensen *et al.*, 2008).

The most remarkable feature uniting all Ecdysozoans is cuticle molting or ecdysis regulated by ecdysteroid hormones, but other morphological apomorphies, such as loss of ciliated epithelia and secretion of epicuticle by the tips of epidermal microvilli (which are both related to the molting process) and loss of primary larvae (Aguinaldo *et al.*, 1997; Schmidt-Rhaesa *et al.*, 1998; Nielsen, 2012) were also proposed. Besides morphological features, an additional apomorphy of Ecdysozoa is the immunoreactivity to horseradish peroxidase by the nervous tissue, found in all ecdysozoans, but no other metazoan studied (Haase *et al.*, 2001). Although, some researchers were initially skeptical in accepting Ecdysozoa (Wägele *et al.*, 1999; Nielsen, 2001; Brusca and Brusca, 2003), it is currently widely accepted by zoologists (Telford *et al.*, 2008; Nielsen, 2003, 2012; Dunn *et al.*, 2014) and certainly the greatest monophyletic radiation of all metazoans.

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See also: Biogeography of Arthropods. Lophotrochozoa, Diversification of

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Intraspecific Coevolutionary Arms Races

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Glossary

Copulatory plug A gelatinous male secretion that hardens in the female reproductive track after mating to prevent/delay female remating. Also called a 'mating plug.'
Cryptic female choice Generically a form of sexual selection where the female is able to influence the sire of her offspring *after* mating. This can be manifested by either a change in overall fertilization success from the sperm of a single male, or preferentially using the sperm of one male over that of another male.
Intersexual conflict An antagonistic coevolutionary process between the sexes of the same species, where an increase in the fitness of males reduces fitness of females, and vice versa.
Interspecific coevolution An evolutionary process between two *different species*, where a change in the fitness of species 'A' directly affects the fitness of species 'B', and vice versa.
Intrasexual conflict An antagonistic coevolutionary process within one sex of the same species, where an increase in the fitness of one male (female) reduces fitness of another male (female), and vice versa.
Intraspecific coevolution An evolutionary process between two individuals of same species/population, where a change in the fitness of individual 'A' directly affects the fitness of individual 'B', and vice versa.
Polyandry A mating system where each female in a population typically mates with multiple males.
Polygynandry A mating system consisting of exclusive mating groups of multiple males and females.
Polygyny A mating system where each male in a population typically mates with multiple females.
Promiscuity A mating system where each individual in a population has multiple mating partners and is characterized by a lack of any pair-bonding between mating partners.
Sperm competition A form of sexual selection where the sperm of two or more males are simultaneous residents in a female's reproductive tract and compete for fertilizations.
Traumatic insemination The practice where a male pierces the body of a female with his phallus and injects his sperm directly into her abdomen.

Introduction: Intraspecific Arms Races

Simply stated, coevolution is the process where two populations evolve directly in response to changes in each other. Typically coevolution is thought of as occurring between two different species, where a change in the fitness of species 'A' directly affects the fitness of species 'B'. Similarly, changes in the fitness of species 'B' directly affect the fitness of species 'A.' Given its general form, coevolution can either occur as a positive or negative process depending on the fitness effects that each species has on the other.

In mutualistic coevolution, each species influences the fitness of the other in a positive way. Evolutionary advances in species 'A' increase the fitness of species 'B'; evolutionary advances in species 'B' increase the fitness of species 'A.' Therefore, over evolutionary time, both players can become increasingly dependent on the other, resulting in mutualism. Examples include the coevolution between insectivores and herd animals, plants and pollinators, and eukaryotes and mitochondria. In contrast, antagonistic coevolution occurs when increases in fitness of one species reduces the fitness of the other species, and vice versa. As the fitness of species 'A' increases, the fitness of species 'B' decreases, and as the fitness of species 'B' increases, the fitness of species 'A' decreases. This ongoing antagonistic relationship is often referred to as a 'coevolutionary arms race,' with each species evolving strategies to mitigate the harmful effects of the other, potentially leading to never-ending cycles of adaptation and counter-adaptation. Common examples of these arms races include the evolutionary dynamics between predators and prey, hosts and parasites, and plants and herbivores.

While the above formulations are frequently applied to interactions between separate species (interspecific coevolution), the same evolutionary dynamics can occur between individuals within a species (intraspecific coevolution). When applied to the different sexes within a species, it seems logical that mutualistic coevolution could operate, with male fitness increasing along with increasing female fitness, and vice versa. Although feasible, this mutualistic intersexual coevolution is only associated with strict life-long monogamy between a male/female pair. Under these conditions, the fitness of a male equals the fitness of his mate, so any reduction in female fitness will result in an equal reduction in male fitness.

Strict monogamy is extremely rare, however, and when one or both sexes have multiple mating partners (i.e., polyandrous, polygynous, polygynandrous, and promiscuous mating systems), the evolutionary interests of males and females diverge, creating sexual conflict. This conflict occurs when the optimal outcome of sexual interactions differs for males and females (Rice and Holland, 1997), causing each sex to evolve strategies that maximize their own fitness, even if they are costly to their mates. Just as mutualistic coevolution can operate within a species, so, too, can antagonistic coevolution. Within a species, antagonistic coevolution results from the diverging

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reproductive interests of males and females who are dependent on one another to reproduce. This situation is analogous to many species of parasites that depend on a host to reproduce: just as a parasite can evolve strategies that increase its fitness at a cost to its host, males and females can evolve strategies that are costly to their mates. In both situations, selection should act to minimize the costs experienced by the host/harmed sex, resulting in antagonistic coevolution.

While it may seem counterintuitive that individuals would evolve strategies that harm members of their own species, let alone their own mating partners, it important to remember that different individuals within a species have different evolutionary interests. Selection acts on the individual, not on a species or population, so it is possible for an individual to increase his/her fitness at the expense of others. Intraspecific coevolutionary arms races can involve any individuals that have divergent optimal fitness strategies, and can include conflict between competing males or competing females (i.e., intrasexual conflict), conflict between parents and their offspring, conflicts between siblings, and conflict between the sexes (i.e., intersexual conflict). Here, we focus specifically on coevolutionary arms races between males and females over mating and fertilization, describing the male–female interactions that are likely to lead to these arms races, and including examples from a variety of mating systems.

Coevolutionary Arms Races Over Mating

Intersexual arms races can evolve whenever male and females interact, and sexually antagonistic coevolution commonly involves the optimal mating rate of males and females within a population. This conflict over mating rate is rooted in anisogamy (the production of gametes of different sizes by males and females); while males tend to produce numerous small and relatively inexpensive gametes (sperm), females tend to produce fewer large and relatively expensive gametes (eggs). Male reproductive success (the number of offspring a male can sire) is, thus, primarily limited by his number of successful fertilizations, whereas female reproductive success is limited by the number of her eggs. Bateman (1948) was the first to demonstrate that this imbalance can lead to conflict over mating frequency. In the fruit fly, *Drosophila melanogaster*, male reproductive success increased much more rapidly with each successive mating than did female reproductive success; the optimal mating rate for males is thus substantially higher than the optimal mating rate for females. Under these conditions, a trait that increases male mating success will be favored by selection even if it is harmful to females (Parker, 1979), and females will then be selected to minimize this harm by mating less often.

Conflict over mating frequency occurs whenever the optimal mating rate is different for males and females, which is the case for the majority of species. As we describe above, the optimal mating rate for males is often higher than it is for females, and this conflict leads to a variety of coevolutionary arms races between males and females.

Grasping and Anti-Grasping Structures

In order for fertilization to occur in many species, a male must physically interact with a female (mount) and insert an intromissive organ (i.e., penis/phallus) inside, or near, the female's reproductive tract. This often leads to overt struggles between males and females over whether or not a male's mating attempt will be successful, with females struggling to actively dislodge mounting males (Blyth and Gilburn, 2011; Rowe *et al.*, 1994). In several species of water striders, males have evolved specialized structures that they use to grasp females once they have mounted, thus reducing their likelihood of being dislodged (Rowe *et al.*, 1994). Males of some species have evolved elongated genitalia that serve a clasping function (Arnqvist and Rowe, 2002b), while males in other species have evolved specialized antennae with hooks or spikes that fit into a groove next to the female's eye (Khila *et al.*, 2012). In these species, females have evolved a number of different 'anti-grasping' structures, such as abdominal spines that aid in dislodging males (Arnqvist and Rowe, 2002b). These male and female structures reflect the current state of the arms race: species of water striders in which females have smaller abdominal spines also show elevated mating rates, likely because these females are less able to dislodge mounting males (Arnqvist and Rowe, 2002a).

A similar male grasping structure is seen in the sagebrush cricket, a species in which females mount the males to mate. During mating, females chew on their mate's fleshy hind wing and ingest hemolymph that seeps from the wounds. This sexual cannibalism provides a nutritive benefit to females, but is harmful to males, reducing their future mating success (Johnson *et al.*, 1999; Sakaluk *et al.*, 2004). Females prefer to mate with virgin males that have intact hind wings (Eggert and Sakaluk, 1994), and will mount males to determine the status of their hind wings, dismounting without mating if the hind wings are not intact. In response to this female behavior, males have evolved a specialized abdominal pinching organ referred to as a 'gin trap' that clasps mounted females and prevents them from dismounting until they mate (Sakaluk *et al.*, 1995).

Male Harassment and Female Resistance

Instead of using specialized grasping structures, males of many species use behavioral adaptations to coerce females to mate. Perhaps the most widespread male approach is to persistently court and harass females that are resisting mating attempts (Clutton-Brock and Parker, 1995). Persistent male harassment that is harmful to females has been documented across a diverse range of species, including seed beetles (den Hollander and Gwynne, 2009), guppies (Magurran and Seghers, 1994), mallard ducks (Titman and Lowther, 1975), and sheep (Reale *et al.*, 1996).

In the fruit fly (**Figure 1**), *D. melanogaster*, females exhibit a variety of rejection behaviors toward an undesirable courting male (Spieth, 1952). Males often continue to pursue females in spite of these rejection behaviors, and this persistent male courtship is harmful to females, shortening their life span (Partridge and Fowler, 1990) and reducing female lifetime fecundity (Rice *et al.*, 2006). Moreover, this conflict over mating can intensify when males are able to detect differences in female fitness (i.e., via body size; Byrne and Rice, 2006; Edward and Chapman, 2012; Long *et al.*, 2009) and direct their antagonistic harassment toward higher quality females,

Figure 1 A male fruit fly (*Drosophila melanogaster*). *Drosophila* males exhibit a number of sexually antagonistic traits, including harassment and toxic seminal fluid proteins. Wikimedia Commons http://en.wikipedia.org/wiki/Drosophila_melanogaster#mediaviewer/File:Drosophila_melanogaster_-_side_(aka).jpg

such that these females suffer proportionally more harm than lower quality females (Long *et al.*, 2009). Importantly, females show genetic variation in their ability to resist the harm inflicted by males; this variation accounts for ~17% of the total variation in female fitness (Linder and Rice, 2005) and is largely mediated by how resistant females are to male mating attempts (Lew *et al.*, 2006).

In the red-backed water strider, males use a more specialized behavior to coerce females to mate. In this species, females have evolved a specialized shield over their vulvar opening that acts like a drawbridge; this gives females control over mating frequency, as they must expose their genitalia for copulation to proceed. Mating occurs on water, and when females refuse to expose their genitalia to mounted males, the males tap the surface of the water with their mid-legs (Han and Jablonski, 2009). This male behavior attracts aquatic predators; because females are directly on the water surface, they are more likely to be attacked by these predators (**Figure 2**). This male intimidation is very effective: females expose their genitalia in response to male water tapping, and do so more quickly when the risk of predation is higher (Han and Jablonski, 2010).

Figure 2 Male coercion in red-backed water striders (*Gerris gracilicornis*), where the male attracts potential predators by 'tapping' the surface of the water until the female submits to a mating. Chang Seok Han http://changshan.egloos.com

Figure 3 Traumatic Insemination in the bedbug (*Cimex lectularius*), where the male bypasses the genital tract of the female and instead uses hypodermic genitalia to inject sperm directly into the female's abdomen. Wikimedia Commons http://en.wikipedia.org/wiki/File:Traumatic_insemination_1_edit1.jpg

Traumatic Insemination

Males of some species have evolved a unique way to circumvent female resistance to mating attempts. Instead of attempting to mount and copulate with a female via her genitalia, these males penetrate the female body wall using specialized 'hypodermic' genitalia and inject sperm directly into the female's body cavity. This 'traumatic insemination' has evolved several times in invertebrates, but is most widely studied in bed bugs (**Figure 3**), where it has been shown to increase male fertilization success but decrease female longevity and reproductive success (Stutt and Siva-Jothy, 2001). In response to this harmful male adaptation, females have evolved a counter-adaptation that minimizes these costs. The 'spermalege' is a specialized region of the female abdomen with a thickened cuticle that lies directly over a pocket on the inner surface of the abdominal wall (Carayon, 1966) that mitigates female injury and defends against pathogens (Morrow and Arnqvist, 2003; Reinhardt *et al.*, 2003).

Coevolutionary Arms Races Over Fertilization

Given the imbalance in gamete investment between the sexes (i.e., males produce many 'cheap' sperm, while females produce few 'expensive' eggs), females have a vested interest in ensuring that the highest quality males fertilize their eggs. This process is generally referred to as sexual selection, and results in males competing for access to females or territories and/or females being selective about their mating partners. The most direct way for females to ensure fertilization only occurs by preferred males is to be choosy about their mates. However, as discussed above, females do not always have complete control over which males they mate with. When females mate with multiple males, however, female choice can continue after mating if females can 'choose' which sperm to use or store, a

Figure 4 The phallus of the seed beetle (*Callosobruchus maculatus*). Males with a phallus that is more damaging to females sire more offspring. Wikimedia Commons http://en.wikipedia.org/wiki/Callosobruchus_maculatus#mediaviewer/File:Callosobruchus_maculatus_penis.jpg

process called 'cryptic female choice' (Eberhard, 1996; Thornhill, 1983).

Female orb spiders (*Argiope keyserlingi*) typically mate with multiple males throughout their life, and consume the males shortly after mating has started. Despite the fact that males vigorously attempt to escape, most are captured and consumed. Capture of the male by the female immediately terminates copulation, and hence can be used by females to control mating duration. It has been found that females preferentially delay capture of smaller males, thus allowing them to fertilize more eggs, and giving females control of offspring paternity (Elgar *et al.*, 2000). This cryptic female choice creates the potential for coevolutionary arms races between the sexes to continue after mating.

Sperm Competition

In the same way that female choice can continue inside the female reproductive track after mating, so too can male competition: when females mate with multiple males, their sperm must compete with one another for fertilizations. Sperm competition is ubiquitous among nonmonogamous mating systems, and has led to a number of diverse adaptations in males to increase their fertilization success (Birkhead and Møller, 1998; Parker, 1970). These male adaptations often lead to conflict with females over which sex controls offspring paternity (Stockley, 1997).

The genitalia of male seed beetles (*Callosobruchus maculatus*) (**Figure 4**) possess a large number of spines that penetrate the reproductive track of females during copulation and cause internal injuries (Crudgington and Siva-Jothy, 2000; Hotzy and Arnqvist, 2009). Males with longer genital spines cause more injuries to females than males with shorter spines. While the mechanism is unknown, when females mate multiply, males with longer spines sire significantly more offspring than those with shorter spines, despite the increase in female harm (Hotzy and Arnqvist, 2009). Similar genital-induced harm to females is seen in dung-flies (Blanckenhorn *et al.*, 2002), but has not been linked to an increase in sperm competition.

Figure 5 A copulatory plug in a female Richardson's ground squirrel (*Urocitellus richardsonii*). Copulatory plugs are common in rodents, and serve to prevent or delay female remating. Wikimedia Commons http://en.wikipedia.org/wiki/Rodent#mediaviewer/File:Mating_plug.jpg

Copulatory plugs are particularly common in insects, reptiles, and some mammals. The components of the plugs are transferred in the ejaculate during mating, and then harden into a plug that fills the female reproductive track (**Figure 5**). Copulatory plugs are larger and stronger in species with higher levels of sperm competition (Ramm *et al.*, 2005), and females cannot remate while the plug is present. Eastern gray squirrel and fox squirrel females physically remove the plug shortly after mating (Koprowski, 1992), while female house mice secrete enzymes after mating that degrade the plug (Dean *et al.*, 2011).

Forced Copulations

One of the most extreme outcomes of persistent male harassment is a forced copulation, whereby a female is physically overpowered, or rendered unable to resist the mating attempt of a male(s). It is in these extreme cases when it would be most advantageous for a female to prevent fertilization from occurring. Forced copulations are particularly common in waterfowl, where they appear to have generated sexually antagonistic genital coevolution (Brennan *et al.*, 2007). For example, male muscovy ducks have corkscrew-shaped penises, while females have anti-corkscrew vaginas with bends and blind alleys (Brennan *et al.*, 2009). This complex female vaginal morphology limits penile access during forced copulations, such that they result in very few fertilizations.

When given the choice, female feral fowl prefer to mate with dominant males over subordinate males. However, the majority of copulations are forced in this species, so females are rarely able to choose their mates (Pizzari and Birkhead, 2000). Instead, females have evolved a morphological counter-adaptation to this male behavior. Females are able to eject ejaculate from their reproductive track after mating with subordinate males, allowing them to preferentially store and use sperm from dominant males.

Toxic Seminal Proteins in *Drosophila*

When a male mates with a female, he not only transfers sperm, but also a seminal fluid containing a cocktail of different seminal fluid proteins. In *Drosophila*, these proteins are usually called accessory proteins (Acps), and they serve a variety of roles. *D. melanogaster* males transmit at least 138

different Acps (Findlay *et al.*, 2008), and many of these proteins undergo rapid evolutionary change, indicating that they are under strong positive selection (Swanson *et al.*, 2001). While the function of the majority of these proteins is unknown, several seem to aid in sperm competition (Chapman, 2001; Wigby *et al.*, 2009), and at least one has been identified as a contributor to the male–female arms race. Sex peptide (Acp70A) increases oogenesis (Kubli, 2003) and directly affects female behavior and physiology. In addition to increasing female fecundity after mating, sex peptide also makes females less receptive to subsequent mating attempts (Peng *et al.*, 2005; Wolfner, 2002), increases female locomotor activity, decreases the time females spend sleeping (Isaac *et al.*, 2009), and increases female feeding rates (Carvalho *et al.*, 2006). While all of these actions serve to greatly increase the male's relative fitness, sex peptide is toxic to females, shortening female life span (Chapman *et al.*, 1995) and lowering female lifetime reproductive success (Wigby and Chapman, 2005). Other Acps show similar involvement in sperm competition (Fiumera *et al.*, 2005) and fecundity induction (Herndon and Wolfner, 1995), so several Acps have the potential to be involved in a postmating arms race in *Drosophila*.

Conclusions: Who Wins an Intraspecific Arms Race?

Given the cyclical nature of antagonistic coevolutionary arms races, can one participant be considered the 'winner'? When applied to an interspecific context (i.e., predator/prey or parasite/host interactions), whichever population can more rapidly overcome the adaptations of the other will move ahead in the arms race and 'win.' Although this win is often temporary, in extreme cases one population can cause the local extinction of the other. If the prey/host species is 'victorious,' then its population will persist and no longer be negatively impacted by the predator/parasite species. If the predator/parasite 'wins,' then either the prey/host population will go locally extinct, or continue to exist in lower numbers than would occur in the absence of the predator/parasite. Extinction of the prey/host, however, does not necessarily mean the demise of predator/parasite, as they may be able to shift to new prey/host species.

Unlike a traditional coevolutionary arms race between two different species, an intraspecific arms race cannot have a 'winner,' even over shorter time periods: males cannot exist without females as reproductive partners, and vice versa. So, while males do not necessarily increase in frequency in the population, the alleles that improve their relative performance do. Similarly, while counter-adaptation does not necessarily cause an increase in the number of females in a population, it will cause an increase in the frequency of the associated resistance alleles. It is this interlocus conflict between the harming and resistance alleles that drives intraspecific antagonistic coevolution and leads to interesting, yet conflicting, potential evolutionary outcomes.

As alleles that increase male fitness at the expense of female fitness accumulate in the population, the fitness of the population as a whole also decreases (because the mean population fitness is limited by the mean female fitness; Holland and Rice, 1999). Therefore, if female resistance alleles do not accumulate sufficiently quickly, it is possible that populations with high levels of conflict will go locally extinct. Although theory suggests that intraspecific antagonistic coevolution increases the extinction risk of populations/species (Kokko and Brooks, 2003), there is only limited empirical support from phylogenetic studies in birds (Morrow and Pitcher, 2003) and mammals (Morrow and Fricke, 2004).

Alternatively, the back-and-forth nature of an intraspecific arms race may promote speciation. Given that any new male-benefit adaption arises through random mutation, it is very unlikely that the same, specific adaptation will arise independently in two separate populations. Female counter-adaptations that reduce or remove the associated male-induced harm will generally need to be specific to the male adaptation. Therefore, each population within a species proceeds down its own unique evolutionary trajectory, such that after each cycle of adaptation and counter-adaptation, populations can become phenotypically distinct. As these differences accumulate, populations can become reproductively isolated, potentially leading to speciation. In addition to being theoretically possible (Gavrilets, 2000; Parker and Partridge, 1998; Rice, 1998), there is supporting evidence that sexual conflict leads to speciation in insects (Arnqvist *et al.*, 2000), but not in birds (Morrow *et al.*, 2003).

Here we have focused specifically on arms races as they pertain to the separate sexes (intersexual) in the context of mating and fertilization, but there are numerous other ways in which antagonistic coevolution can operate within a species. Just as males have adaptations to coerce females to mate, males also have to compete for these matings with other males, and females may need to compete with one another for access to resources or breeding grounds. These intrasexual conflicts can lead to a wide variety of 'solutions,' including different morphologies and/or alternative behaviors. Similar conflicts can occur between parents and their offspring or between siblings over the amount of resources allocated to each offspring (Trivers, 1974; Godfray and Parker, 1992), and between males and females over the amount of parental care each provides to their offspring, if this care comes at the expense of future mating/reproductive opportunities (Westneat and Sargent, 1996). Despite being members of the same species, or even the same population, different individuals have selfish genetic interests. As a result, arms races that occur within species may be equally as important as arms races between species for adaptive evolutionary change.

See also: Antagonistic Interspecific Coevolution. Sexual Conflict

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Invasion Biogeography

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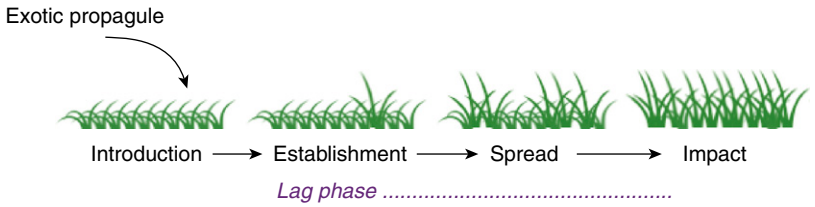
An invasive species is a nonindigenous species that is expanding its range and that causes some impact, either environmental or economic. Researchers debate as to whether impact is important for the definition, for example, some argue invasive species include all nonindigenous widespread species, or of these, only those that are also expanding their range (Sax *et al.*, 2007; Valéry *et al.*, 2008; Colautti and Lau, 2015). Regardless of the definition, two features characterize invasive species, colonization of new ecological environments and subsequent geographical spread. Only relatively recently have researchers recognized that biological invasions necessarily involve not only ecological, but also evolutionary processes. This understanding is not solely academic. Knowledge of how invasive species evolve and what limits their evolution provides insights into predicting whether and how species will respond to global change, especially changes in climate and land/water use.

In 1965, Herbert G. Baker and G. Ledyard Stebbins published the *Genetics of Colonizing Species* (Baker and Stebbins, 1965). This volume provided the 'ah-ha moment' for many biologists to realize that evolutionary forces were important in the geographical spread of species, and in particular, the role of genetic mechanisms, which were becoming better understood at that time. It was already recognized even since Darwin that certain ecological features were critical in species range expansion, including ecological attributes of either the invasive species themselves, or the invaded habitats, and/or their interaction (Elton, 1958; Sax *et al.*, 2007). For example, commonly cited ecological characteristics of invasive species include high dispersal, rapid generation time, and generalist life style, among others, while characteristics of invaded habitats include disturbance and species poor communities with few competitors and predators (Williamson, 1996). With their volume, Baker and Stebbins brought new genetic insights into what determines the success of colonizing species, especially by linking approaches including detailed natural history, novel methods of genetic characterization, observations from natural experiments, and common garden manipulations. For the 50th anniversary of the Baker and Stebbins volume, a group of researchers reexamined the genetics of colonizing species (Barrett, 2015; Bock *et al.*, 2015), which along with an earlier, and in retrospect now visionary, perspective by Lambrinos (2004), provides a framework for this review.

Biological invasion is best thought of as a process, with several identifiable key stages (**Figure 1**; Kolar and Lodge, 2001;



Evolutionary process	Introduction	Establishment	Spread	Impact
Migration	Dispersal traits Pathways Numbers of propagules Multiple colonization events Life history variation	Bet-hedging Hybridization	Dispersal within range New population structure Hybridization	Dispersal within range
Drift		Founder events Inbreeding		
Mutation			New variation Genetic rearrangement	
Selection		Preadaptation Climate matching Phenotypic plasticity	Local adaptation Life history evolution	Life history evolution

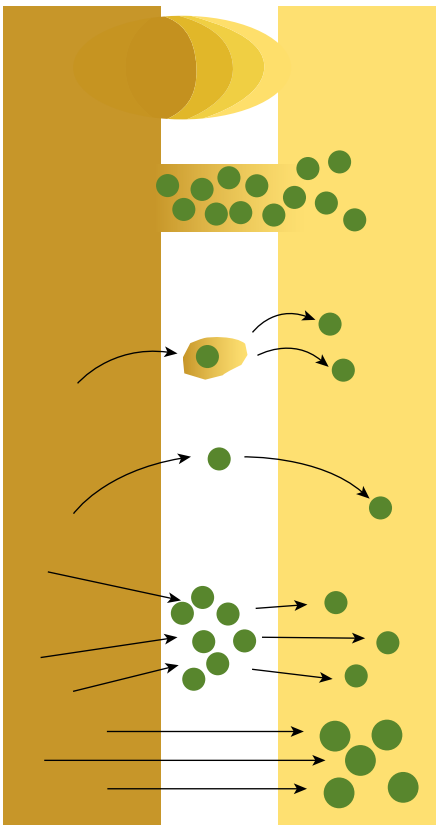
Figure 1 Stages of invasion and associated evolutionary processes. Redrawn from Kolar, C.S., Lodge, D.M., 2001. Progress in invasion biology: Predicting invaders. Trends in Ecology & Evolution 16, 199–204; Sakai, A.K., Allendorf, F.W., Holt, J.S., 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32, 305–332.

Sakai *et al.*, 2001; Lambrinos, 2004). In the invasion process, organisms are introduced, either naturally or anthropogenically, and a fraction of those become established. Of the established species, a proportion spread, and of those, only some species cause some type of environmental or economic impact. Often, but not always, invasive populations may take some time to reach a population size that is noticed or causes a significant problem, a period called the 'lag phase' (Figure 1). At each stage in this process, ecological and evolutionary forces operate as filters, resulting in only a small proportion of species at each stage in the process making it to the next stage. This phenomenon has been termed the '10's rule,' noting an arbitrarily small 10% of species making it to the next stage (Williamson, 1996). Evolutionary forces involved in the invasion process include migration, drift, mutation, and selection, all of which can cause changes in the frequencies of genes within populations.

The invasion process itself places some constraints in dictating how the forces of evolution can interact and when. For example, both drift and selection require genetic variation on which to act, yet many invasions are thought to have originated by a small set of founding propagules, presumably with a small and random sample of the genetic variation of the source population. One might naturally ask, how important then can drift and selection be for the success of invaders, given initial low genetic diversity? This is a central evolutionary challenge facing invasion biologists.

Introduction Phase

Which species are introduced depends on dispersal, termed migration in a genetic/evolutionary context. Some organisms disperse on their own, some are vectored by other organisms, and some disperse in association with human activities. Spiders ballooning on threads of silk are an example of natural dispersal, with individuals being predictably among the first to arrive on newly created islands (Gillespie *et al.*, 2012); widow spiders that move around the world associated with produce, such as bananas, illustrate the effects of anthropogenic factors in dispersal. Of critical importance is how many individuals arrive in the new habitat, either at one time or sequentially over time. This magnitude of dispersal from a source area to the new habitat over time is termed propagule pressure – i.e., how many individuals arrive. Propagules can disperse in many ways reflecting the nature of available habitat, but also the mechanism of introduction (Figure 2). In addition to propagule pressure, patterns of life history and ability to disperse are important in the introduction phase, with the result being that some types of species repeatedly arriving at new localities, and their



	Description
Leading-edge dispersal	A gradual change in range is initiated by individuals colonizing new areas from the edge of the range
Corridor	A physical connection of suitable habitat forms, linking a portion of the original range to another area
Jump dispersal	Propagules disperse over sometimes substantial distances, but still with a connection between the new and original ranges
Extreme long-distance disperal	Propagules move far beyond the dispersal range seen over ecological time scales
Mass dispersal	A dispersal route is established such that many individuals can move from different parts of the range to many new sites
Cultivation	Propagules are actively moved and provided with resources to establish and persist

Figure 2 Types of modes of dispersal. Redrawn from Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2013. Invasion Ecology. New York: John Wiley & Sons.

representation can be nonrandom, especially in remote locations (Carlquist, 1966).

Establishment Phase

Carlquist (1966) noted, "Difficulties of establishment seem much greater than those of transport. To establish, the number of founding individuals and their genetic make up becomes important." It is well known that small numbers of founders carry only a subset of the genetic variation in the source population, and such founder events may lead to negative effects associated with eventual establishment, such as inbreeding. Many studies are now showing that multiple founder events by the same species are common for invasive species (Bock *et al.*, 2015). Further, when these colonists come from different places, the genetic pool in the invasive population can be sizable. A now classic example is that of multiple colonizations of a Caribbean lizard, *Anolis sagrei*, (**Figure 3**) from different locations in the Caribbean into Florida (Kolbe *et al.*, 2004). When *Anolis* individuals from these different sources mixed, the genetic diversity in the resulting invasive population was highly significantly greater than any of the sources individually. Such admixture of individuals from multiple populations may be far more important than realized in contributing to genetic variation in invasive species, and may allow invasive populations to have high levels of genetic variation early in the invasion process. In a similar way, invasive species can mix genetically, or hybridize, with indigenous species or other invaders. Examples of invasive species exhibiting hybridization include, *Spartina* plants, *Rhagoletis* flies, *Anas* dabbling ducks, and *Bemisia* whiteflies among others (Vellend *et al.*, 2007).

Another key to establishment is the extent to which invasive species are already adapted to the novel habitat. This adaptation can be a result of the new environment closely matching the source, for example, in climate variables, or similarities in the biotic composition, including symbionts, competitors or predators. Alternatively, conditions in the source environment may have 'preadapted' the organism for high fitness in a new different set of environmental conditions found in the novel habitat, such as Mediterranean species that invade areas of similar climates worldwide (Barrett, 2015).

Figure 3 *Anolis sagrei*, photo credit: Wikipedia.

Another form of preadaptation is phenotypic plasticity, in which species can naturally cope with a diverse set of natural conditions, as seen for at least some traits of the soapberry bug, when presented with an introduced plant. Phenotypic plasticity also provides the opportunity for an expression of diverse phenotypes upon which selection can act, perhaps in different environments (Lande, 2015).

As with introduction, those species that establish are not a random subset of the set of possible species in a source community. Indeed, one predictor of whether a species is likely to be invasive is whether it has been observed to be invasive elsewhere (Lonsdale, 1999; Bock *et al.*, 2015), and such serial invasions are common. What studies of such species are not able to observe are all the introductions that failed to establish, which is no doubt is a considerably high number (Carlquist, 1966). Thus, similar to a 'non-reporting bias' in statistical sampling, we observe only those species that are able to establish for whatever reasons. Because monitoring the fate of unsuccessful propagules under natural conditions is very difficult, understanding the importance of this non-reporting bias of successful invasive species is extremely difficult.

The importance of genetic variation in establishment remains elusive and, for reasons noted above, genetic variation may not be limiting. While theory predicts genetic variation is necessary for adaptation and even preadaptation, many successful invaders have little genetic variation (Sax *et al.*, 2007; Bock *et al.*, 2015); for example, the spreading Japanese knotweed in Britain appears to be a single genetic clone. Additionally, an invasive species that has already been selected for coping with limited genetic variation in a previous invasion elsewhere may not necessarily require genetic variation to be successful in a novel habitat.

Spread Phase

Once established, invasive species typically expand their range geographically. Individuals may move to new areas of the same type of environment, or to different types of habitats, where there may be selection as a result of the new environmental conditions. Populations in different areas may become differentiated genetically, either through drift or through local adaptation (Vellend *et al.*, 2007). Genetic variation may again be increased when individuals from such populations hybridize upon later contact. By contrast, if founding populations are small and deleterious mutations accumulate in expanding populations, expansion success could be limited; this little studied phenomenon is termed expansion load (Peischl *et al.*, 2015). Several other related questions remain unanswered, including the relative importance of adaptation at this phase compared to preadaptation, the importance of local adaptation in farther geological spread, and the extent to which population structure of invasive populations resembles that of species with a longer history in the environment (Gillespie *et al.*, 2008; Barrett, 2015).

During this phase of invasions, populations are necessarily growing. Often the time from introduction to the time of a significant population size can be long, for example, 10s or even 100s or more of generations, resulting in the 'lag phase' noted above (**Figure 1**). There are both ecological and

Figure 4 Garlic mustard, *Alliaria petiola*, photo credit: Wikipedia.

evolutionary explanations for this lag phase. Population growth of invasive species can result from invasive populations responding sufficiently in numbers to new biotic pressures including competitors and predators, or in overcoming *Allee effects* at small population size. Large-scale monitoring programs measuring the same characteristics across a wide invasive range, such as implemented for the garlic mustard, *Alliaria petiola* (Colautti *et al.*, 2014; **Figure 4**) offer a comparative framework for understanding the role of novel environments. It may also be that additive genetic variation has increased since establishment through a variety of possible mechanisms, including mutation, genome rearrangements, and horizontal transfer of novel genetic elements. New genomic tools offer unprecedented opportunities to understand the relative importance of each genetic mechanism in the success of invasive species.

Selection is also critically important at the phase of geographical spread; selection can act to favor those individuals that are able to make it to new environments, and/or to make it to these environments first. For example, extensive studies of the cane toad, *Rhinella marina*, show selection for morphology and life history traits that favor dispersal, especially at the invasion front (Phillips and Shine, 2006; Shine, 2010; **Figure 5**). The process of geographic spread itself creates conditions that may accelerate evolution, through drift, selection, or genetic rearrangements (Kirkpatrick and Barrett, 2015).

Impact

Eventually, invasive populations build in numbers and geographic area to have a noticeable impact on ecological communities or on human activities with economic consequences. Here, selection can act on life history variation, including

Figure 5 Cane toad, *Rhinella marina*, photo credit: Wikipedia.

dispersal, in response either to biological or environmental conditions in the novel habitat, or anthropogenic conditions, such as pest control. For example, resistance to pesticides including antibiotics is well known throughout the diversity of life, including microbes, plants, and animals, allowing many organisms to spread uncontrolled.

Invasion Biogeography

The study of biological invasions can offer insights into the dynamic nature of patterns of biogeography (Sax *et al.*, 2007). While much remains to be understood, some patterns are beginning to emerge. First, while colonizations and large-scale biotic exchange have been ongoing in geological time, human activities have accelerated the rate of exchange. Not only is turnover more rapid (Burns, 2015), but also species have become more homogenized among regions, as has species richness among regions (Sax and Gaines, 2006; van Kleunen *et al.*, 2015). The rate at which these effects have occurred, including the development of global patterns of biodiversity suggest that long-term explanations of global biodiversity are not necessary – for example, traditional latitudinal gradients of biodiversity may have a more recent explanation. Second, the number of indigenous species seems to be correlated, suggesting that biological communities are not saturated. However, this result is complicated (Sax and Gaines, 2006). For example, more species are invading low temperate latitudes, although with smaller geographical ranges. By contrast, fewer species are invading at higher latitudes, though with larger geographic ranges. Islands appear to be disproportionally affected (Sax and Gaines, 2008). Finally, the impact and spread of biological invasions depends on the species involved and their interactions. Climate and dispersal limitation explain only a portion of the distribution of invasive species, suggesting a key role of species interactions (Capinha *et al.*, 2015).

Summary

Perhaps the most important evolutionary insight from the study of invasive species is that rapid adaptation of invaders is common (Bock *et al.*, 2015). It appears that genetic variation is

not necessary limiting, which can be the result of many possible mechanisms, including nonrandom sampling of invasive propagules, multiple colonization events, hybridization, preadaptation, phenotypic, plasticity, mutation, and novel genetic mechanisms (Vellend *et al.*, 2007). A second main insight is that each of the phases of the invasion process is associated with important evolutionary, as well as ecological forces, and both contribute to the success of invasive species. Third, biological communities are not saturated, such that even in the most diverse assemblages species are able to establish and persist (Sax *et al.*, 2007).

Looking forward, studies of invasive species have much to offer in understanding biological evolution associated with novel environments, especially in understanding the limits of evolutionary response to global change. Such systems offer natural experiments, in which the same species is introduced to multiple environments, but also the opportunity for experimentation through purposeful, controlled introductions, such as in biological control, 'common garden' designs, and controlled studies of selection. New genomic tools promise to offer insights into genetic mechanisms associated with invasion, particularly with respect to the importance of mutations, novel genetic variation, and genetic rearrangements. Interactions of species are paramount. The tricky part, as noted by Baker and Stebbins (1965), is how to combine these approaches.

See also: Biogeography, Conservation. Dispersal Biogeography. Invasive Species, Evolution and

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http://cisr.ucr.edu
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http://www.invasivespeciesinfo.gov/index.shtml
USDA National Invasive Species Information Center (NISIC).

Invasive Species, Evolution and

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Glossary

Admixture Population composed of individuals from previously separated source populations.
Biotic homogenization Increasing genetic and taxonomic similarity between regions
across the globe which is promoted by species invasions.
Invasion vector Mechanism of human transport of nonnative species from their native, source region to a nonnative, recipient region.
Invasive species Nonnative species that have been introduced by humans and have established and spread in their introduced range.
Propagule pressure The total number of introduced individuals, which is a function of number of introduction events and number of propagules released during each event.

Background

The study of species invasions is important both from the perspective of biodiversity conservation and because it provides general insights into ecology and evolution (Sax *et al.*, 2005, 2007). One of the most important and defining characteristics of invasive species is that their evolutionary history with species in the invaded region is relatively short (Strauss *et al.*, 2006a,b; Sorte *et al.*, 2010a). To explore the role of evolution in species invasions in more detail, it is first important to define invasive species, the steps species must undergo to become invasive, and the scope of the invasive species problem.

Defining Invasive Species

Invasive species are defined as nonnative species that have been introduced by humans and have established and spread in their introduced range. These last two characteristics – establishment and spread – imply impacts on native communities, although impacts have been quantified for only a small proportion of introduced species (e.g., Williams and Smith, 2007). In some cases, when the term invasive is synonymous with weedy, some subset of native species could also fit this definition (Vigueira *et al.*, 2013). This article focuses on nonnative invasive species; however, certain evolutionary concepts related to invasive species will also apply to native pests.

The Invasion Pathway

Species invasions occur via a stepwise process known as the invasion pathway (**Figure 1**; see also Theoharides and Dukes, 2007). First, individuals of a species are transported by humans from the source region in their native range to the recipient region in their nonnative range via an introduction vector. This transport can be either intentional (e.g., plants sold in the horticultural trade; Mack and Lonsdale, 2001) or unintentional (e.g., hitchhikers in commercial shipping vessels; Westphal *et al.*, 2008). Second, colonization occurs if the transported individuals are able to survive and reproduce in the nonnative range. Third, the introduced species is considered established if it has formed a reproducing and self-sustaining population. The final step in the invasion pathway is secondary spread, in which the species extends its range within the nonnative region. By definition, an invasive species must not only become established in its new location, but it must also expand its range beyond the original introduction site.

Whether or not a species is successful in becoming invasive depends upon its ability to pass through filters imposed by the different stages of the invasion pathway (**Figure 1**). For example, before even arriving in their new territory, future invasive species must find – or be chosen for – transport and

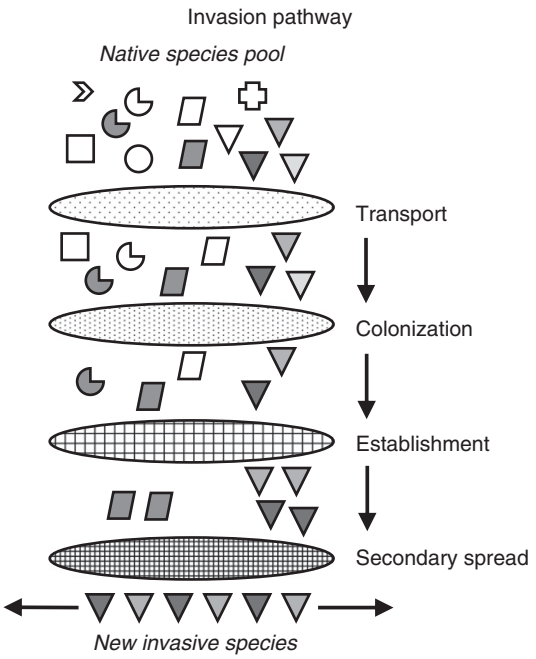

Figure 1 In order to become invasive, species (different shapes) must pass through four stages (listed on the right). Each stage acts as a filter to decrease the species pool as well as genetic diversity (indicated by shading in the shapes) within each species.

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survive the journey. Once in the new habitat, invasion success depends on the ability to tolerate climatic conditions, attain resources, and avoid consumption in the nonnative range under a potentially novel set of abiotic and biotic conditions. The sequential narrowing of the potential invasive species pool is illustrated by the tens rule, which suggests that only 10% of introduced species become established, and only 10% of these become invasive (Williamson, 1996). Actual proportions rarely conform to such a simple scheme and are taxon-specific; however, establishment and subsequent invasiveness only occur in the minority of introductions (Jeschke and Strayer, 2005).

Scope of the Invasion Problem

Despite the fact that most species do not become invasive, those that do can have extremely large ecological impacts and economic costs (Pimentel *et al.*, 2005), and the invasion problem is increasing (Butchart *et al.*, 2010) concurrent with globalization. There were approximately 120 000 nonnative species in the United States, Europe, Australia, South Africa, India, and Brazil as of the census by Pimentel *et al.* (2002) (**Figure 2**), and the control of – and damages by – invasive species in those countries cost more than $300 billion per year. Even a relatively small-scale introduction of the killer alga, *Caulerpa taxifolia*, one of the world's '100 worst' invasive species, near San Diego, California, USA, cost >$6 million for local eradication (Williams and Grosholz, 2008).

In ecological terms, invasive species have driven declines in native populations, as well as extinctions. The impact of each invasive species is expected to increase directly with range size, population size, and per capita effect (Parker *et al.*, 1999). Although extinction causes are often equivocal and occasionally disputed, invasive species are implicated as a leading cause of animal (particularly bird and fish) extinctions (Gurevitch and Padilla, 2004; Clavero and Garcia-Berthou, 2005). In fact, invasive species are considered one of the primary threats to global biodiversity across terrestrial and aquatic ecosystems (Sala *et al.*, 2000; Millennium Ecosystem Assessment, 2005) and, more specifically, to an estimated 42% of species on the threatened and endangered species lists (Pimentel *et al.*, 2005). Extinctions are particularly likely on islands, where potential for avoidance of invasive predators via niche displacement or rapid evolution is relatively low (Mooney and Cleland, 2001). For example, the invasive brown tree snake appears to have driven extinctions of native birds on Guam, in the tropical western Pacific Ocean (Savidge, 1987). The invasion problem is becoming exacerbated as introductions are not only continuing but are increasing in many regions (Cohen and Carlton, 1998; Millennium Ecosystem Assessment, 2005), leading to rapid biotic homogenization (see Olden *et al.*, 2004).

Evolutionary Characteristics of Invasive Species

Introduced species can be prone to founder events and population bottlenecks imposed by filters in the invasion pathway, leading to declines in genetic diversity within nonnative populations as compared to their native, source populations. In a review of 80 species of animals, plants, and fungi, diversity of alleles and heterozygosity were significantly lower in introduced populations than in source populations, although decreases were less than 20% (Dlugosch and Parker, 2008; also see Wares *et al.*, 2005). Furthermore, Dlugosch and Parker (2008) found a U-shaped relationship between genetic

Figure 2 Invasive species are a global issue, leading to economic, ecological, and evolutionary impacts on native ecosystems. Examples of recent invaders include (a) the Cuban tree frog, invasive in the USA (Florida and Hawaii) and throughout the Caribbean; (b) a suite of invasive tunicates that are now found in coastal waters worldwide and foul docks, boats, and shellfish; and (c) the cordgrass *Spartina alterniflora* which has hybridized with a native species, *Spartina foliosa*, and is shown here invading San Francisco Bay, California, USA.

(allelic) diversity and time since first introduction, suggesting that selection and genetic drift continue the loss of genetic diversity during the first several decades after colonization. Over longer time scales, multiple introductions can cause genetic diversity to rise again via the mechanisms discussed below. Thus, it is perhaps not surprising that a lag phase often occurs between the colonization and spread stages in the invasion pathway (see Crooks, 2005), in which population growth and subsequent spread are inhibited either directly by low population sizes or by low level of genetic variation within the populations.

Propagule pressure – or the total number of introduced individuals – is one of the best supported correlates of invasion success (Kolar and Lodge, 2001; Lockwood *et al.*, 2005; Colautti *et al.*, 2006) and can counteract the effect of founder events on genetic diversity within introduced populations (Roman and Darling, 2007). Simberloff (2009) reviewed examples – from birds to ungulates – of cases where introductions failed until the number of introduced individuals exceeded a minimum threshold. Increased propagule pressure is associated with increased population sizes and increased genetic diversity within populations (Simberloff, 2009). Furthermore, multiple introductions may lead to the formation of admixtures, new populations composed of individuals from previously separated source populations. Such introduced admixture populations can have equivalent (Dlugosch and Parker, 2008) or even increased levels of genetic diversity as compared to individual source populations (Kolbe *et al.*, 2004; Gillis *et al.*, 2009).

In addition to the invasion process influencing genetic diversity, there is some evidence that genetic diversity also influences invasion success. For example, genetic diversity has been linked to colonization ability in the plant *Arabidopsis thaliana* (Crawford and Whitney, 2010) and productivity and clonal spread in a perennial grass (Lavergne and Molofsky, 2007). It is important to note, however, that there are a number of counter-examples to the trends presented above. Successful invasions of a broad range of species – including a European solitary bee to North America and the North American muskrat to Europe – are thought to have derived from only one to a few introduced individuals (see Simberloff, 2009). There are also examples where invasion success was associated with decreases rather than increases in genetic diversity (e.g., Schmid-Hempel *et al.*, 2007), particularly when loss of genetic variation increased the frequency of a genotype that proved to be beneficial (e.g., increase population sizes) in the nonnative habitat (e.g., Tsutsui *et al.*, 2000).

Evolution of Invasive Species

The invasion process drives evolution in invasive species via both non-selective and adaptive evolutionary mechanisms. The genetic characteristics of invasive species, discussed above, are indicators of the types of non-selective mechanisms at work. Low genetic diversity may indicate the influence of genetic drift, the random change in allele frequencies that more strongly impacts smaller populations. Genetic drift is likely in founder populations, including of introduced species, due to their small sizes (Sakai *et al.*, 2001). High genetic diversity, on the other hand, has been related to the number of separate introduction events occurring over time (Dlugosch and Parker, 2008). Gene flow can be high when introduced individuals are sampled from a large geographic area and also when multiple introductions create admixtures, with increased propagule pressure decreasing the negative impacts of genetic drift and increasing potential for adaptive evolution in the nonnative range. Invasive success can be increased by hybridization both within and between species, including between natives and nonnative (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009). For example, hybrids of the introduced cordgrass *Spartina alterniflora* and native species *Spartina foliosa* grow larger and are more invasive than either of the parent species in San Francisco Bay, California, USA (Grosholz, 2002; **Figure 2**).

Adaptive evolution is promoted in invasive species as they experience strong selection pressures at each stage in the invasion pathway. Initial transport might favor individuals that associate with and survive human transport, whereas secondary spread has been associated with increases in reproduction (Colautti and Barrett, 2013) and innate dispersal ability (Phillips *et al.*, 2006). For example, cane toads with longer legs are the first to arrive at and colonize new populations in Australia, and this shift in toad morphology could explain why the invasion front is extending faster over time (Phillips *et al.*, 2006). Within its nonnative habitat, a successful new invader must be capable of surviving environmental conditions, attaining resources, and avoiding predation. Novel environments and biotic interactions can select for shifted climatic tolerances (e.g., Sexton *et al.*, 2002; Lee *et al.*, 2003; Sorte *et al.*, 2011) or increased predator defense (e.g., Miehls *et al.*, 2014). On the other hand, some invasive species experience relaxed selection in the nonnative habitat due, for example, to release from their native enemies or competitors (the enemy release hypothesis (ERH); Keane and Crawley, 2002) or encounters with naïve prey (Cox and Lima, 2006). The evolution of increased competitive ability (EICA) hypothesis describes how this relaxation could allow a reallocation of resources from, for example, predator defense to competitive traits such as faster growth rate (Blossey and Nötzold, 1995). A review of pair-wise experiments suggested that invasive plants were better competitors than native species (Vilà and Weiner, 2004) and a meta-analysis of several hundred species indicated that invasive species had higher values for performance-related traits than non-invasive and native species (Van Kleunen *et al.*, 2010). However, the role of evolution in these competitive and trait differences is unknown, and there are many counter-examples. For example, Seabloom *et al.* (2003) found that native perennials were better competitors than invasive annual grasses, and they attributed the greater dominance of invasive species in their system to differential propagule pressure.

Although there is an increasing number of observations consistent with adaptation, it is important to note that natural selection is not the only explanation for observed phenotypic differences (Keller and Taylor, 2008). Phenotypes are reflections of both genotypes and environmental conditions, and many reports of phenotypic variation are based solely on observational data used to compare individuals of a species from within versus outside its native range or between multiple invasive populations. To determine whether phenotypic

variation is driven by genetic differences versus environmental plasticity, researchers often employ transplant – or 'common garden' – experiments in which the environmental factor is removed or accounted for in the experimental design (e.g., Sexton *et al.*, 2002; Lee *et al.*, 2003; Colautti and Barrett, 2013 cited above). Parker *et al.* (2003) used common-garden experiments to compare morphological traits across 10 populations of an invasive weed inhabiting a range of elevations across the Sierra Nevada Mountains, California, USA. They found that the great majority of phenotypic variance was at the individual (not family or population) level, indicating that differences between samples collected from the field were primarily due to phenotypic plasticity. There is even evidence that invasive species have higher levels of phenotypic plasticity than noninvasive species, as indicated by a meta-analysis of 75 plant species pairs (Davidson *et al.*, 2011). Clearly, population-level differences of invasive species across their native and nonnative ranges could reflect influences of myriad processes, including genetic drift, gene flow, hybridization, phenotypic plasticity, and natural selection.

Invasive Species as Drivers of Native Species Evolution

Observations across time (before and after invasion) and space (within vs. outside invasive species' ranges) have uncovered evidence of invasive species impacts on native species, including cases of rapid evolution in native populations (Mooney and Cleland, 2001; Lambrinos, 2004; Strauss *et al.*, 2006b; Vellend *et al.*, 2007). As an example, Phillips and Shine (2006) suggested that native black snakes in Australia have evolved increased resistance to cane toad toxin and decreased prey preference for the toads after less than 23 generations. Invasive species have also impacted native populations via positive interactions. A native checkerspot butterfly in Nevada, USA incorporated the invasive European weed *Plantago lanceolata* into its diet, and breeding studies indicated a genetic basis to the butterfly's feeding preference (Singer *et al.*, 1993). In some cases, utilizing novel resources requires further adaptation, and a native Australian soapberry bug has evolved longer mouthparts in order to feed on an invasive vine (Carroll *et al.*, 2005). Hybridization between invasive and native species appears to be widespread (Mooney and Cleland, 2001), such as the *Spartina* cordgrass example, above. Invasive species can even promote hybridization between two native species by providing a novel resource and, thus, leading to novel niche overlap between native species that would otherwise not meet nor reproduce (Schwarz *et al.*, 2005). It is, therefore, clear that invasive species have driven evolution of native species although, as for the evolution of the invasive species themselves, the relative contribution of genetic adaptation to observed changes is often unknown.

Implications for the Future: Applying an Evolutionary Perspective to Invasive Species Management

In the words of Charles Elton, called the 'father' of invasion biology, "we are seeing one of the great historical convulsions in the world's fauna and flora" (Elton, 1958, p. 31). Invasive species are contributing to the homogenization of both species and genetic material on a global scale, including driving extinctions of native species and diversification in their invaded habitats. They are an ecological, economic, and evolutionary threat of our own making: while we facilitated invasions initially, we now spend billions of dollars on their control. In so doing, we are driving adaptation of characteristics that allow the invasive species to avoid control and persist in their invaded range (Lee, 2002), including mimicry of the crops they invade and resistance to herbicides and pesticides (Vigueira *et al.*, 2013). Furthermore, we are indirectly contributing to the increased threat of invasive species by our role in driving global climate change, which appears to favor invasive species over native species (Sorte *et al.*, 2010b, 2013).

Future attempts to prevent, control, and eradicate invasive species could benefit from the incorporation of an evolutionary perspective (Whitney and Gabler, 2008). Although protocols are increasingly being implemented to prevent unintentional introductions of nonnative species, intentional imports continue, including through the horticultural trade (Bradley *et al.*, 2012). Importation suitability should be informed by Weed Risk Assessments, which, in the version used by the Australian government, includes population biology characteristics such as hybridization potential and reproductive strategies. Many invasive species start out as seemingly innocuous species introductions, and eradication efforts would ideally start during the establishment and lag phases. Efforts to prioritize nonnative species for control and eradication could be aided by an understanding of population genetics and ecological interactions, in order to identify species that are likely to become invasive, cause ecological and evolutionary damage (i.e., hybridization, extinctions, etc.), and evolve resistance to control methods (Allendorf and Lundquist, 2003). Finally, issues of invasive species developing evolved resistance should be acknowledged, with control practices including fluctuation of multiple herbicides, pesticides, or biological control agents, and potentially assisted spread of nonresistant genotypes (Stockwell *et al.*, 2003). A multipronged approach to invasive species management that incorporates an evolutionary perspective will help us to meet future challenges as invasion rates continue to increase (Millennium Ecosystem Assessment, 2005; Simberloff *et al.*, 2013).

See also: Ecological Fitting and Novel Species Interactions in Nature. Pest Management, Evolution and

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Land Animals, Origins of

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Glossary

Coprolite Fossil feces are called coprolites.
Cryptobiosis The ability of an animal to enter a state of reduced metabolism in order to overcome adverse environmental conditions.
Detritivore An organism which eats detritus is called a detritivore.
Epifauna Organisms which live above (i.e., not within) the substrate are called epifauna.
Hexapoda Hexapoda is a group of arthropods including insects, springtails, and their close relatives.
Interstitial Living between grains of sediment is called interstitial.
Isotonic Solutions with the same concentration of solute, so that osmosis does not occur.
Metazoa Multicellular animals are called Metazoa.
Osmosis The passage of water from a solution of higher concentration to one of lower concentration is called osmosis.
Phylum (pl. phyla) A major group of animals with a distinctive body plan; e.g., Arthropoda, Mollusca.
Tetrapod A vertebrate animal with legs (rather than fins) is called a tetrapod.

Introduction

Terrestrialization – the colonization of the land habitat from the sea by plants and animals – was the third most important event in the history of life on Earth, after the origin of life and the development of multicellularity. Here, we look particularly at the origin of land animals. For many people, that brings to mind fish acquiring legs and crawling out of the mud for the first time onto dry land. However, the first tetrapods were preceded onto land by invertebrate animals many millions of years previously. Moreover, what constitutes a terrestrial animal? How much of its life cycle must it spend on land to be considered part of the terrestrial fauna?

There is no doubt that land animals originated in the sea; the isotonicity between the solute concentrations of animal cells and sea water is a testament to this. Osmotic concentration in terrestrial animals is a useful clue to the route they took onto land. Not many animals have managed to move from the sea to the land: of over 30 phyla, only the vertebrates, arthropods, mollusks, and annelids have significant numbers of macroscopic terrestrial representatives. A greater number of phyla include very few terrestrial species (e.g., platyhelminths; Figure 1), cryptobiotic representatives, or internal parasites on terrestrial organisms. The body plans of some highly successful marine phyla have apparently precluded their terrestrialization; these include the sipunculid, echiuroid, and priapulid worms; cnidarians; lophophorates; chaetognaths; pogonophores; hemichordates; and echinoderms. No phylum originated on land, and no major terrestrial taxon has become extinct, as far as we know.

Physiology

In order for marine animals to colonize the land, a number of physiological barriers need to be overcome. These include: changes to methods of respiration, water management and osmoregulation, digestion, temperature control, reproduction, dispersal, sensory perception, and support and locomotion. Water is essential to life, as a medium for biochemical reactions, and for the transport of cell solutes, for example. It is the variability of its availability on land that is problematic for terrestrial life – inundation can be as fatal as dehydration for a

Figure 1 Terrestrial flatworm (Platyhelminthes: *Bipalium* cf. *rauchi*, Singapore); © the author.

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Figure 2 Salamander (Tetrapoda, Lissamphibia: *Salamandra atra*, Switzerland); © the author.

Figure 3 Millipede (Diplopoda: *Anadenobolus monilicornis*, Barbados); © the author.

land animal. Four groups of land animals can be defined based on their management of water availability.

- Aquatic organisms avoid the problem by living in interstitial water in soils; these include microscopic nematodes, protozoans, and micro-arthropods.
- Cryptic forms differ from those in the first group in being larger, but similarly inhabit environments of constantly high humidity, such as soil and tropical forest litter. Included in this group are earthworms, leeches, flatworms (Figure 1), isopods, slugs, insect larvae, some amphibians (Figure 2), and myriapods (Figure 3).
- Poikilohydric (desiccation-tolerant) animals require high humidity to function, but can tolerate desiccation by drying out and rehydrating when conditions become favorable again. Cryptobiotic rotifers, mites, and tardigrades occur in this group; also included are animals with desiccation-tolerant resting stages such as the eggs of fairy shrimps.
- Homoiohydric organisms have achieved the true conquest of the land by the use of waterproof cuticles, transport systems, and osmoregulation. In this group are most tetrapods, insects (Figures 4 and 5), arachnids (Figures 6 and 7), and some isopods and mollusks (Figure 8).

Another important barrier to terrestrialization is the necessity to change from obtaining oxygen from water to

Figure 4 Beetle (Hexapoda, Coleoptera: *Stenocara gracilipes*, Namibia); © the author.

Figure 5 Butterfly (Hexapoda, Lepidoptera: *Neptis* sp., Yunnan, China); © the author.

Figure 6 Spider (Arachnida, Araneae: *Lasiodora* sp., Brazil); © the author.

Figure 7 Scorpion eating a cricket (Arachnida, Scorpionida: *Isometrus maculatus*, Singapore); © the author.

Figure 8 Land snail (Pulmonata: *Helix pomatia*, Italy); © the author.

Figure 9 Land crab (Crustacea, Decapoda: Trinidad); © the author.

Figure 10 Lizard (Amniota, Squamata: *Agama aculeata*, Namibia); © the author.

breathing air. Oxygen is more abundant in air (8.65 mol m^{-3}) than in water (0.262 mol m^{-3}), but its availability to animals depends on other factors, such as the rate of diffusion and the efficiency of oxygen-binding molecules in the blood. Many littoral animals can survive out of water for periods, but those that spend their whole lives out of water need lungs rather than gills. The problem is compounded by the fact that the CO_2 and O_2 molecules are larger than the H_2O molecule, thus membranes for gas exchange leak water. This means that respiratory surfaces need to be internalized and valves are required to regulate air flow, spiracles in insects, for example, so that water is not lost.

For support and locomotion on land, small animals such as slugs and worms can use their hydrostatic skeletons, but larger arthropods and tetrapods have evolved a hanging stance for stability, and both groups developed an ankle joint to prevent the newly acquired plantigrade foot from twisting on the ground. Eyes used in air differ from aquatic visual organs because of the differences in refractive index of the two media, and organs of hearing used in air are capable of perceiving higher-frequency sounds than in water. Reproduction and dispersal is much easier in the sea, where gametes can be simply released into the water, and larvae disperse in ocean currents. On land, internal fertilization and courtship is the norm. Crabs (Figure 9) and amphibians (Figure 2), for example, need to find water to breed, but the amniote egg of higher tetrapods (Figures 10 and 11) has removed the dependence on aquatic environments. Insect eggs have a complex coat to prevent both drowning and water loss.

Terrestrial adaptations can be determined in living animals from their anatomy and physiology, but to determine the sequence and timing of events during the major phase of terrestrialization in the Palaeozoic, the fossil record holds the only clues. Complex terrestrial biotas, based mainly on arthropods and plants, had developed by the Devonian period; colonizations by vertebrates, mollusks, and crustaceans followed these early pioneers much later, into already well-established ecosystems.

Figure 11 Armadillo (Amniota, Mammalia: *Dasypus novemcinctus*, Arkansas, USA); © the author.

The Fossil Record

Fossil evidence for the colonization of the land by animals (Figure 12) comes from two sources: indirect evidence from the sedimentary environment, such as fossil soils or traces of terrestrial organic molecules, and trace fossils; and direct morphological evidence from the fossils themselves. Trace fossils are evidence of the existence of organisms without any actual remains; examples include fossil footprints and trackways, worm trails, feeding structures, burrows and nests, resting traces, and chemical signatures. Body fossils, as the name implies, are actual remains of the organism, though these need not consist of the original material, which may have been replaced by other substances and, indeed, impressions of the animal's body in the sediment are also included here.

Trace Fossils

Fossil trackways indicating that aquatic animals hauled themselves out of the water and across subaerially exposed sediments date back to the latest Cambrian period (ca. 488 Ma: MacNaughton *et al.*, 2002). However, whether these animals were habitually terrestrial or were aquatic animals sprinting from one pool to another to survive desiccation is not clear. Moreover, evidence for the sediment being exposed to the air (e.g., mud cracks) does not necessarily tell us whether the tracks were made under water and then exposed, or the mud was already drying and cracking when the trackway was made (Braddy, 2004). Nevertheless, a Cambro-Ordovician origin of terrestrial animals was suggested by Rota-Stabelli *et al.* (2013) using molecular clock analyses of extant Ecdysozoa.

Many of the early Paleozoic trackways have been ascribed to myriapod-like animals, but it would be unwise to accept them as explicit evidence for the existence of myriapods (millipedes, Figure 2, and centipedes, Figure 13) as we know them. It is conceivable that there were other extinct arthropods around at this time with multiple limbs capable of leaving such impressions (see discussion in Dunlop *et al.*, 2013).

Body Fossils

While with trace fossils, sedimentological evidence is used to reveal terrestriality, to interpret body fossils, we need to find morphology that indicates land life unequivocally. For example, a lung is clearly a terrestrial adaptation; but legs need not be: they occur in amphibians, for example. Similarly, it is unwise to assume that a fossil is of a terrestrial animal just because its modern relatives live on land today. For example, while all modern onychophorans live in damp forests, their Paleozoic relatives were marine.

The earliest multicellular animals on land may well have been tardigrades. These tiny 'water bears' are extremophiles; that is, they can live in environments which would be lethal to other animals, including deep oceans, under ice, the tops of the highest mountains, hot springs, and they can even survive the vacuum of space (Jönsson *et al.*, 2008)! These tiny animals undergo cryptobiosis: if the environment becomes extreme, they metamorphose into cyst-like tuns, from which they emerge when circumstances become favorable again. Their oldest fossils are Cambrian in age (ca. 509 Ma: Müller *et al.*, 1995), from marine sediments. However, their propensity to form tuns, which can then be dispersed by the wind, means that they would have easily accessed emergent land surfaces, and likely survived there. Unfortunately, their small size means that they are rare as fossils, and the oldest terrestrial tardigrade fossil comes from amber of Cretaceous age (ca. 80 Ma: Cooper, 1964).

Excluding a very dubious record of an Ordovician mite, the oldest metazoan fossils that can confidently be considered terrestrial are millipedes from the Silurian (ca. 428 Ma) of Scotland (Wilson and Anderson, 2004). This fossil, *Pneumodesmus newmani*, shows spiracles along the side of the body; these are openings of the tracheal system by which the animal would have breathed air. Thus, there is direct morphological evidence of terrestriality. Coprolites occur in rocks of late Silurian age of the Welsh Borderland which have been attributed to detritivorous animals, probably millipedes (Edwards *et al.*, 1995). A few scorpion fossils are known from rocks of middle and late Silurian age (ca. 430–420 Ma). For example, *Dolichophonus loudonensis*, also from Scotland (Laurie, 2012) is the oldest known arachnid, and is about the same age as *Pneumodesmus*. However, there has been a debate for many years about whether the early scorpions were terrestrial or aquatic. An aquatic mode of life for early scorpions was the prevailing opinion for most of the twentieth century but, more recently, this has been questioned because of the lack of obvious morphological evidence. Some, younger, scorpion fossils do seem to have had gills, but it has been argued that these animals migrated into the freshwater habitat from the land. So, it seems that, based on trackways, coprolites, and body fossils, millipedes were the earliest recognizable animals on land.

Ludford Lane

Not far behind the millipedes, in terms of their fossil record, came the centipedes (Figure 13) and arachnids (Figures 6 and 7). Late Silurian fossils from the ca. 419 Ma locality at Ludford Lane, Shropshire, England, consist of tiny pieces of cuticle which are extracted from the sediment using acid (Figure 14). These

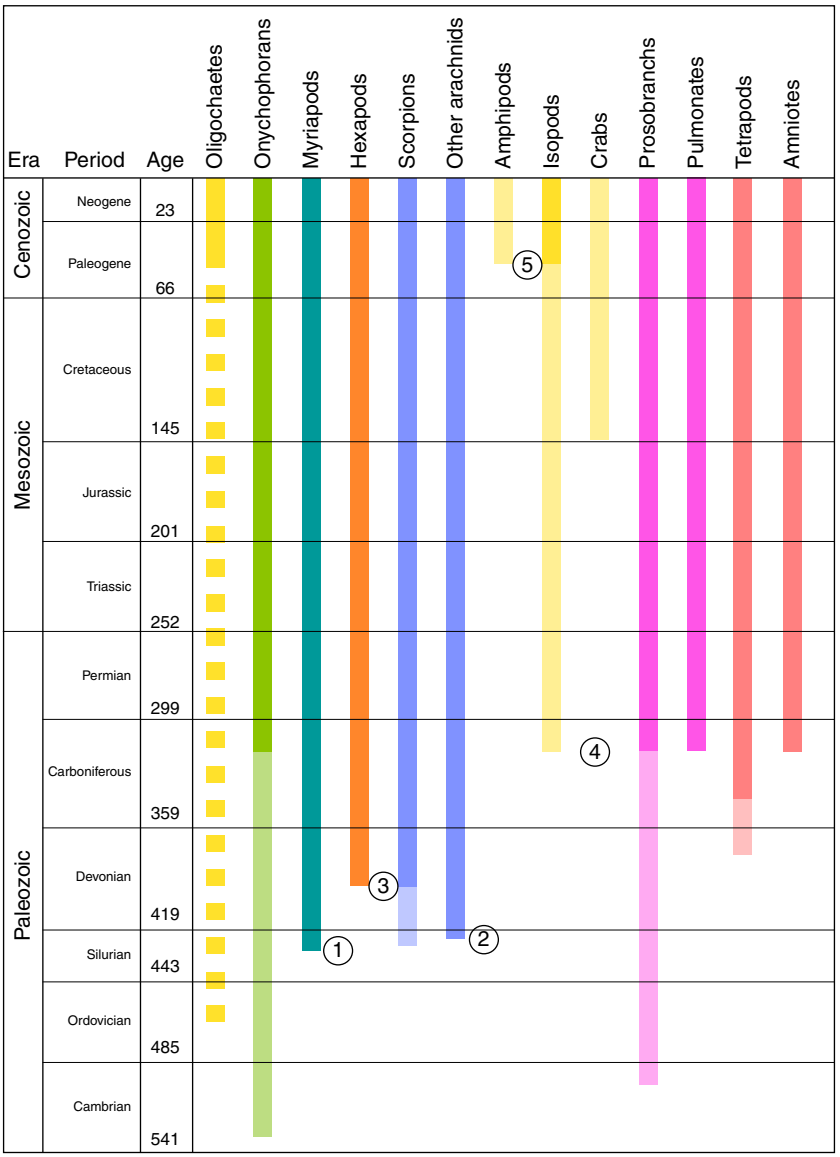

Figure 12 The body fossil record of terrestrial animals. Lighter colors refer to earlier aquatic record; dashed line refers to uncorroborated fossil record. Numbered points refer to important localities in the record of terrestrial animals: (1) Stonehaven, Scotland (earliest land animal, a millipede); (2) Ludford Lane, England (early terrestrial biota, including arachnids and myriapods); (3) Rhynie Chert, Scotland (early terrestrial biota); (4) Late Carboniferous sites such as Mazon Creek, Illinois; (5) Baltic amber; © the author.

fragments include the oldest centipede (Figure 15), probably related to the modern House centipede *Scutigera* (Jeram *et al.*, 1990; Shear *et al.*, 1998). Scutigeromorphs (e.g., Figure 13) are thought to be fairly primitive among centipedes. There is also a millipede belonging to the extinct arthropleurids (Shear and Selden, 1995). Also at Ludford Lane is the oldest non-scorpion arachnid, belonging to the extinct spider-like trigonotarbids (Figure 16). Younger trigonotarbids show book lungs, indicating true terrestriality, so this habitat is also assumed for the Ludford Lane fossil too. The detritivore coprolites, mentioned above, come from this famous locality.

Ludford Lane presents a very early terrestrial ecosystem based on detritus feeders (e.g., millipedes), which were preyed upon by carnivores (e.g., centipedes, arachnids). There is no evidence for herbivorous animals, as we would understand them today: i.e., animals chewing leaves and other green parts of plants. It appears that this type of food web prevailed until the late Paleozoic, by which time the symbiotic fungi and bacteria in the guts of herbivores had developed to allow internal decomposition of green plants (Shear and Selden, 2001).

Rhynie Chert

Returning to Scotland, the next youngest locality bearing terrestrial animals is of early Devonian age: the Rhynie and Windyfield cherts of Aberdeenshire, Scotland, of ca. 410 Ma age. These two adjacent localities preserve an entire terrestrial ecosystem of early plants and animals, in extraordinary

Figure 13 Centipede (Chilopoda: *Thereuropoda longicornis*, Taiwan); © the author.

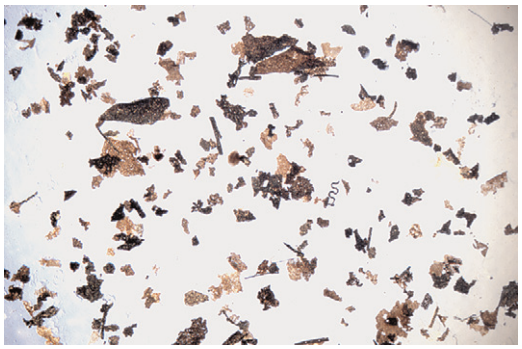

Figure 14 Minute fragments of plant and arthropod cuticle derived by maceration of Ludford Lane sedimentary rocks (Silurian, Ludford Lane, Shropshire, UK) in hydrofluoric acid; © the author.

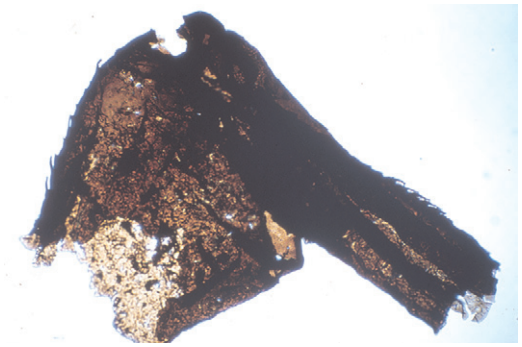

Figure 15 Knee joint of a scutigeromorph centipede (Silurian, Ludford Lane, Shropshire, UK); © the author.

Figure 16 *Palaeotarbus jerami*, the oldest known trigonotarbid arachnid (Silurian, Ludford Lane, Shropshire, UK); © the author.

Figure 17 Piece of Rhynie Chert (Devonian, Aberdeenshire, Scotland) packed with rhizomes of early land plants; © the author.

three-dimensional detail, in siliceous rock produced by a Devonian hot spring (Figure 17). More trigonotarbid arachnids occur here (Figure 18), as well as the oldest mites, for example, *Protacarus crani*, the harvestman *Eophalangium sheari* (Dunlop *et al.*, 2004). Also of significance in the Rhynie fauna are the oldest hexapods (including insects). There is a springtail, *Rhyniella praecursor* (Hirst and Maulik, 1926; Scourfield, 1940), and also the oldest true insect, *Rhyniognatha hirsti*, known only from its mandibles (Engel and Grimaldi, 2004). To complete the fauna known from Rhynie and Windyfield, there are enigmatic euthycarcinoids, centipedes, and another possible hexapod (Fayers and Trewin, 2005).

Gilboa

The Middle Devonian (ca. 390 Ma) Gilboa, New York, locality records the oldest terrestrial fossils in North America. It has

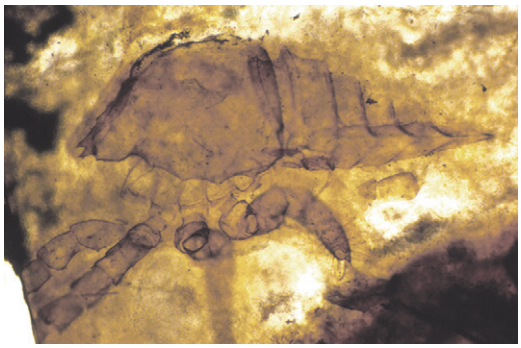

Figure 18 *Palaeocharinus*, a trigonotarbid arachnid from the Rhynie Chert (Devonian, Aberdeenshire, Scotland); © the author.

yielded trigonotarbid arachnids, as well as oribatid and alicorhagiid mites, the oldest pseudoscorpion, and the enigmatic uraraneid arachnids. *Attercopus fimbriunguis* was originally thought to be an odd trigonotarbid (Shear *et al.*, 1987), but was later reinterpreted as the oldest spider (Selden *et al.*, 1991). More recently, it was shown to be an extinct, spider-like animal, and named as a new arachnid order: Uraraneida (Selden and Shear, 2008). *Attercopus* produced silk, but lacked the typical spinneret organs which define true spiders. The Gilboa locality also has centipedes, including another scutigeromorph (Shear and Bonamo, 1988; Shear *et al.*, 1998).

Younger Records

Fossil insects from the Devonian are surprisingly rare. The record of a bristletail from Gaspé, Canada (Labandeira *et al.*, 1988) has been seriously questioned as to whether it is a real fossil or a modern contaminant (Jeram *et al.*, 1990). More recently, Garrouste *et al.* (2012) reported the discovery of a terrestrial insect, *Strudiella devonica*, from the late Devonian (ca. 365 Ma) of Belgium. This find, too, has been disputed (Hörnschemeyer *et al.*, 2013). So, it appears that hexapods, including the insects, did not become common in the fossil record until later in the Paleozoic.

Crustaceans did not get onto land until much later, and even today only a few groups are fully terrestrial. The oldest terrestrial isopods are known as fossils from Eocene (ca. 49 Ma) Baltic amber; the oldest known amphipods are even younger, Miocene in age (ca. 16 Ma), from Mexican amber; and land crabs date back only to Quaternary times (ca. 3–2 Ma).

The review so far has dealt mainly with arthropods. Having a fairly tough cuticle, these fossilize fairly readily, unlike earthworms, for example. It was also in the Devonian that the first vertebrates began to attempt terrestrialization. Tetrapods (limbed vertebrates) had evolved by Devonian times and there was a high diversity, but they are thought to have been aquatic, similar to salamanders of today (Clack, 2009). Following the Devonian, there is a barren time period known as Romer's Gap (after the vertebrate paleontologist A. S. Romer who first recognized it), which stretches from ca. 360–345 Ma into the early Carboniferous period. The first tetrapod fossils to appear after Romer's Gap include terrestrial forms; however, fossils are now appearing which hint at morphological adaptations for land life, at least, from within this gap (Clack and Finney, 2005).

Routes onto Land

It has already been hinted that animals adapted to the extremes of life on the seashore are already pre-adapted to life on land. This route proved the most successful for invertebrate colonizers, and was apparently taken by nemertines, polychaetes, many land mollusks, most crustaceans, chelicerates, and probably myriapods and hexapods (Little, 1983). Evidence comes from study of the osmoregulation abilities of the living animals; that for a marine route onto land for Crustacea and Mollusca is strong, but is less so for other groups. Interstitial environments allow a more gradual transition in salinity from marine to terrestrial than that encountered by the epifauna. Arthropods show relatively high osmotic concentrations which, in the case of the relatively small myriapods and hexapods, can be compared with the high osmotic concentrations in small crustaceans, and so a marine route is suggested. Conversion of the book-gill into the book-lung in arachnids is a good example of the use of an existing aquatic respiratory organ to breathe air.

A freshwater route onto land was apparently used by platyhelminths, annelids, prosobranch mollusks, crayfish, some crabs, and vertebrates (Little, 1983). The correlation between osmotic concentration and routes onto land shown by crustaceans can also be seen in the mollusks. The most successful are the pulmonates (slugs and snails), which have a relatively high osmotic concentration; in contrast, prosobranchs have a low osmotic concentration and are generally restricted to humid tropical forests where fatal desiccation is less likely.

The success of terrestrial vertebrates, having taken the freshwater route onto land, contrasts with those successful invertebrate groups with largely marine ancestors. The larger tetrapods could spend longer on land without the threat of desiccation because of their relatively low surface area to body mass ratio. Also, freshwater origins conferred the ability to osmoregulate efficiently, and a large size and waterproof skin allowed them to overcome the problem of water availability on land. Littoral animals need to breathe air fairly continuously for long periods while awaiting the return of the tide, so many have adapted pre-existing gill structures for air breathing. In contrast, animals in poorly oxygenated freshwater are intermittent air-breathers (e.g., coming to the surface to gulp air) and so many developed new organs to take in large volumes of air at a time. Palaeontological evidence also points to a freshwater route for the terrestrial vertebrates.

Some animal groups evolved from already terrestrial ancestor; hexapods for example, for whom the problems of terrestrialization had already been solved by their ancestors.

Conclusion

It seems likely that, among metazoans, small, cryptobiotic animals were the first to reach land from the sea. Fossil evidence for this is, however, sparse. The oldest recorded land animals are millipedes, which would have lived on detritus from early land plants. There is evidence for these in the form of body fossils and traces fossils (coprolites). Other arthropods

followed, with tetrapods emerging later, possibly within the barren period known as Romer's Gap (ca. 360–345 Ma).

Once on land, animals went on to do quite remarkable things. A number of groups took to the trees, which had appeared in Devonian times, and later the air between the trees. As insects took to the skies, so their main predators, the spiders, followed them up with their elaborate webs. The tetrapods then took to the air: pterosaurs and birds, and later, bats. Finally, many land animals returned to the water; freshwater insects, for example, and marine mammals: the whales and dolphins.

See also: Complexity, the Role of Oxygen in Evolution of. Fungal Evolution: Aquatic–Terrestrial Transitions. Metazoans, Origins of

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Land Vertebrates, the Origin and Evolution of

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Glossary

Anoxic Anoxic refers to depleted oxygen content, lacking or almost lacking available oxygen.
Autopod The distal subdivision of a limb (hand or foot) is called autopod.
Buccal The mouth cavity is called buccal cavity. In buccal pumping, expansion and contraction of the mouth cavity powers breathing, promoting movement of either water or air into the gills or lungs.
Cladogram A diagram showing the branching pattern of relationships between groups and taxa.
Distal Something further away from the body.
Monophyletic Part of a single group containing all the descendents of a single ancestor.
Phylogeny The pattern of relationships between animal species and among monophyletic groupings.
Proximal Something nearer to the body.

Who Are the Land Vertebrates?

Land vertebrates today are a very diverse set of creatures, but their origins go back to the Paleozoic era, when vertebrate animals first ventured onto the land. Since then they have radiated into every continent, and undergone often radical evolutionary changes. They don't only live on land: some have taken to flight (birds, bats), others to swimming (whales, manatees), some have reduced the number of fingers and toes (horses, many salamanders), or lost their legs altogether (snakes, caecilians). Nonetheless, they belong to a single lineage or monophyletic group known as tetrapods. The name means 'four legs.' Their early ancestors evolved four limbs with elbow, wrist, knee, and ankle joints, and which ended in fingers and toes (digits). These limbs could both support their weight against gravity and provide enough flexiblity to allow a walking step. Today, the tetrapods consists of two quite distinct groups: the Amphibia, which includes salamanders, frogs, and caecilians; and the Amniota, which includes mammals, turtles, lizards, crocodiles, and birds (Figure 1). These two groups have been separate for at least 300 million years. They have a rich fossil record that can help us understand their evolutionary history, but to understand how early tetrapods worked it would be ideal to look at modern analogs. However because modern tetrapods and the earliest fossil examples are very different in many respects, it is a challenge to find modern analogs for understanding things like the early tetrapods' physiology and sensory systems, their locomotory capabilities, and their reproductive strategies. While taking information from modern groups into account, we must also make inferences from skeletal features and phylogenetic relationships of the earliest tetrapods and their relatives.

Modern amphibians are highly specialized animals with many unique features (Figure 2). The earliest fossil record of representatives of modern groups is actually relatively recent, from only about 200 million years ago (mya). The earlier history and origin of this group is strongly debated by paleontologists. Today, amphibians are united by the possession of features of the skin and sensory systems that do not fossilize well or at all. They are not defined by their reproductive system. In fact, amphibians as a whole show the most diverse evolutionary strategies to be seen among tetrapods. They are defined by their relationships to each other and to amniotes. Amniotes are defined by possession of egg membranes supporting the embryo during its development (Figure 3). These may be contained in an egg with a shell, or within the mother's body. These membranes do not usually fossilize either. In both cases, for fossils, we have to use skeletal anatomy to work out relationships to modern forms (see any vertebrate biology text book such as Kardong, 2014).

For many fossil species, it is clear where they belong – for example, dinosaurs are evidently related to birds and crocodiles, and snakes are evidently related to lizards. However, for other, usually older species, this is not always straightforward. Early in their evolutionary history, the first creatures with limbs and digits are not obviously related to either modern group, but pre-date the split. Thus, they are neither amphibians, nor amniotes, but simply 'early tetrapods.'

A Phylogeny and Timescale for Tetrapod Origins

Tetrapods, both recent and extinct, belong to sucessively wider groups that includes their ancestors, 'fish' with fins and scales. Tetrapods belong to a group called tetrapodomorphs, and this includes many species that became extinct about 360 mya, at the end of the Devonian period (Figure 4). The group is characterized by having an internal nostril called a choana, and by having bones equivalent to the two forearm bones – radius and ulna – of tetrapods (Figure 5). It is seen for example in the tetrapodomorph *Eusthenopteron* (Figures 6 and 7). In turn, this group sits within the lobe-finned fishes or sarcopterygians, characterized by fins that are attached to the body via a single bone, equivalent to the tetrapod humerus and femur. The earliest lobe-fins are found in rocks about 420 mya, near the beginning of the Devonian. Today, the only other living lobe-fins are lungfishes and coelcanths. The sarcopterygians are quite distinct from the main array of fishes in waters today, the ray-finned fishes, or actinopterygians.

At the present time, the earliest skeletal fossil record of tetrapods that had digits dates from around 380 mya in the Late Devonian (Ahlberg, 1998), although possible trackways

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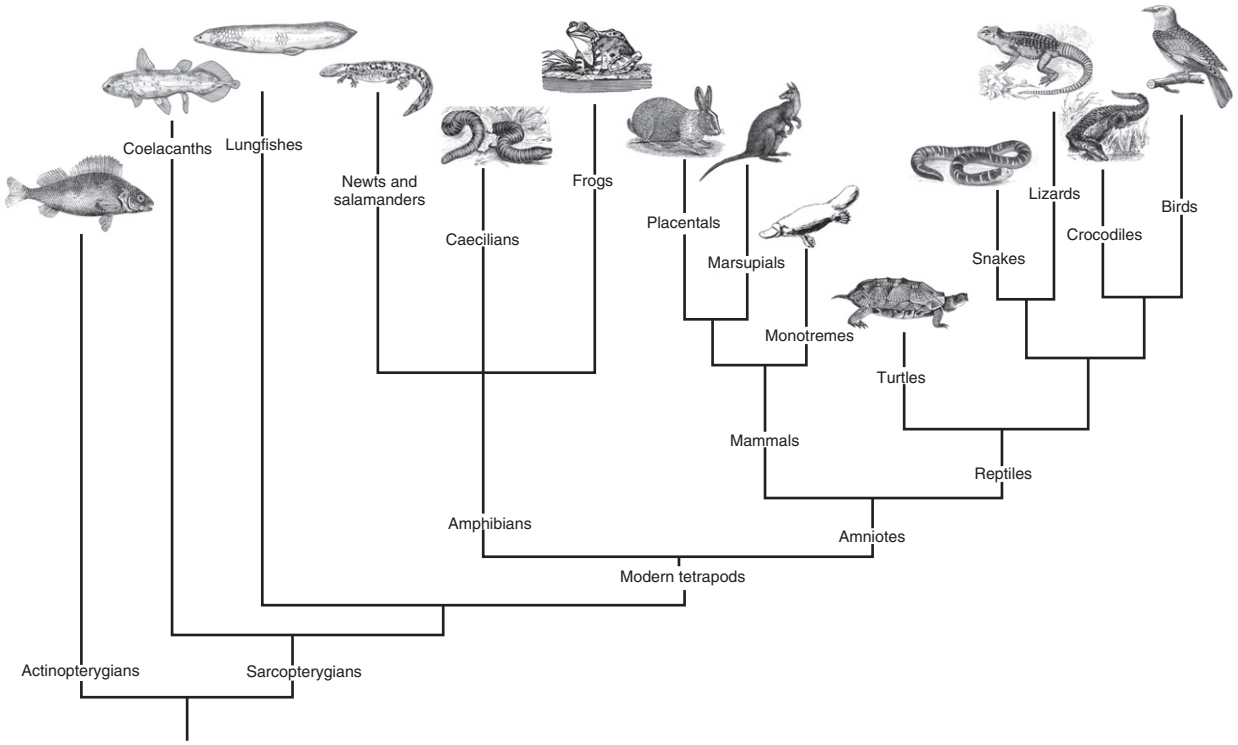

Figure 1 Family tree of bony vertebrates, showing relationships between modern tetrapods and other moden sarcopterygians. There is no signficance to the vertical lengths of the branches other than to accommodate the images.

Figure 2 Photograph of a salamander, a modern amphibian.

made by tetrapods have been found dating from nearer 390 mya in the Middle Devonian (Niedzwiedzki *et al.*, 2010). The earliest modern-type foot with five digits has been found in rocks about 345 mya in the Early Carboniferous.

What Were Conditions Like during the Late Devonian?

The Late Devonian was a time of biotic crisis caused by glaciations in the southern hemisphere. In the rocks there is a record of black shales formed in anoxic water conditions, the cause of which is disputed, but which is indicative of some great changes in environmental conditions. Falls and rises in sea levels coincident with glaciations and their melting are one possible explanation, or the evolution of large and deciduous

Figure 3 Photograph of a turtle hatching. A turtle is a modern amniote, having an egg with a protective shell.

plants is another (McGhee, 2013). Global temperatures are thought to have been relatively cool during this time interval. It is known that in the latter stages of the Late Devonian, known as the Famennian stage, oxygen levels were at or possibly above those of the present-day atmosphere, although earlier in the Devonian, levels had been much lower.

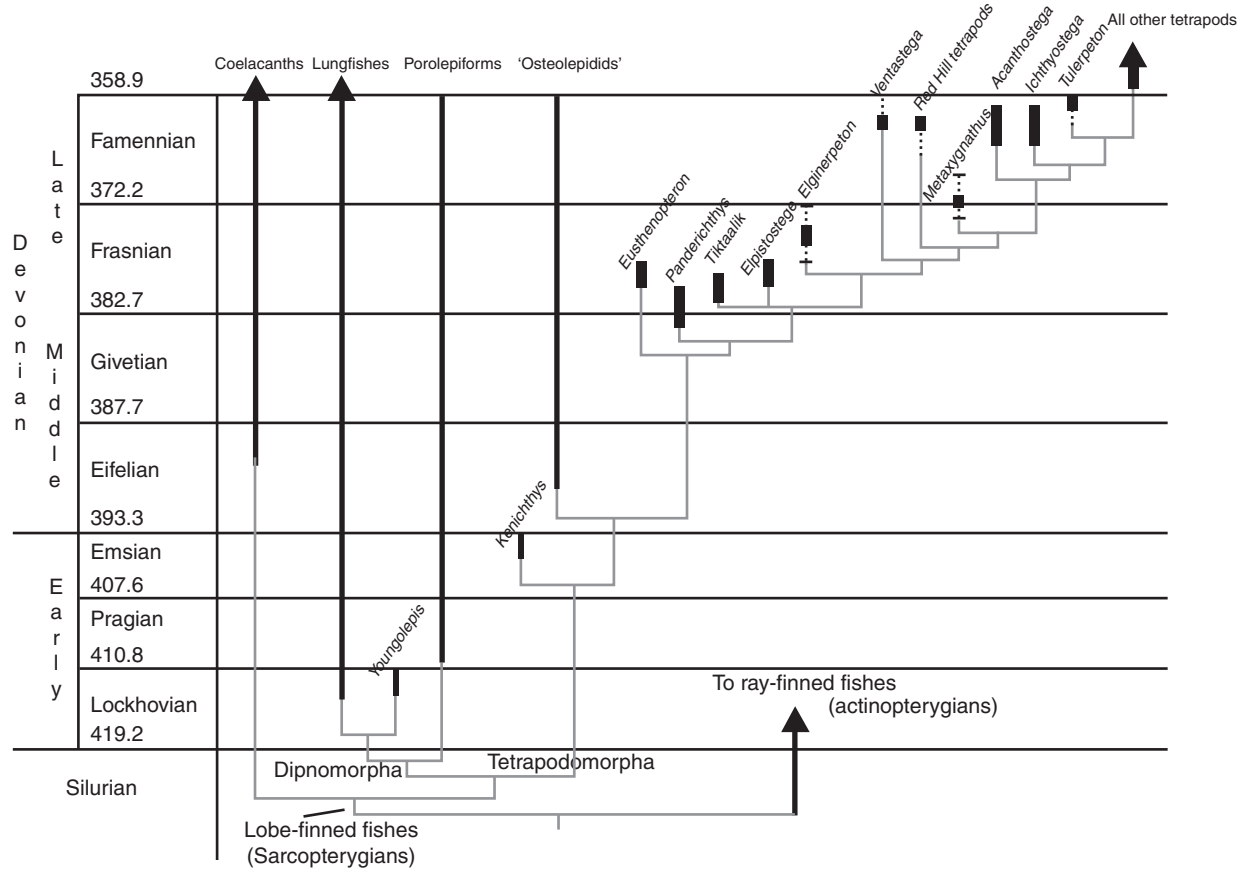

Figure 4 A timescale of the Devonian period with a cladogram of sarcopterygians superimposed. Heavy bars show the time ranges of the groups or individual taxa, and a terminal arrow indicates the modern survivors. Reproduced from Clack, J.A., 2012. Gaining Ground: The Origin and Evolution of Tetrapods, second ed. Indiana: Indiana University Press. Material appears courtesy of Indiana University Press. All rights reserved.

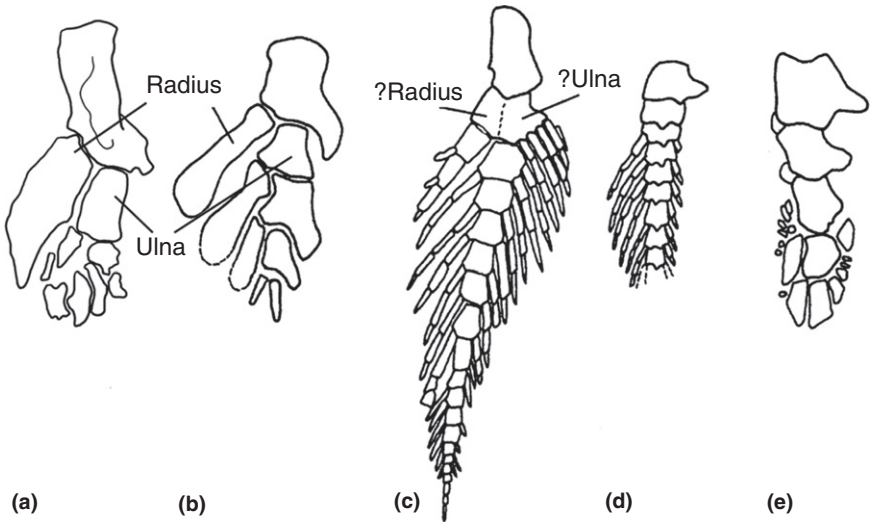

Figure 5 Fin skeletons of the lobed fins of sarcopterygian groups: (a) the Devonian tetrapodomorph *Panderichthys*, (b) the Devonian tetrapodomorph *Eusthenopteron*, (c) the modern lungfish *Neoceratodus*, (d) the Devonian porolepiform *Holoptychius*, (e) the modern coelacanth *Latimeria*. Homologs of the radius and ulna of tetrapods are indicated. Reproduced from Clack, J.A., 2012. Gaining Ground: The Origin and Evolution of Tetrapods, second ed. Indiana: Indiana University Press. Material appears courtesy of Indiana University Press. All rights reserved.

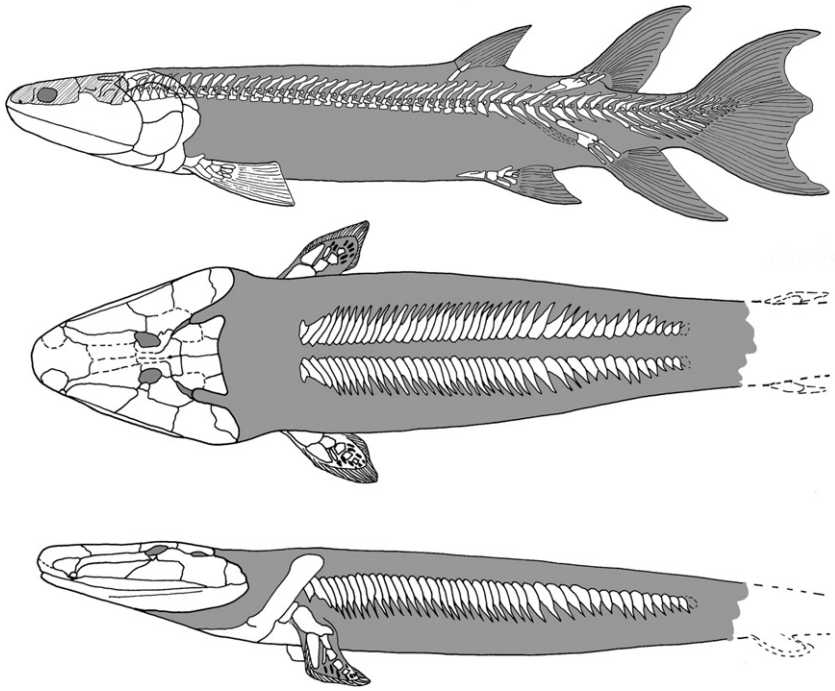

Figure 6 Two Late Devonian tetrapodomorphs: Top, *Eusthenopteron*. Middle and lower, *Tiktaalik* in dorsal and lateral views respectively. Reproduced from Clack, J.A., 2012. Gaining Ground: The Origin and Evolution of Tetrapods, second ed. Indiana: Indiana University Press. Material appears courtesy of Indiana University Press. All rights reserved.

Figure 7 Photograph of *Eusthopteron foordi* fossil. University Museum of Zoology (UMZC) specimen GN 784.

What Stimulated the Evolution of Land-Going by Vertebrates?

This is a question that is often asked, but is hard to answer definitively. In fact there was probably not a single cause, but the interaction of many influences. Some ideas include the fact that land had been colonized earlier by plants, and by the Late Devonian, these included large tree-like forms and a diversity of ground-covering species. These must have provided shelter from desiccation for emerging early tetrapods. In the undergrowth, many invertebrates were to be found, providing food for the tetrapods, in an environment devoid of any predators. In the water were large predatory fish that could have preyed on the early tetrapods or their young, perhaps pushing the tetrapods to the water's edge (McNamara and Selden, 1993). Prior to the emergence of tetrapods with limbs, the low oxygen regime of the Early and Middle Devonian stages might have given advantage to air-breathing forms living in waters that were anoxic (Clack, 2007).

Among Lobe-Fins, What Advantages Were Crucial to the Move onto Land?

It is sometimes mistakenly assumed that breathing air was the great advance of early tetrapods, but there is evidence to suggest that air-breathing to some degree was found in all bony vertebrates, including ray-fins, during their early evolution. Lobe-fins appear to have specialized in this, being bimodal breathers using both gills and lungs, as do modern lungfish. Thus tetrapods were already air-breathers before they redeveloped their fins into limbs.

The internal fin skeleton of lobe-finned fishes (Figure 5) is built of robust bony elements that were not characteristic of ray-finned fishes. The type of bone is called endochondral, in which the bone replaces a cartilaginous precursor. It allows the development of new features as it is remodeled in response to different conditions. This kind of bone is also readily adapted to form the vertebral column and ribs that are greatly developed in tetrapods. The single bone forming the most proximal element of the fin or limb is joined to the body via a ball and socket joint that allows a wide range of motion at the shoulder or hip. It also facilitates the alternate movement of fore- and hind limbs on opposite sides of the body, typical of tetrapods, but also seen in lungfishes and coelacanths (It is also seen independently in some bottom-dwelling sharks.). This may have been an advantage to animals living in shallow waters, or in an ambush predator, moving one limb at time, slowly advancing through the weedy undergrowth.

What Were the Earliest Tetrapods and Their Immediate Ancestors Like?

The fossil record suggests that the tetrapodomorph lobe-fins were mainly large species, reaching a meter or more in length. Some, such as *Tiktaalik*, from the early Late Devonian (**Figure 8**), indeed are known that were at least 2.75 meters long (Daeschler *et al.*, 2006; Shubin *et al.*, 2006; Shubin, 2009). They typically had large heads in relation to their bodies as compared with modern amniotes, and they appear to have been rather flat. There is an argument about whether the flattening is due to compression during fossilization, but certainly the breadth of the head is unlikely to be exaggerated in that way.

Fishes use their gill skeletons to ventilate their gills, and early tetrapods almost certainly used the same muscles and bones to ventilate their air bladders in a form of buccal pumping. Modern amphibians still use this mechanism. This form of breathing necessitates a large buccal cavity to acquire as much air as possible with each movement, making sense of the large and broad heads of these early tetrapods and their ancestors.

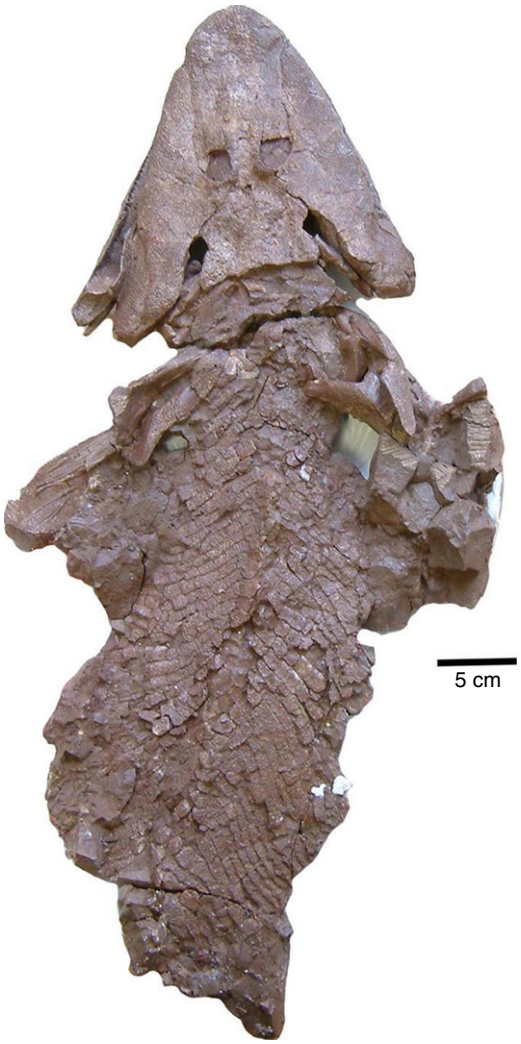

Figure 8 Photograph of *Tiktaalik roseae* (photograph by J. A. Clack).

Primitively, vertebrates used their noses for only olfaction, as is the case still in ray-fins and chondrichthyans (sharks and rays). They have two nostrils on each side of the head, one for water in, and one for water out after it has passed over the sensory tissue in the nasal capsule. In tetrapodomorphs, the port for water out, now called the choana, moved inwardly to lie inside the mouth. This can be seen in the early Late Devonian fish *Eusthenopteron* (Porro *et al.*, 2015). What advantage that conferred is not clear, but it laid down the conditions that were conducive to air-breathing in later forms. It allows air to pass into the mouth and thence into the pharynx and air bladder (i.e., lung).

Tetrapodomorph fishes and early tetrapods had skulls reinforced by dermal bone, formed directly in the skin. In the fishes, this covering was attached at the top to similar bones in the shoulder girdle, forming a smooth hydrodynamic profile. The cheek plates were mobile, and a slot lower down between the cheek and shoulder allowed water that had passed over the gills to flow out. This meant that the head was effectively kept in line with the body. In tetrapods with limbs, this connection was lost and the joining bones disappeared. The head could thus be moved relative to the body, both up and down and side to side. The beginnings of a neck was formed. This is seen in the tetrapodomorph *Tiktaalik* (**Figure 8**).

The proximal element of the forefin, the humerus, was particularly robust, and bore processes and flanges to which substantial muscles must have attached. Articulating with this bone were the equivalent of the radius and ulna, although their proportions were different from what they became in tetrapods. The radius was a long narrow bone and the ulna was a short stout one. Beyond those lay a series of smaller elements whose pattern varied according to the species, but which like the other bones were robust with substantial joint surfaces (**Figure 5**).

One major difference between tetrapodomorph fish and tetrapods with limbs and digits is the size of the hind fin or limb and its associated pelvic girdle. In the fish, as in almost all fishes today, the pelvic fin and girdle were all small elements, much smaller than the forefin and shoulder girdle. At some point in their evolution, the tetrapods with limbs enlarged the hind limb and girdle, joining the latter to the vertebral column. The beginnings of changes to the pelvis can now be seen in *Tiktaalik* although the fin skeleton itself including the femur equivalent is not known (Shubin *et al.*, 2014).

The earliest tetrapods in which the more distal bones are true digits are from the late Late Devonian, and are best known in three taxa: *Acanthostega*, *Ichthyostega* (**Figure 9**), and *Tulerpeton*. In this case, the most distal elements are more organized, with the elements arranged in succession with joints between them, as in modern digits. Essentially that is the definition of a digit. However, unlike modern tetrapods, these animals all had more than five digits, namely, eight, seven, and six respectively (Clack, 2012). In *Acanthostega*, the joints of the fore- and hind limbs were not capable of bearing weight, nor of rotating at the wrist of ankle (**Figure 9**). Effectively, they were paddles. In *Ichthyostega*, the hind limb was also a paddle-like structure, although the hand remains unknown (Coates and Clack, 1990; Coates, 1996). In both these forms, the

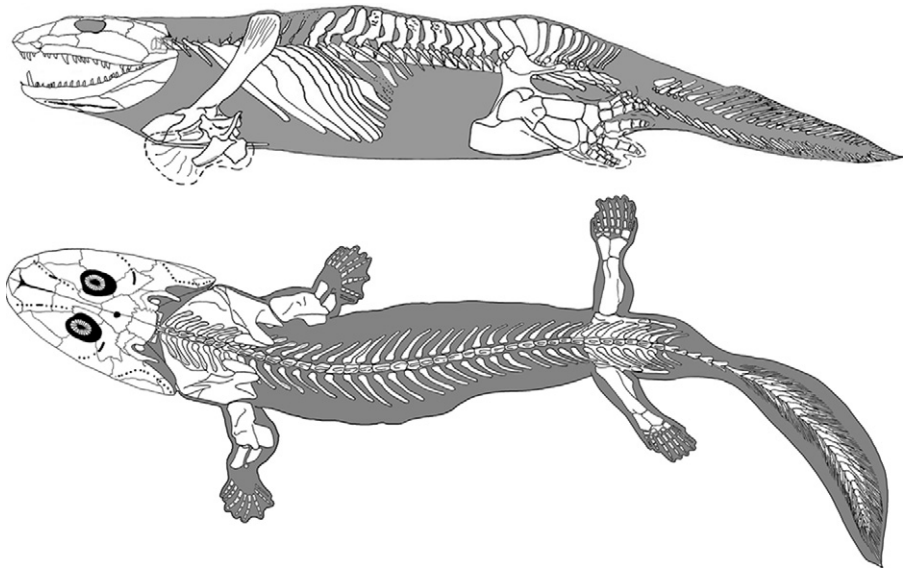

Figure 9 Two Late Devonian tetrapods: Top, *Ichthyostega*. Lower, *Acanthostega*. Reproduced from Clack, J.A., 2012. Gaining Ground: The Origin and Evolution of Tetrapods, second ed. Indiana: Indiana University Press. Material appears courtesy of Indiana University Press. All rights reserved.

Figure 10 A Photograph of the skull and a life model of *Acanthostega gunnari*. The skull is about 120 mm long.

femur and humerus were of similar sizes (Figures 9–11). These two also retained bony fin rays along the tail, features otherwise only seen in fish.

How Did These Early Tetrapods Move?

Given their paddle-like nature, the limbs of these early tetrapods suggest that the animals were mainly aquatic. That is particulary true of *Acanthostega*, and it seems very likely that even if it made forays onto land, it would not have supported its body off the ground, but used a limb-assisted wriggle. Some salamanders use this form of locomotion today, especially for fast movement. The giant Japanese salamander is an example of this, and in fact from the point of view of size and habits is quite a good model for *Acanthostega*.

Recent work on *Ichthyostega* using cutting-edge computer techniques has allowed an assessment of the range of movement of the limbs permitted by the bone morphology. This study showed that the shoulder and hip joints were highly restricted in their range of motion, in a way that suggested the forelimbs were used to provide the main propulsive force. The humerus bore massive muscles holding the forearm more or less horizontal, while the elbow joint was relatively mobile, and took the weight of the forequarters of the animal. The hind limb would move effectively as a paddle when the animal was swimming, but on land would be used only as a stabilizer. It also emerged that it was likely to have used a gait in which both forelimbs moved in concert rather than alternately, producing a shuffling motion. The nearest analogy seems to be with mudskippers rather than any modern tetrapod (Pierce *et al.*, 2012, 2013).

The overall picture seems to suggest one of experimentation with ways of moving on land, and that walking in the conventional sense was not an immediate development.

When Did True Terrestrial Locomotion and Terrestrial Living Develop?

As far as we can tell from the current fossil record, the earliest development of the five digited – pentadactyl – limb did not appear until the Early Carboniferous. A fossil from the earliest Carboniferous, the Tournaisian stage, of Scotland shows a five-digited autopod, although whether it came from a fore- or hind limb cannot be established. Slightly later but also in the

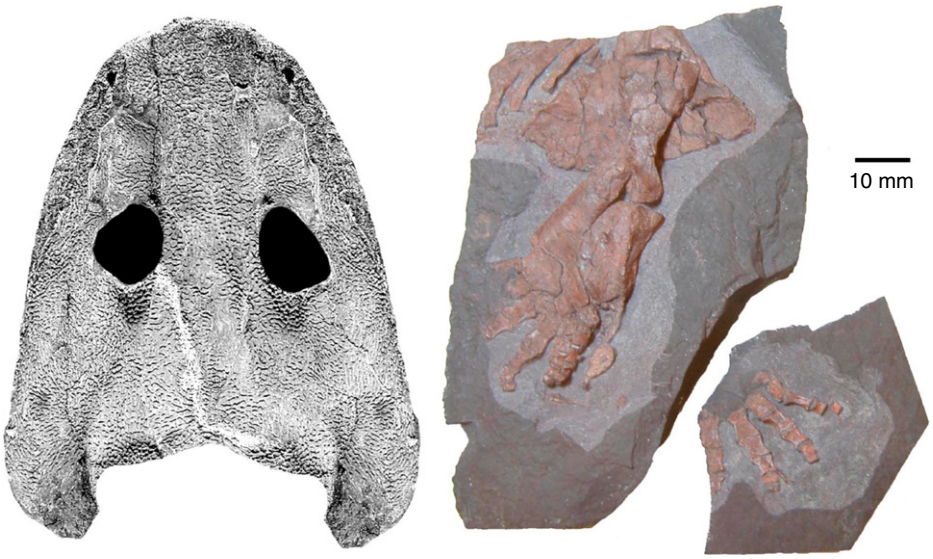

Figure 11 Photographs of the skull and hind limb fossil of *Ichthyostega stensioi* (photographs from UMZC archive).

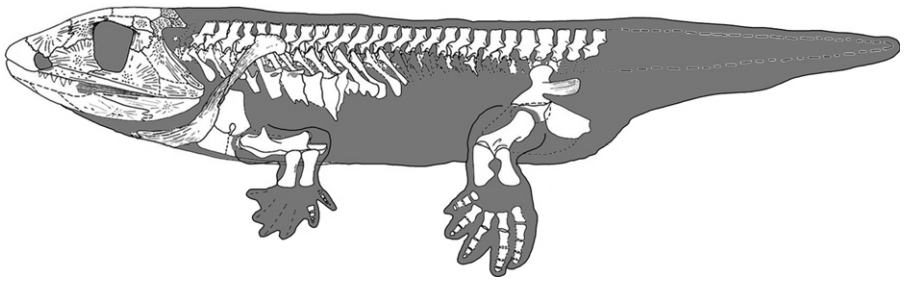

Figure 12 The Early Carboniferous tetrapod Pederpes. Reproduced from Clack, J.A., 2012. Gaining Ground: The Origin and Evolution of Tetrapods, second ed. Indiana: Indiana University Press. Material appears courtesy of Indiana University Press. All rights reserved.

earliest Carboniferous, the skeleton of the tetrapod *Pederpes* (Figure 12) had a pentadactyl hind limb with proportions and shape like that of later Carboniferous tetrapods (Clack, 2012). It had asymmetrical bones in its ankle that suggest a reorientation of the foot so that it pointed forwards rather than to the side as in the earlier forms. Nonetheless it still seemed to have had more than five digits on the forelimb (Clack, 2002).

Looking at the embryonic development of fore- versus hind limbs of modern animals, and combining this with what we see in fossil forms suggests that following the evolution of an enlarged hind limb and associated hip girdle, the hind limb 'took over,' developed ahead of the forelimb and began to provide the main propulsive force for walking and for raising the body off the ground (Coates *et al.*, 2008). However, the morphology of the humerus compared with the femur suggests that prior to that, it was the forelimb that was the major component in motion, raising the front of the body out of the water. The humerus is a much more complicated bone than the femur, and is much more comparable to that of the fish equivalent than is the femur. A gradual sequence of changes to the humerus can be traced across the transition, whereas the femur seems to have been a separate development. Evidence from embryonic development and from genetics supports the idea that fore- and hind limbs were not homologs, but have separate embryonic origins.

What we can say is that by the end of the earliest Carboniferous Tournaisian stage, diverse tetrapods were present. New fossils from the United Kingdom and Canada show a range of sizes and morphologies (Smithson *et al.*, 2012; Anderson *et al.*, 2015), and numerous trackways from Nova Scotia document diverse forms of locomotion from both very large (say 1–2 m length animals), to very small ones (say the size of a small lizard) (Figure 13).

As to 'why five' for the number of digits, the combined ability to have limbs that can bear weight with joints that allow the flexibility for walking may have restricted the possibilities for the form of the wrist or ankle around which five was the most comfortable fit.

Although it is clear that tetrapods could walk on land in a conventional way by the end of the Tournaisian stage, some may argue that full terrestriality was only achieved once the amniotic egg had been developed, allowing the animals to dissociate themselves from the need to reproduce in water. Unfortunately, it is very difficult to determine when this happened, or even what mode of reproduction these early tetrapods might have used.

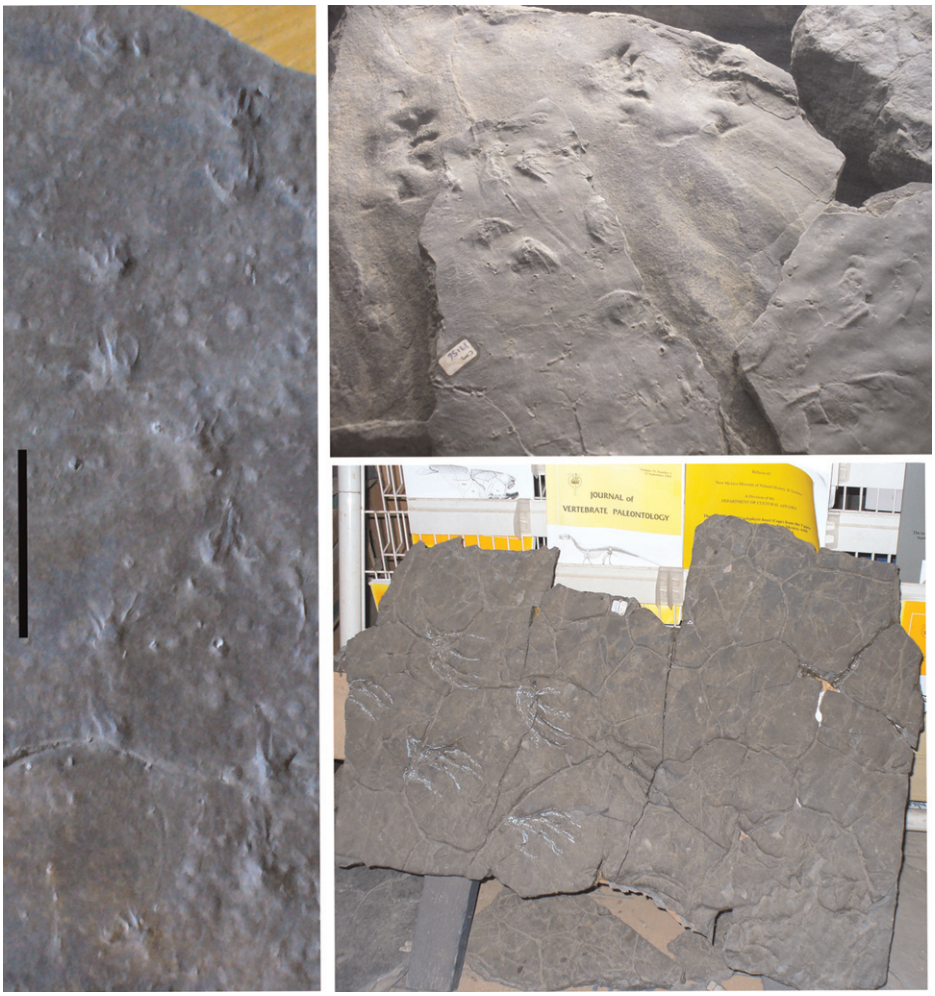

Figure 13 Photographs of Early Carboniferous tetrapod trackways in the Blue Beach Museum, Nova Scotia, showing trackways of different sizes and styles. Scale on the left hand side photograph is 50 mm. Scale on the upper right photograph is given by the museum label, about 18 mm maximum dimension. Scale on the lower right is given by the journal cover, 210 mm across (photographs by J. A. Clack).

See also: Amniotes, the Origin of. Land Animals, Origins of. Vertebrates, the Origin of

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Lichen-Forming Fungi, Diversification of

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Glossary

Ascoma A fruiting body bearing meiosporangia (sporangia in which meiosis occurs) known as asci.
Ascospore Meiospore of ascomycetes is called ascospore.
Columella Sterile tissue in the hymenium of some tropical crustose lichens.
Crustosen Crust-like growth form of the vegetative thallus in lichens.
Cupulate exciple It is a type of ascomatal margin forming a cupula.
Diaspore A vegetative propagule.
Endolichenic Growing inside a lichen.
Foliose Leaf-like growth form of the vegetative thallus in lichens.
Fruticose Growth form with pendulous or upright branches of the vegetative thallus in lichens.
Heterokont Having two morphologically different flagellae.
Hymenium A layer consisting of meiosporangia and sterile elements.
Isidium Corticated outgrowth of the thallus surface for vegetative dispersal of both partners in a lichen.
Lichenicolous Growing on a lichen.
Neoendemic A species that originated recently and is indigenous to a certain environment.
Periderm Cork-like outer layer of a plant stem.
Perithecium Pear-shaped ascoma opening with a small pore.
Photobiont Algal or cyanobacterial partner in a lichen symbiosis.
Soredium Powdery, non-corticated structures on the thallus surface for vegetative dispersal of both partners in a lichen.
Synapomorphy Shared derived character.
Thallus Plant-like vegetative body that lacks differentiation of stem, leaves, and roots.

Lichen-forming fungi form stable symbiotic associations with photosynthetic partners, such as algae and/or cyanobacteria. The fungi mostly belong to Ascomycota and to a lesser extent to Basidiomycota. Most algae in lichen symbioses belong to green algae, but heterokont (Stramenopiles) algae, such as brown algae or yellow-green algae, are also known to form stable associations with fungi. In addition, bacteria and additional fungi (endolichenic and lichenicolous) are found in the lichen symbiosis but their roles in the symbiosis are currently not fully understood. Lichen-forming fungi are a successful group, with almost 20% of all known fungi forming lichen associations, and they occur in all ecosystems: from the polar regions to the tropics. They are able to grow on all sorts of terrestrial substrates, including rocks, soil, wood, bark, and also living leaves. They are unusual for fungi in forming extensive vegetative structures, so-called thalli that have crustose, foliose, or fruticose growth forms (Figure 1). The vegetative structures provide space for the photosynthetic partners that provide nutrients in the form of sugar or sugar alcohols to the fungal partner.

Although some molecular clock-based studies suggested an origin of lichens (Heckman *et al.*, 2001) as early as the Precambrian, other studies suggest a later evolution (Lücking *et al.*, 2009; Beimforde *et al.*, 2014). Part of the problem is the uncertainty with interpreting the fossil species *Paleopyrenomycites devonicus*, which has been widely used to calibrate the fungal tree of life (Berbee and Taylor, 1993; Taylor *et al.*, 1999; Taylor and Berbee, 2006; Berbee and Taylor, 2010). The interpretation of the morphological structures has varied, with the ascoma-type and ascus interpreted differently, either as

Figure 1 Growth forms of lichens. (a) and (b). Crustose lichens ((a) – *Ochrolechia* on siliceous rocks, (b) – *Lecanora* on mosses and detritus). (c) and (d). Foliose lichens ((c) – *Menegazzia* on bark, (d) – *Hypogymnia* on twigs). (e). Fruticose lichen (*Cladia* on soil). Photos (c) and (d) by Todd Widhelm, photo (e) by Sittiporn Parnmen.

being a perithecium with unitunicate asci placing them in the derived Sordariomycetes (Taylor and Berbee, 2006) or as a perithecioid ascoma with operculate asci placing them in the early diverging Pezizomycetes (Lücking *et al.*, 2009). The earliest fossils of lichens are from the lower Devonian (Taylor *et al.*, 1997), which is consistent with a later evolution of this type of fungal symbiosis. In fact, there are no lichenized taxa in basal groups of Ascomycota or Basidiomycota (Lutzoni *et al.*, 2004; Schoch *et al.*, 2009; Prieto and Wedin, 2013), which together with the mostly mycorrhizal Glomeromycota (Redecker and Raab, 2006) form the crown group of fungi (**Figure 2**). The latter group and all other fungal phyla lack any lichenized species.

Within Ascomycota, stable, symbiotic relationships with algae and/or cyanobacteria evolved within the derived Leotiomyceta probably during the Carboniferous (Schwartzman, 2010; Prieto and Wedin, 2013). Although the major Ascomycota lineages with lichenized species originated then, successive waves of diversification in the Jurassic and Cretaceous created the diversity at higher phylogenetic levels (Amo de Paz *et al.*, 2011; Prieto and Wedin, 2013). This subsequently gave rise to the current species diversity that originated between the Eocene and Pleistocene (see below). The diversification of major clades in lichenized fungi, especially in Lecanoromycetidae and Ostropomycetidae, is probably correlated with the major diversification events in angiosperms (Prieto and Wedin, 2013). The angiosperms provided many new environments for epiphytic lichens. Three of the four most diverse families of lichenized fungi, Parmeliaceae (c. 2800 spp.), Graphidaceae (c. 2100 spp.), and Ramalinaceae (c. 800 spp.) are especially diverse on angiosperm bark (Jaklitsch *et al.*, 2015). Although substrate specificity is rare in lichens, a number of species are confined to similar substrates in terms of bark pH, water capacity, and bark hardness (Brodo, 1973). Recent estimates suggest the origin of angiosperms either in the lower Jurassic to lower Cretaceous (Bell *et al.*, 2005; Bell *et al.*, 2010) or in the Triassic to early Jurassic (Clarke *et al.*, 2011).

It is unclear whether lichenization within Leotiomyceta originated once (Lutzoni *et al.*, 2001) or several times (Gargas *et al.*, 1995; Schoch *et al.*, 2009). Experiments suggest that there is a latent capacity for mutualism in fungi and algae (Hom and Murray, 2014). In an experiment, obligate mutualism between the non-symbiotic model organisms *Saccharomyces cerevisiae* (yeast) and *Chlamydomonas reinhardtii* (alga) was induced in an environment requiring reciprocal carbon and nitrogen exchange. This capacity for mutualism was shown to be phylogenetically broad, including other *Chlamydomonas* and yeast species. These experiments showed that under specific conditions, environmental change induces free-living species to become mutualists. Also some fungi occurring in boreal ecosystems were shown to be facultatively lichenized

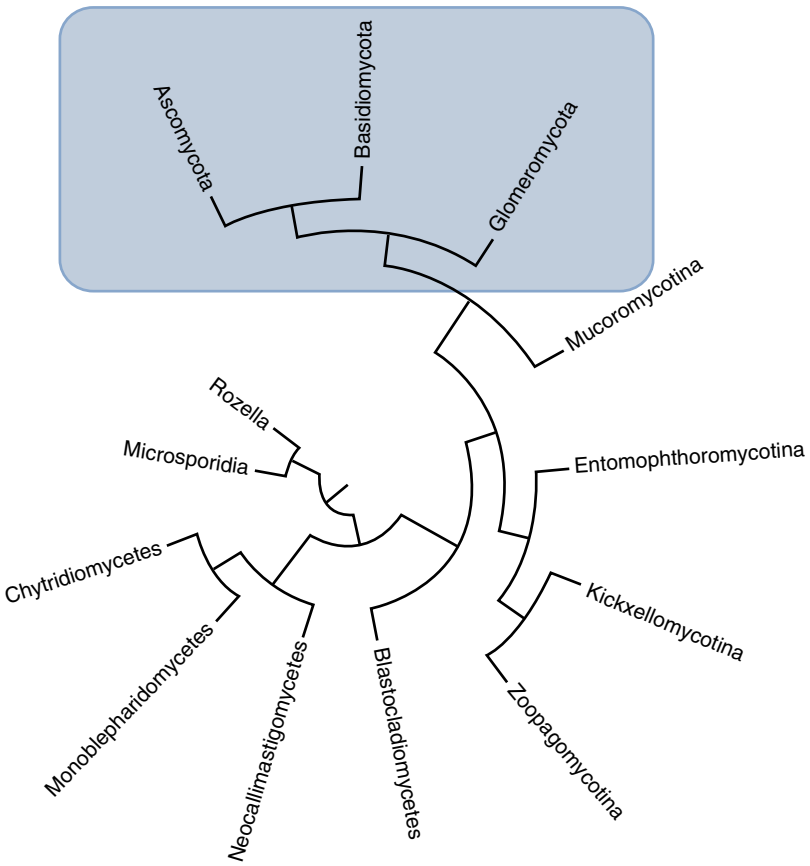

Figure 2 Cartoon tree of the phylogenetic relationships of phyla and other major clades within the fungal kingdom. The crown group of fungi indicated by a blue box.

(Wedin *et al.*, 2004). Phylogenetic analyses showed intermixed groups of lichenized and saprotrophic specimens, suggesting that these related species can undergo their sexual lifecycle either as lichens or as saprotrophs. The flexibility of nutritional modes allows separate individuals to exploit different niches during forest succession. These studies suggest that there is more plasticity in nutritional modes of fungi than previously thought. This is not surprising, given that nutritional modes (parasitism vs. mutualism) and interkingdom host switches have been demonstrated to be common at an evolutionary scale in fungi (Spatafora *et al.*, 2007; Arnold *et al.*, 2009). Some fungi growing on lichens, so-called lichenicolous fungi, have originated from lichenized ancestors, such as the genera *Nesolechia* and *Phacopsis*, which evolved during the Miocene (Divakar *et al.*, 2015). It was even hypothesized that lichenicolous fungi may represent a 'halfway house' to explain evolution of non-symbiotic fungi from lichenized ancestors (Lutzoni *et al.*, 2001; Arnold *et al.*, 2009).

The Evolution of Current Species Diversity in Space and Time

A number of studies have demonstrated that diversification events occurred during periods of climatic changes. For example, in the temperate to boreal crustose genus *Biatora*, the main diversification occurred during phases of climatic cooling when new types of forest vegetation and the arctic-alpine vegetation evolved (Printzen and Lumbsch, 2000). Likewise, the diversification of *Montanelia* was estimated to have occurred during a period of cooling and the development of marked thermal seasonality during the Miocene (Divakar *et al.*, 2012). In the genus *Melanohalea* it was shown that Pleistocene glaciations were not inherently unfavorable for some species and even indicated that some populations were able to expand during Pleistocene glacial cycles (Leavitt *et al.*, 2012a). Molecular data also suggest that closely related species may have experienced different demographic histories. In the genus *Xanthomendoza*, two closely related species with arctic-alpine to montane temperate distributions were studied (Leavitt *et al.*, 2013a). Whereas the data suggested a Late Pleistocene population expansion in *Xanthomendoza montana*, there was evidence for a long-term stability in the demographic history of the bipolar *Xanthomendoza borealis*, which suggests that the latter species was able to survive climatic oscillations without major contraction of its population size. In a study focusing on the phylogeography of *Ramalina menziesii*, a lichen occurring along the west coast of North America, from Baja California to Alaska, multiple lineages were found within the species (Sork and Werth, 2014). Localized lineages were found to be ancient, while some recent lineages were found to be widespread and some of the populations such as the ones in Baja California, were relatively isolated. This study provided evidence that both population persistence and dispersal contribute to the wide range of a genetically diverse species.

The fossil record for lichenized fungi is rather poor and largely restricted to Paleogene amber (Kaasalainen *et al.*, 2015). However, molecular clock approaches have been widely used to address the question of when the current diversity of species found in lichenized fungi evolved. An early study on *Biatora*, which occurs in temperate to boreal habitats mainly of the northern hemisphere, showed that species predominantly diversified during the Eocene and Oligocene (Printzen and Lumbsch, 2000). However, there is a growing body of evidence that a large portion of the current species diversity in lichen-forming fungi is much younger and most studies indicate major diversification happened during the Neogene. During the Neogene period the climate became cooler and drier and mountain systems developed with the uplift of the Himalayas, Alps, and Rocky Mountains, resulting in the alteration of air circulation and weather patterns. These processes likely had a major impact on diversification patterns. In the temperate-to-boreal genus *Melanelixia* (Leavitt *et al.*, 2012b) and the mostly Neotropical genus *Oropogon* (Leavitt *et al.*, 2012c) the main diversification was estimated to have occurred during the Miocene. Interestingly, in these two genera, cryptic species, which are delimited based on DNA sequence divergence, were detected that originated during the Miocene. This indicates that phenotypically cryptic species in lichenized fungi can be relatively ancient and do not necessarily represent recent divergence events. Diagnosable phenotypic differences may be absent even millions of years after their divergence, suggesting that our understanding of morphology and chemistry in these organisms is poor.

The main diversification during the Miocene and Pliocene was found in the genera *Flavoparmelia* (Del-Prado *et al.*, 2013), *Melanohalea* (Leavitt *et al.*, 2012a), *Montanelia* (Divakar *et al.*, 2012), the *Xanthoparmelia pulla* group (Amo de Paz *et al.*, 2012), and the Macaronesian species of *Nephroma* (Sérusiaux *et al.*, 2011). The former three genera all belong to the family Parmeliaceae, which is one of the largest families of lichen-forming fungi (Thell *et al.*, 2012; Kraichak *et al.*, 2015a). *Melanohalea* and *Montanelia* have their centers of diversity in temperate to arctic-alpine regions of the northern hemisphere, whereas *Flavoparmelia* – although being a cosmopolitan genus – has its center of diversity in Australasia. The latter genus occurs in temperate to subtropical areas mostly on tree bark. The *Xanthoparmelia pulla* group, which also belongs to Parmeliaceae, occurs worldwide in areas dominated by a Mediterranean (winter rain) climate. Traditionally, these species were distinguished using phenotypical characters and were assumed to have a subcosmopolitan distribution. However, molecular data, similar to results in the *Parmelina quercina* group (Arguello *et al.*, 2007), demonstrated that distinct lineages were correlated with geographical distributions (Amo de Paz *et al.*, 2012). Five major clades were found, two clades occurring in South Africa and one clade in the Mediterranean basin, Macaronesia, and California, whereas two clades had disjunct distributions, occurring in Australia and South America, and California and South America, respectively. These disjunctions were explained by long-distance dispersal (in the most recent California–South America disjunction it was estimated at 3.44 Ma). In a few genera of Parmeliaceae, molecular data suggest that the diversification predominantly occurred even more recently. In the fruticose genus *Letharia*, which occurs in temperate to boreal areas of western North America, continental parts of Eurasia, and montane areas of northern Africa, the major speciation was estimated to have occurred during the Pleistocene (Altermann *et al.*, 2014).

The genus *Xanthoparmelia* is the most speciose among lichenized fungi with over 800 species (Thell *et al.*, 2012). A clade of *Xanthoparmelia* species occurring in North America has been shown to have diversified mainly during the Pleistocene (Leavitt *et al.*, 2013b). Recently diverged lineages are generally more difficult to separate using molecular data due to incomplete lineage sorting, requiring a large sampling of markers (Leavitt *et al.*, 2013b; Altermann *et al.*, 2014). Whereas this has been a limiting factor examining these species complexes using Sanger sequencing, it is likely that next generation sequencing will allow more of these recently evolved complexes to be thoroughly studied.

The studies addressing diversification events of species complexes in lichens suggest that lichen-forming fungi are not unique, in that diversification is similar in land plants clades of similar age (Linder, 2008). However, lichenized fungi tend to have wider distributional ranges than land plants. This is true although molecular data have demonstrated that cryptic species are commonly found hidden within phenotypically circumscribed species (Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011) and helped to revise species delimitation in these fungi. The wider distributional ranges are likely due to the higher distributional capacity of the relatively small ascospores or vegetative diaspores (soredia or isidia) that allow dispersal of the photosynthetic partner simultaneously with the fungal partner. Indeed, long-distance dispersal has been shown to have occurred commonly in lichenized fungi (Divakar *et al.*, 2010; Geml *et al.*, 2010; Otálora *et al.*, 2010; Amo de Paz *et al.*, 2011; Fernandez-Mendoza *et al.*, 2011; Amo de Paz *et al.*, 2012; Leavitt *et al.*, 2012a; Parnmen *et al.*, 2012).

Local diversification has been found in a clade of *Umbilicaria* species endemic to the central Andes (Hestmark *et al.*, 2011). All endemic species found in this region share a common ancestor in the *Umbilicaria vellea* group that has a worldwide distribution and contains several asexually reproducing species. The authors interpreted independent reversals to sexual reproduction as an explanation for different ascoma morphologies in this monophyletic endemic lineage. In a different study on the genus *Nephroma*, all species endemic to the north-west African archipelago of Macaronesia were shown to represent neoendemics that originated from a common ancestor shared with a widely distributed species (Sérusiaux *et al.*, 2011). The endemics are estimated to have reached the archipelago via long-distance dispersal.

Adaptive Radiation and Explosive Diversification

Across the entire tree of life, large disparities can be seen in species richness. Explosive diversification (Gittenberger, 1991; Givnish, 2015) or adaptive radiation (Osborn, 1902; Gavrilets and Losos, 2009; Rundell and Price, 2009) are well known to cause dramatic differences in species numbers among clades, and prominent examples of radiations include African cichlid fishes (Seehausen, 2006), Darwin finches (Grant, 1981; Lamichhaney *et al.*, 2015), or the Hawaiian silverswords (Baldwin and Sanderson, 1998). Adaptive radiation is usually explained as being driven by divergent selection caused by competition among closely related, ecologically similar species (Givnish, 1997). In lichenized fungi our understanding of the impact of trait evolution on diversification is not well understood and the few studies addressing this issue focus on specific genera. Despite the fact that some hyper-diverse families, such as Parmeliaceae, have distinct morphological synapomorphies, such as the cupulate exciple (Divakar *et al.*, 2013, 2015), the relationship of these traits and diversification are not well understood. However, the remarkable phenotypic disparity in the genus *Cladia* (including crustose, foliose, and fruticose species) has been explained by adaptive radiation (Lumbsch *et al.*, 2010) in temperate Australia. Explosive diversification has resulted in hyper-diverse groups, such as the genera *Ocellularia*, *Parmotrema*, *Usnea*, and *Xanthoparmelia* (Kraichak *et al.*, 2015a,b). The former genus belongs to a hyper-diverse family but even within the family, *Ocellularia* has a significantly accelerated rate of diversification (Kraichak *et al.*, 2015a,b). In this genus, the accelerated diversification rate was significantly associated with the presence of a unique sterile tissue in the hymenium (**Figure 3**) that often covers most parts of the ascomata, the so-called columella (Kraichak *et al.*, 2015b; Rivas Plata and Lumbsch, 2011). Hence the columella was interpreted as a key innovation, which is a trait that enabled a lineage to proliferate (Simpson, 1953). The function of the columella, however, is less clear and this underlines the poor state of knowledge of trait evolution in lichenized fungi. Hypotheses of its function include being a 'battering ram' to push through the outer periderm of these tropical epiphytic lichens (Redinger, 1936) or defense against fungivores, since the structure fills almost the entire hymenium and hence potentially protects the ascospores (Lücking and Bernecker-Lücking, 2000).

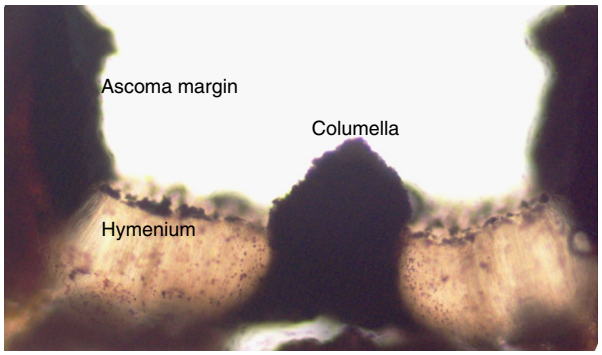

Figure 3 Cross section through an ascoma of the genus *Ocellularia* showing the columella, a sterile dark structure in the center of the hymenium.

Association of Rates of Diversification and Evolutionary Rates

Similar to the way rates of diversification fluctuate over time and among clades, differences in evolutionary rates have been shown to be common across the tree of life (Langley and Fitch, 1974; Britten, 1986; Arbogast *et al.*, 2002; Bromham and Penny, 2003). A number of causes have been invoked to explain the observed differences, most commonly body size and metabolic rate (Martin and Palumbi, 1993; Bromham, 2002), generation time (Gu and Li, 1992; Ohta, 1992), symbiotic association (Lutzoni and Pagel, 1997; Woolfit and Bromham,

2003), or environmental conditions (Bromham and Cardillo, 2003). Disparities of evolutionary rates have also been shown among lichen-forming fungi (Lumbsch *et al.*, 2008; Otálora *et al.*, 2013). In addition, theory also predicts a correlation between rates of substitution changes and diversification (Jobson and Albert, 2002). In the presence of a founder effect (Mayr, 1963), i.e., when speciation takes place in small, peripheral populations, genetic drift will cause rapid genetic change resulting in longer branches in phylogenetic trees (Pagel *et al.*, 2006). Evidence from various organismal groups support the correlation of evolutionary rates and rates of diversification (Barraclough *et al.*, 1996; Barraclough and Savolainen, 2001; Webster *et al.*, 2003) and has also been shown for Ascomycota (Wang *et al.*, 2010). Approximately a third of all Ascomycota species are lichen-forming. However, currently the frequency of speciation that experienced founder effects is poorly understood.

The Diversity of the Photobiont in the Lichen Symbiosis

Most studies to date have focused on the fungal partner in the lichen symbiosis. However, there is evidence for strong phylogenetic signals in the occurrence of photobionts in clades of lichenized fungi (Rambold *et al.*, 1998; Dahlkild *et al.*, 2001; Helms *et al.*, 2001; Persoh *et al.*, 2004; Buckley *et al.*, 2014; Lindgreen *et al.*, 2014; Leavitt *et al.*, 2015). At the same time, it was shown that some lichenized fungi are able to adapt to different habitats by forming symbiotic relationships with ecologically distinct photosynthetic partners (Del Campo *et al.*, 2010, 2013). It was hypothesized that ecological diversification and speciation of lichen symbionts in different habitats includes a transient phase consisting of associations with more than one photobiont in individual thalli (Del Campo *et al.*, 2013) and that such a diversification might be promoted by different physiological backgrounds. An alternative interpretation sees the ability to form association with ecologically different photobionts as an adaptive strategy to expand the potential geographical range of a species (Sadowska-Des *et al.*, 2014). Studies addressing photobiont diversity and selectivity of the fungal partner, however, are currently in their infancy and additional studies will undoubtedly shed more light in the complex interplay of partners in these fascinating symbiotic systems.

See also: Endophytic Microbes, Evolution and Diversification of. Mutualism, the Evolutionary Ecology of

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Life Histories, Axes of Variation in

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Glossary

Allometric relationship A linear relationship on the log–log scale between a life history trait and body mass. Across species, this corresponds to evolutionary allometry. The intercept is the allometric coefficient and varies a lot across taxons and life styles. The slope of the relationship is the allometric exponent and varies little across taxons or life styles, being mostly dependent on the dimension of the trait.

Biological (or physiological) time A measure of perceived time by a given species in opposition to the absolute time unit. Biological time is scaled to body mass. A given absolute time period (say a month) is perceived as being much longer in small species than in large ones.

Dimensional analysis A standardized analysis of the relationships between different biological traits. The standardization is based on three fundamental dimensions (length, volume, and time) and their associated measurement units (e.g., centimeter, kilogram, and years). The dimensional analysis tracks the dimensions and the measurement units when performing calculations or comparisons among traits.

Lifestyle A description of the way of life characterizing a species. This mostly includes the type of diet, the mating tactic, and the habitat features individuals within a species share.

Pace of life A synonymous term for slow–fast continuum. Often used in a broader perspective including not only life history traits, but also physiological and behavioral traits.

Variability is an essential property of living organisms. Both Darwin (1859) and Fisher (1930) understood that evolution required variation. Biologists have been assessing the causes and the consequences of the variability they observe at all stages of biological organization, from the cell to the ecosystem, since the very first studies of life history. The diversity of life history traits, defined here as any phenotypic trait that influences the trajectory of a given individual from birth to death, is one of the most striking and intensively studied topics in evolutionary biology. Let us consider mammals as an example of the wide range of variation. This vertebrate class, which includes about 4600 extant species (Wilson and Reeder, 2005), displays tremendous variation in life history traits. While the gigantic blue whale (*Balaenoptera musculus*), the largest animal to have ever existed on earth, weighs 172 t, the tiny shrew (*Suncus etruscus*) only weighs 1 g. In addition to large variation in body mass (Silva and Downing, 1995), mammals display a tremendous variation of life histories. For example, the gestation time of the African elephant (*Loxodonta africana*) is 660 days, while in voles (*Microtus* sp.) it can be as short as 20 days (Hayssen *et al.*, 1993). Bats (Chiroptera, Rhinolophidae family) give birth to offspring weighing almost one-third of the mother's weight, while in polar bears (*Ursus maritimus*), offspring at birth are only about one-thousandth of their mother's body mass (Hayssen *et al.*, 1993). The chimpanzee (*Pan troglodytes*) gives birth to a single offspring every 4 or 5 years while the tenrec (*Tenrec ecaudatus*) can produce up to 32 embryos within a single litter (Hayssen *et al.*, 1993). Also, small marsupial mice belonging to the genus *Antechinus* reproduce only once during their lifetime (semelparity), while a long-lived species like roe deer (*Capreolus capreolus*) can reproduce up to 15 times (iteroparity). Finally, the European water vole (*Arvicola terrestris*) lives for less than 6 months in the wild, whereas some individuals of Bowhead whale (*Balaena mysticetus*) can survive for more than a century. This extreme variability is highly structured across species with some strong associations with life history traits. This covariation corresponds to life-history strategies (Stearns, 1976, 1980), which are organized along major axes of life history variation. For example, within the mammals, the African elephant has a long gestation time (in fact the longest ever reported), large size, long inter-birth intervals (by giving birth only every 4 years on average), produces a large single offspring at each reproductive attempt, has a delayed age at first breeding and a long life. In contrast, voles have a short gestation time, produce several litters each year each consisting of several offspring, have an early age at first parturition and a short life. This type of covariance defines an axis of life history variation that is frequently defined as the slow–fast continuum of life histories, by far the most intensively studied axis of life history variation, especially in vertebrates (but not only; see also Blackburn, 1991 on Hymenoptera and Franco and Silvertown, 1996 on plants). What is the origin of the slow–fast continuum concept? What is the current empirical evidence supporting the existence of this axis of life history variation in vertebrates? What are the structuring factors shaping the slow–fast continuum of life histories? What are the ecological and evolutionary consequences of this continuum? Are there other axes of life history variation than the slow–fast continuum? From a selected review of the literature published over the past

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50 years, we will try to provide answers to these questions. We will also identify gaps in our current understanding of life history variation.

A Brief History of the Origin of the Slow–Fast Continuum

Identifying the origin of a concept is difficult when several authors have contributed to our current understanding of it. This is exactly the case for the slow–fast continuum. The concept of the slow–fast continuum has no single, unique origin. As the main axis of life history variation, the slow–fast continuum is rooted in history of life history theory. In this respect, seminal work by Lack (1947) on clutch size in birds, Dobzhansky (1950) on latitudinal differences in the way natural selection operates in *Drosophila*, and Cole (1954) on the number of breeding attempts over the lifespan have all contributed to the emergence of the slow–fast continuum concept. However, the foundation of the slow–fast continuum is more precisely based on the concept of life history trade-offs and as such, is grounded in the principle of allocation proposed by Cody (1966) (itself partly inspired by Lack's works). This principle of allocation states that organisms have a limited amount of time or energy available for expenditure, so that allocating more to one biological function (e.g., growth, reproduction, or repair) should lead to the allocation of less energy to other functions. Thereby, the slow–fast continuum can be interpreted as the range of possible solutions to the trade-off between reproduction and survival.

In addition to these pillars of current life history theory, two important, but often overlooked papers, were published in 1962. First, Stahl (1962) called for more dimensional analyses (see **Box 1**) in biology when he stated "In order to define biological similarities in a meaningful way it is necessary to review the subject of dimensional analysis, which forms part of the basis for similarity theory" (p. 205). In particular, he identified that "two principles play a key role in biological analysis: conservation of volume and synchronism of times" (p. 209). Volume (like body mass or birth mass) and time (like age at first reproduction or lifespan) are the dimensions of traits most often included in empirical analyses of the slow–fast continuum. In the same year, MacArthur (1962) proposed a model extending Fisher's (1930) work to the density-dependent case, in which "the carrying capacity of the environment, K, replaces fitness as the agent controlling the action of natural selection" (p. 1897). This work set the basis for r-K selection (originally defined by MacArthur and Wilson (1967)) that led Pianka (1970) to launch his "r-K continuum" (p. 592). Pianka's r-K continuum provides the first axis of life-history variation with similarities and differences, to the slow–fast continuum (see below).

However, perhaps the easiest way to identify the origin of the slow–fast continuum is to simply identify the first use of this term. It was coined for the first time by Stearns (1983) in his pioneering comparative analysis of mammalian life histories when he wrote "This suggests that both size and phylogeny have significant independent effects on patterns of covariation in life-history traits, and that both effects contribute to the ordering of mammal species onto a "slow-fast" continuum" (p. 186). Regardless of when the term was born, or the historical contributions that led to our development of life history theory, empirical analyses of slow–fast continuum have accumulated over the last three decades.

Box 1 What is dimensional analysis?

Dimensional analysis was devised by physicists, but can be defined in an evolutionary biology context as a standardized analysis of the relationships between different biological traits. The standardization is based on three fundamental dimensions (length, volume, and time) and their associated measurement units (e.g., centimeters, kilograms, and years). The dimensional analysis tracks the dimensions and the measurement units when performing calculations or comparisons among traits. The problem of converting metrics from one dimensional unit to another is thus a main objective of dimensional analyses.

All life history traits can be related to a fundamental dimension, or derived units. For instance, gestation and incubation periods, weaning or hatchling periods, age at first reproduction, and lifespan are all measured in units of times (i.e., days, weeks, months, or years), antler size of deer is measured in units of length (centimeters or meters), and body mass has a dimension of volume measured in units of mass. Such an heterogeneity in dimension has obvious practical consequences. For instance, the dimensional analysis implies an allometric exponent to one-third (not 1) when regressing antler size against body mass (on a log–log scale) to assess the type of allometry (i.e., positive, negative, or isometry). Indeed, physical constraints lead us to expect isometric relationships among traits sharing the same dimension. The dimensional analysis thus provides the theoretical background for constructing dimensionless numbers (e.g., ratio between age at first reproduction and lifespan) that are expected to be size-independent (see Charnov, 1993).

Empirical Analyses of Life History Variation in Vertebrates

We will now review the evidence in support of this slow–fast continuum, the way the continuum is identified, and its biological meaning. We conducted a literature search in ISI Web of Knowledge using first the topic keywords 'slow–fast or fast–slow' and then 'pace of life' and, in both cases, refined the research domains to 'Zoology' and 'Evolutionary Biology.' We added the term 'pace of life' because it has become a general term for measuring the speed of a life cycle in ecological (e.g., Wiersma *et al.*, 2007) and physiological (Hille and Cooper, 2015) contexts. We identified 253 and 96 papers respectively and screened the summaries to select 29 studies of vertebrates in which at least one covariation among life history traits was tested (**Table 1**). We also identified 12 studies reporting analyses of a measure of species-specific positions on the slow–fast continuum in relation to other biological processes (**Table 2**). It is noteworthy that we did not identify any analysis in amphibians and that most studies focused on only one vertebrate class (only Herrando-Pérez *et al.*'s (2012) study encompassed a wide range of species including mammals, birds, reptiles, and fishes).

Table 1 Review of comparative analyses in which the slow–fast continuum of life histories across species has been investigated using only traits measuring biological times ('Time metrics') or both time metrics and traits measuring other dimensions ('Non-time metrics'). The taxonomic group analyzed (with its associated sample size in brackets), the method used to assess the slow–fast continuum, the support for the existence of a slow–fast continuum, and the reference, are provided. As traits measured as counts per unit of time (like annual fecundity) have the dimension of time, they are included as time metrics (see Allainé *et al.*, 1987 for further details). Corrections for allometric and/or phylogenetic constraints are displayed in italics. The studies are sorted in chronological order of publication

Time metrics	*Non-time metrics*	*Taxon/method*	*References*
Gestation time Age at eye opening Duration of lactation Inter-birth interval Number of litters per year Age at first reproduction Longevity	Offspring mass Number of offspring *Adult mass*	Mammals ($N=65$) PCA PC1 accounting for 68.1% of life history variation *Corrected for allometric constraints* *Replicated at different taxonomic levels* **Support**	Stearns (1983)
Gestation time Age at first reproduction Number of offspring per season	Neonatal mass Neonatal mass/Adult mass Litter size Litter size × Neonatal mass/Adult mass *Adult mass*	Lagomorphs ($N=22$) PCA PC2 accounting for 36.1% of life history variation **Support**	Swihart (1984)
Gestation time Age at weaning Estrous cycle length Age at first reproduction Age at maturity (both sexes) Lifespan Inter-birth interval	Adult mass (males) Neonatal mass Neonatal brain mass Adult brain mass *Adult mass*	Primates ($N=135$) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Analyzed at the taxonomic level of subfamily* **Support**	Harvey and Clutton-Brock (1985)
Gestation time Age at weaning Age at independence Lifespan Age at first reproduction Inter-birth interval	Litter size Birth mass Litter mass *Adult mass*	Carnivores ($N=112$) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Replicated at different taxonomic levels* **Support**	Gittleman (1986)
Incubation time (per egg) Time to fledging Age at first reproduction	Clutch size Egg mass *Adult mass*	European birds ($N=191$ genera) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Analyzed at the taxonomic level of genus* **Support**	Saether (1987)
Gestation time Inter-birth interval Age at first reproduction Lifespan r-max	Neonatal mass Litter size *Adult mass*	Primates ($N=58$) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* **Support**	Ross (1988)
Adult survival Age at first reproduction	Clutch size	European birds ($N=107$) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Replicated at different taxonomic levels* **Support**	Saether (1988)
Gestation time Age at weaning Period of maternal care	Adult brain mass Litter size Neonatal mass Neonatal brain mass	Mammals ($N=712$) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Replicated at different taxonomic levels*	Read and Harvey (1989)

(*Continued*)

Table 1 Continued

Time metrics	*Non-time metrics*	*Taxon/method*	*References*
Age at first reproduction Period as independent juvenile Inter-birth interval Lifespan Reproductive lifespan Annual fecundity	Litter mass Basal metabolic rate Litter body growth rate Litter brain growth rate Annual biomass production *Adult mass*	**Support**	
Annual fecundity Age at first reproduction Adult life expectancy		Mammals (N=80) Birds (N=114) PCA PC1 accounting for 74% (mammals) and 85% (birds) of life history variation *Corrected for allometric constraints* **Support**	Gaillard *et al.* (1989)
Juvenile mortality Gestation time Lactation time Period of maternal care Inter-birth interval Period of adolescence Age at first reproduction Lifespan Reproductive lifespan Annual fecundity	Litter size Neonatal mass Litter mass Body growth rate Annual litter mass Lifetime output (number of offspring) Lifetime output (total litter mass)	Bivariate linear regressions (log–log scale) Mammals (N=48) *Corrected for allometric constraints* *Replicated at different taxonomic levels* **Support**	Promislow and Harvey (1990)
Age at first reproduction Annual survival	Body growth rate	Reptiles (N=16 snakes and 20 lizards) Bivariate linear regressions (log–log scale) *Corrected for phylogenetic inertia* **Support**	Shine and Charnov (1992)
Adult length Hatchling length Clutch frequency Age at first reproduction	Hatchling mass Length at maturity Clutch size	Lacertids (N=16) PCA PC1 accounting for 73.7% of life history variation *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **No Support**	Bauwens and Uriarte-Diaz (1997)
Brood frequency Age at first reproduction Annual mortality	Clutch size *Adult length*	Lizards (N=90) PCA PC1 accounting for 52.5% of life history variation *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support**	Clobert *et al.* (1998)
Gestation time Age at weaning Period of maternal care Age at first reproduction Period as independent juvenile Lifespan Reproductive lifespan Litter frequency Annual fecundity	Brain mass Litter size Neonatal mass Litter mass Resting metabolic rate *Adult mass*	Insectivores (N=63) Bivariate linear regressions (log–log scale) *Corrected for phylogenetic inertia* **Partial Support**	Symonds (1999)
Incubation time Age at first reproduction	Clutch size Egg mass Nestling growth rate	Birds (N=34) PCA PC1 accounting for 71% of life history variation	Ricklefs (2000)

(*Continued*)

Table 1 Continued

Time metrics	*Non-time metrics*	*Taxon/method*	*References*
Survival to maturity Annual fecundity Annual adult mortality	*Adult mass*	*Corrected for allometric constraints* **Support**	
Age at first reproduction Lifespan	Slope of the fecundity-length relationship Egg volume Fecundity at maturity	Fishes (N=84 populations/49 species) PCA PC1 accounting for 51.8% of life history variation *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support**	Rochet *et al.* (2000)
Lifespan Age at weaning Gestation time Annual fecundity	Neonatal mass Mass at permanent exit from the pouch Mass at weaning Litter size *Adult mass*	Metatherians (N=161) Bivariate linear regressions (log–log scale) *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support (but for gestation time)**	Fisher *et al.* (2001)
Gestation time Inter-birth interval Age at weaning Age at first reproduction	Litter mass Neonatal mass *Adult mass*	Mammals (N=267) Factor analysis PC1 accounting for 40% of life history variation *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support**	Bielby *et al.* (2007)
Age at first reproduction Age at last reproduction Juvenile survival Annual adult survival Annual fecundity		Rodents (N=43 populations/29 species) PCA PC1 accounting for 84% of life history variation **Support**	Dobson and Oli (2007a)
Age at first reproduction Age at last reproduction Juvenile survival Annual adult survival Annual fecundity		Mammals (N=143) PCA PC1 accounting for 81.7% of life history variation *Corrected for allometric constraints* *Replicated at different taxonomic levels* **Support**	Dobson and Oli (2007b)
Age at first reproduction Inter-birth interval Lifespan	Fecundity (number of offspring per clutch/litter) Offspring mass *Adult mass*	Fishes (N=46) Mammals (N=100) Birds (N=302) PCA PC1 accounting for 66% (fishes), 78% (mammals), and 60% (birds) of life history variation *Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support**	Jeschke and Kokko (2009)
Annual fecundity Nursing care period Lifespan	Clutch size Number of broods Egg mass Laying date *Adult mass*	Passerines (N=68) PCA PC1 accounting for 34.8% of life history variation *Corrected for phylogenetic inertia* **Support**	Reif *et al.* (2010)
Gestation time Age at weaning Inter-birth interval	Brain size *Adult mass*	Lemur (N=24) PCA PC1 accounting for 67.8% of life history variation *Corrected for phylogenetic inertia* **Support**	Catlett *et al.* (2010)
Gestation time Lactation time	Litter size Birth mass	Carnivores (N=85) Bivariate linear regressions (log–log scale)	Paemelaere and Dobson (2011)

(*Continued*)

Table 1 Continued

Time metrics	*Non-time metrics*	*Taxon/method*	*References*
Period of maternal care Age at first reproduction Age at independence Inter-birth interval Lifespan	Litter mass *Adult mass*	*Corrected for allometric constraints* *Corrected for phylogenetic inertia* **Support when uncorrected for body size but No support when corrected**	
Age at first reproduction Annual fecundity Lifespan	*Adult mass*	Mammals (N=152) Birds (N=225) Reptiles (N=37) Fishes (N=115) PCA PC1 accounting for 65.4% of life history variation *Corrected for phylogenetic inertia* **Support**	Herrando-Pérez *et al.* (2012)
Lifespan Age at 50% maturity Spawning interval Spawning duration	Growth coefficient Length at 50% maturity Fecundity at maturity Slope of fecundity-length relationship Relative fecundity (number of oocytes per gram) *Adult length*	Scombrids (N=42) PCA PC2 accounting for 23% of life history variation **Support**	Juan-Jorda *et al.* (2013)
Age at first reproduction Gestation time Lactation time Lifespan	Neonatal mass Litter size *Adult mass*	Mammals (N=41) PCA PC1 accounting for 66.5% of life history variation *Corrected for phylogenetic inertia* **Support**	Swanson and Dantzer (2014)
Nestling period	Clutch size Egg size relative to adult mass Body growth rate Absolute latitude	Birds (N=9) PCA **Support**	Stager *et al.* (2014)
Lifespan Age at first reproduction Annual fecundity	Length at maturity Maximum body size Offspring size	Barents Sea Fishes (N=76) PCA and RDA PC1 (PCA) and PC2 (RDA) accounting for 64.2% and 13.3% of life history variation, respectively *Corrected for allometric constraints* (RDA) *Corrected for phylogenetic inertia* **Support**	Wiedmann *et al.* (2014)

To assess covariation among life-history traits, multivariate analyses are a reliable method. However, although the first analysis performed by Stearns (1983) relied on a Principal Component Analysis (PCA), subsequent works in the 1980s and 1990s typically used a series of bivariate linear regressions. The justification for not using multivariate analyses was generally based on two main arguments (see e.g., Harvey and Clutton-Brock, 1985): the necessity of having complete information for all traits in each species in the analysis, and the difficulty of interpreting a statistical combination of traits. Accumulation of life history data over the last 20 years has reduced the problem of missing data, as has the development of new methods for dealing with missing data in multivariate analyses (Josse and Husson, 2011). Problems of interpreting principal components of PCA in a biological context only occur when a heterogeneous set of traits in terms of dimension are included in analyses. These issues can easily be dealt with by accounting for the dimensionality of traits (see below). In recent years, as revealed by an examination of **Table 1**, PCA has become the rule when analyzing axes of life history variation, with all but two studies published since 2000 based on multivariate analyses. The move to PCA can even be detected within the work of the same group of researchers (e.g., Oli, 2004 vs. Dobson and Oli, 2007a,b, 2008).

Most of the available studies reported statistically significant covariation among life history traits and thereby supported the existence of a slow–fast continuum, providing clear evidence that it corresponds to the primary main axis of life history variation across species in mammals, birds, reptiles, and fishes (**Table 1**). The position of a species on the slow–fast continuum ranges from species with high adult survival and low reproductive output (the slow end of the continuum

Table 2 Studies reporting analyses of a measure of species-specific positions on the slow–fast continuum in relation to other biological processes. The metric used to assess the position of a given species on the continuum, the taxonomic group studied, the biological process analyzed, and the reference are all listed in the table. The studies are ordered chronologically. All metrics used but one (in italics) had a dimension of time

Metric	*Taxon*	*Biological process*	*References*
Dental development	Hominin	Evolution	Dean *et al.* (2001)
F/α ratio (annual fecundity/age at first reproduction)	*Mammals*	*Demography*	Oli (2004)
Generation time	Mammals and birds	Senescence	Jones *et al.* (2008)
Age at first reproduction	Muroid rodents	Exploratory behavior	Careau *et al.* (2009)
Nonlinear combination of annual fecundity, age at first reproduction, and annual adult survival	Mammals and birds	Senescence	Péron *et al.* (2010)
Age at sexual maturity	Mammals, birds, amphibians, and reptiles	Senescence	Ricklefs (2010)
Generation time	Marine fishes	Temporal variation in demography	Bjorkvoll *et al.* (2012)
Annual fecundity	Birds	Perception of predation risk	Hua *et al.* (2013)
Generation time	Mammals	Placentation type	Garratt *et al.* (2013)
Generation time	Mammals and birds	Age-specific contributions to fitness	Saether *et al.* (2013)
Age at which cumulative loop elasticity reaches 50% of total elasticity	Carnivores	Management strategies	Van de Kerk *et al.* (2013)
Generation time	Mammals	Transient dynamics	Gamelon *et al.* (2014)

illustrated by Primates in mammals) to species with low adult survival and high reproductive output (the fast end of the continuum illustrated by lagomorphs in mammals). This axis of life history variation associates with principle of resource allocation (i.e., the Cody (1966) Principle of Allocation) supporting the hypothesis that a trade-off between survival and reproduction does shape the diversity of life histories across species within classes, and even orders, of vertebrates. In this narrow sense, it suggests that there is a universal slow–fast continuum of life history variation across vertebrates. Remarkably, in the most detailed analysis of the slow–fast continuum in vertebrates published to date ($N=46$ fish, 100 mammal, and 302 bird species), Jeschke and Kokko (2009) came to the opposite conclusion, writing "In other words, there is no universal fast-slow continuum" (p. 872). So, how can we reconcile what appears to be the contrasting conclusions of Jeschke and Kokko (2009) with our conclusions from a more up-to-date review of the literature? A likely explanation involves the variation among empirical studies in the approaches they use and subtle differences in the questions asked between early and current analyses. Different empirical analyses have corrected for different confounding factors and have worked with different life history traits, and these differences need to be taken into account before a clear take-home message of the existence of a slow–fast continuum within vertebrate lineages emerges.

Problems of Dimensionality When Assessing a Slow–Fast Continuum

Studies correcting for body mass and phylogeny have revealed allometric and evolutionary constraints, but even after having corrected for these processes, a slow–fast continuum of life histories still persists (**Table 1**). Body mass obviously has a major influence on all biological processes (Peters, 1983; Calder, 1984) and accounts for most of the variation observed in life history traits across species. Interestingly, while the slow–fast continuum shows up at both absolute and mass-dependent scales, the relative contribution of traits and the ranking of a given species along this continuum generally differ quite markedly. For instance, cetaceans obviously live in the slow line by displaying late age at first reproduction, low fecundity and long lifespan. However, for a given size, cetaceans rank among the fastest species in relation to the very large size they can reach in their marine habitat (Gaillard *et al.*, 1989). On the other hand, within a given lineage, the relative ranking of species on the continuum remains rather constant independent whether allometry is accounted for or not. The effects of phylogeny are also quite obvious, but often strongly covary with ecological factors. For instance, all bats belong to the order Chiroptera and share strong similarities including a remarkably long lifespan for their mass and their ability to fly. The phylogenetic inertia, which generates statistical problems of nonindependence among data points, has been increasingly accounted for (**Table 1**). However, the empirical evidence so far accumulated indicates that the phylogenetic inertia does not markedly influence the detection and the strength of the slow–fast continuum. The question of accounting for (or not) variation in body mass and phylogeny prior to analyse life history variation has focused most of the attention of evolutionary biologists (see, e.g., Jeschke and Kokko, 2009). However, the problem of dimensionality (**Box 1**) has been overlooked, even though it is likely a more crucial issue for interpreting life history variation from comparative analyses of life history traits.

Broadly speaking, Pianka's (1970) r-K continuum was the first formally proposed axis of life history variation. In addition to the trade-off between survival and reproduction, this continuum also includes in the covariation body mass, population size, climate, and intra- and interspecific competitions. In its original form it was a rather poor example of an axis of biological covariation in terms of dimensionality! Among other flaws not detailed here (see Stearns, 1977; Boyce, 1984 for further details), the r-K continuum of Pianka confuses the underlying pattern of life history variation with density-dependence, a process potentially involved to explain the pattern. Stearns (1976) was the first to start to separate the underlying pattern from the density-dependent component when he opposed the 'r-selection label' to the 'K-selection label,' which provides *sensu stricto*, the first slow–fast continuum. In the early 1980s, use of the concept of physiological time (that because "metabolic rate and longevity change with body size, time itself may be scaled", Lindstedt and Calder, 1976 (p. 91)) to life history analysis led some biologists to the view that observed covariation among traits measured in time units is an allometric consequence rather than a direct product of natural selection (Lindstedt and Calder, 1981; Calder, 1984). This explains why evolutionary biologists, starting with Stearns (1983), then searched for the existence of a slow–fast continuum of life histories after correcting for allometric constraints, providing unambiguous support for it (e.g., Harvey and Clutton-Brock, 1985; Gittleman, 1986; Saether, 1987; Gaillard *et al.*, 1989; Read and Harvey, 1989). A lively debate about whether allometric constraints or natural selection processes determine most of the observed life history covariation then took place in the late eighties (see, e.g., Read and Harvey, 1989). As allometric variation is itself under natural selection processes, such a separation between allometry and adaptation is highly disputable. Nowadays, it is widely accepted that both allometry and natural selection are acting to determine observed variation in life history. Thus, studying the slow–fast continuum requires consideration of the crucial problem of dimensionality (**Box 1**).

In all but three studies (Gaillard *et al.*, 1989; Dobson and Oli, 2007a,b), a mixture of traits with different dimensions were analyzed, leading researchers to question the interpretation of a slow–fast continuum, because common sense tells us that the slow–fast continuum is obviously based on the concept of time. As recognized in other research areas, including paleontology (see, e.g., Robson and Wood, 2008), life history traits can be classified into two broad categories: traits that describe the timing of life history events like age at first reproduction, gestation time, or longevity (corresponding to physiological times) and traits like mass, or growth rates, that are not biological times, but are correlated with biological times (as notably observed by Stahl (1962)). While the concept of biological similarity (Stahl, 1962) relies upon the use of time (e.g., gestation time, age at maturity, longevity) and time frequencies (e.g., heart beats/minute, annual fecundity, annual survival) to measure a time scale, it does not permit the inclusion of volume traits (like body, birth, or brain mass), nonstandardized number (like litter size or clutch size), or changes of volume over time (like growth rate). So far, most studies of the slow–fast continuum have included litter size or clutch size (**Table 1**) although these traits do not have the dimension of time. It was well established that litter size varies non-monotonously across mammalian species before any empirical analysis of slow–fast continuum was performed (Tuomi, 1980). The right metric for measuring the reproductive output in the context of a time scale is the fecundity per time unit (e.g., annual fecundity, Allainé *et al.*, 1987) because some small species produce several litters per reproductive season and some large species only reproduce every second, third, or fourth year. The use of clutch/litter size instead of annual fecundity in Jeschke and Kokko (2009) is likely to account for most of the differences they reported between analyses accounting for body mass or not. Moreover, among the common pitfalls, lots of analyses of the slow–fast continuum include redundancy in life history traits. Such nonindependence between traits is likely to alter both the assessment of relationships in bivariate analyses (as nicely pointed out almost 30 years ago by Sutherland *et al.*, 1986) and the relative contribution of life history traits to the slow–fast continuum by deviating the first major axis of the PCA towards redundant variables. Our literature survey thus points out that problems of dimensionality occur in most empirical studies (see **Table 1**), which likely explain differences of interpretation among studies and prevent a reliable understanding of the slow–fast continuum.

Importantly, when the dimensionality of all traits is restricted to physiological times and any trivial redundancy among traits is avoided or accounted for by using multivariate analyses, traits equally contribute to the slow–fast continuum, which supports the concept of biological similarities (Gaillard *et al.*, 1989; Dobson and Oli, 2007b). Only considering physiological times included in PCA performed so far also provides remarkable support for this remark (**Table 3**). Therefore, to answer clearly the question raised by Jeschke and Kokko (2009), body mass should not be included when assessing the slow–fast continuum of life history. We thus share their suggestion 'to reserve the term "fast–slow continuum' to the raw data,' but only when traits with a dimension of time are considered (p. 876).

What Shapes the Slow–Fast Continuum of Life Histories?

Three main factors have been identified that shape the slow–fast continuum: body mass, phylogeny, and ecology. Western (1979) was the first to recognize that life history variation can be partitioned into an allometric component, and one or several non-allometric components. All analyses have identified a major role of allometric relationships in generating variation in life history traits across species (e.g., Peters, 1983; McMahon and Bonner, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Brown and West, 2000 for reviews). As a rule of thumb, about 50% of life history variation observed across species can be statistically explained by variation in body mass, as long as the range of body sizes included in analyses spans several orders of magnitude. The trade-off between reproduction and survival persists when body mass effects are accounted for in analyses, indicating that the diversity of life history strategy is not solely due to differences in size (Stearns (1983), Gaillard *et al.* (1989), and Read and Harvey (1989) on mammals,

Table 3 Loadings of the traits with a dimension of time on the axis of life history variation interpreted as a slow–fast continuum in a set of empirical PCA studies reporting the loadings of each trait on the slow–fast continuum. In virtually all instances (exceptions in bold), all traits positively covary (same sign for physiological times and opposite sign for time frequencies) and the loadings are remarkably similar, providing a clear support for the concept of biological similarities

Trait 1	*Trait 2*	*Trait 3*	*Trait 4*	*Trait 5*	*References*
0.91	0.90	− 0.86	0.77	0.89	Stearns (1983)
0.74	0.96	− 0.88			Swihart (1984)
	0.82	− 0.79	0.79[a]		Clobert *et al.* (1998)
0.26			0.84	0.78	Bielby *et al.* (2007)
	0.90		0.94	0.67	Jeschke and Kokko (2009) – Fish raw
	0.89		0.86	0.15	Jeschke and Kokko (2009) – Fish corrected
	0.90		0.87	0.73	Jeschke and Kokko (2009) – Mammals raw
	0.83		0.47	0.73	Jeschke and Kokko (2009) – Mammals corrected
	0.90		0.81	0.52	Jeschke and Kokko (2009) – Birds raw
	0.62		0.39	**− 0.42**	Jeschke and Kokko (2009) – Birds corrected
0.40			0.41	0.43	Catlett *et al.* (2010)
0.58	− 0.20		− 0.39	− 0.28	Juan-Jorda *et al.* (2013)
− 0.86	− 0.86		− 0.81	− 0.73	Swanson and Dantzer (2014)

[a]Presented as − 0.79 for annual mortality in the original paper.

Trait 1: Gestation (Stearns, 1983; Swihart, 1984; Bielby *et al.*, 2007; Catlett *et al.*, 2010; Swanson and Dantzer, 2014); Spawning duration (Juan-Jorda *et al.*, 2013).

Trait 2: Age at first reproduction (Stearns, 1983; Swihart, 1984; Clobert *et al.*, 1998; Jeschke and Kokko, 2009; Swanson and Dantzer, 2014); Age at 50% maturity (Juan-Jorda *et al.*, 2013).

Trait 3: Annual fecundity (Swihart, 1984; Clobert *et al.*, 1998); Number of litters per year (Stearns, 1983).

Trait 4: Annual survival (Clobert *et al.*, 1998); Age at weaning (Bielby *et al.*, 2007; Catlett *et al.*, 2010); Lifespan (Stearns, 1983; Jeschke and Kokko, 2009; Juan-Jorda *et al.*, 2013; Swanson and Dantzer, 2014).

Trait 5: Inter-birth interval (Stearns, 1983; Bielby *et al.*, 2007; Jeschke and Kokko, 2009; Catlett *et al.*, 2010; Juan-Jorda *et al.*, 2013; Lactation time (Swanson and Dantzer, 2014).

Swihart (1984) on lagomorphs, Harvey and Clutton-Brock (1985) on primates, Gittleman (1986) on carnivores, Saether (1987) on European birds, Saether and Gordon (1994) on ungulates).

Phylogeny also has an influence on the ranking of species on the slow–fast continuum. For instance, bats and primates in mammals, and Procellariiformes in birds, have much slower life histories than predicted by their size. While early studies accounted for the possible confounding effects of phylogeny by replicating the analyses at different taxonomic levels (e.g., Stearns, 1983) or performing analyses at a higher taxonomic level (like genera, e.g., Saether, 1987), methodological advances in phylogenetic methods have allowed phylogenetic inertia to be appropriately corrected for in life history analyses (Freckleton *et al.*, 2002), including PCA (e.g., Phylogenetic PCA, Revell, 2009, 2010).

The ecological factors shaping variation on the slow–fast continuum have not yet been clearly identified, which looks a bit paradoxical given that the original Pianka (1970) r-K continuum explicitly associated covariation among life history traits with ecological factors. According to Fisher *et al.* (2001), "most studies have failed to find robust ecological correlates of life history diversity in birds, mammals, and other taxa" (p. 3538). Some studies have identified habitat type (i.e., aerial, terrestrial, and marine, Gaillard *et al.*, 1989; Pontier *et al.*, 1990) or diet (Saether and Gordon, 1994; Fisher *et al.*, 2001) as structuring factors. Recently, stronger evidence that the lifestyle influences the pace of life of a species has been proposed. For example, evidence is accumulating that tropical birds have a slower life history than their temperate counterparts, likely as a function of lower basal metabolic rates (Wiersma *et al.*, 2007; Jimenez *et al.*, 2014) and smaller organ size (Wiersma *et al.*, 2012). For a given size, arboreal mammals live longer than those that live on the ground (Shattuck and Williams, 2010), and flying mammals outlive their nonflying relatives (Healy *et al.*, 2014). In addition, energy-saving strategies have been reported to shape species-specific pace of life. Thus, hibernation slows the pace of life in mammals (Turbill *et al.*, 2011). However, the current lack of evidence for strong structuring effects of ecological correlates might be explained by the simplicity of the metrics used to assess ecological correlates.

Evolutionary Consequences of the Slow–Fast Continuum

As rightly noted by Ricklefs (2000), analyses of life history variation have, for many years, contributed to "divorcing life-history from its environmental context" (p. 13). While the search for more refined ecological factors that likely shape the ranking of species on the slow–fast continuum has still to be performed, evidence is mounting concerning the evolutionary consequences of a given position on the slow–fast continuum. Indeed, several studies have assessed the effect of the species-specific position along the continuum on different biological processes like demography (Saether *et al.*, 2013), senescence (Jones *et al.*, 2008), or parent–offspring conflicts via placentation types (Garratt *et al.*, 2013). They clearly showed that slow- and fast-living species should markedly differ in demographic responses to human disturbance or climate change. Most of these studies ranked species on the continuum with an appropriate measure of time, with generation time, the weighted mean age of mothers in a stable population, being the most frequently used metric. In addition to closely matching a species position on the slow–fast continuum

identified through the use of the first component of a PCA conducted on time-related traits (correlation coefficients of 0.903 in mammals, Gaillard *et al.*, 2005), generation time is functionally the most relevant metric at the population level. Generation time proportionally decreases with increasing r-max (i.e., the maximum population growth rate a given species can reach in absence of any resource limitation, Caughley, 1977) across species, leading the product between r-max and generation time (i.e., the per-generation growth rate) to be a dimensionless number (*sensu* Charnov, 1993). Thus, within a lineage and in absence of specific adaptations, the per-generation growth rate is expected to be a constant independent of body mass. Moreover, generation time defines the scale at which genes pass from parents to offspring. It consequently obviously correlates with how quickly a lineage can evolve (Okie *et al.*, 2013) with species at the slow end of the continuum evolving at a slower rate than those at the fast end (Martin and Palumbi, 1993; Galtier *et al.*, 2009; Bromham, 2011). We could thus expect slow-living species to be more resistant to environmental perturbations but to be less able to respond to such perturbations (either by demographic or evolutionary changes) than fast-living species (Morris *et al.*, 2008; Gamelon *et al.*, 2014). Therefore, the position of the species along the slow–fast continuum of life history variation has important consequences from both ecological and evolutionary viewpoints.

Are There Other Axes of Life History Variation Than the Slow–Fast Continuum?

The slow–fast continuum identified from multivariate analyses only accounts for approximately half of the variation observed in life history traits, meaning that, for a given position on the slow–fast continuum, species are organized according to some other sources of covariation. This was recognized since Stearns (1983) identified a precociality–altriciality continuum after accounting for the slow–fast continuum. Subsequent analyses provided similar findings by identifying other axes of life history variation, involving covariations among some reproductive traits. For example, a gradient of allocation of reproductive effort over the lifetime going from semelparity to marked iteroparity accounted for about 10% of the demographic variation observed among mammalian species, once the effects of allometry and of the pace of life were accounted for (Gaillard *et al.*, 1989). On the other hand, Bielby *et al.* (2007) pointed out the existence of an offspring size–offspring number trade-off in mammals after accounting for the slow–fast continuum. These authors questioned the general relevance of the slow–fast continuum based on the argument that (1) there is more than one axis of variation in life histories across species, so that species can be fast or slow in different ways (Bielby *et al.*, 2007), and (2) strongly correlated traits in some taxonomic groups are poorly correlated in others. For example, the loadings of weaning age on the slow–fast continuum in Artiodactyls was only half those reported in other mammalian groups. While it is evident that the slow–fast continuum only corresponds to one axis of life history variation as demonstrated by our literature review (**Table 1**), the conclusion that the covariation of traits shaping the slow–fast continuum differs among groups does not hold. The detailed life history analysis performed by Bielby *et al.* (2007) involved traits with different dimension (e.g., time, volume, non-standardized number), and this heterogeneity in dimension among traits shaping the axes of life history variation prevents interpretation of axes of variation as corresponding to slow–fast continuums. As reported in the **Table 3**, when restricted to physiological times and frequencies, the slow–fast continuum is remarkably constant across taxonomic groups. Again, instead of mixing traits with different dimensions, we recommend looking for a series of axes of life history variation based on comparable traits (i.e., meeting the concept of biological similarity), which will be easy to interpret. For instance, performing a comparative analysis including a wide range of physiological times including developmental and demographic traits would offer a powerful way to assess the full complexity of life history variation.

Perspectives: Beyond Interspecific Analyses and Toward an Integrated Pace of Life Syndrome

The study of the axes of life history variation has primarily been performed at the interspecific level. However, these axes cannot account for all the variability of life history traits. For a given species, for a given ecological type, and for a given taxonomic position, variation in life history is still important. It is nowadays widely recognized that considerable variation in life history occurs among populations within a given species, and also among individuals within a population. Until now, whether the fundamental trade-off between survival and reproduction shapes a slow–fast continuum at these lower levels of biological organization has yet to be investigated. In roe deer, it has been shown that declining populations live at a slower pace of life than colonizing ones (Nilsen *et al.*, 2009). Similarly, heavily hunted wild boar (*Sus scrofa*) populations exhibited fastest life-history speed than lightly hunted ones (Servanty *et al.*, 2011). However, a recent comparative demographic analysis among eight contrasted populations of a lizard species (*Sceloporus grammicus*) did not find any evidence for a slow–fast continuum (Pérez-Mendoza *et al.*, 2013). Further work is clearly required to understand such patterns of variation.

While available analyses of life history variation have mostly been performed in an ecological context, a more holistic view is required, and this has started to emerge. For instance, a recent study aiming at understanding the life history differences between tropical and temperate birds, has suggested that a physiological slow–fast continuum is also existing (Hille and Cooper, 2015). In addition, the increasing popularity of personality analysis in ecology allows identification of behavioral syndromes (Sih *et al.*, 2004) that can be related to individual life history variation. The works of Careau *et al.* (2009) and Hua *et al.* (2013) relating the pace of life with exploratory behaviors of muroids and the perception of predation risk, respectively, and by Patrick and Weimerskirch (2014) on the links between personality and senescence rates among individuals provide insightful examples of what can be done in the next future. Finally, while available analyses of life history variation have mostly been performed in an ecological context, a more global view is required and has started to

emerge. The search for a better understanding of life history differences between tropical and temperate birds has led researchers to include physiological traits, suggesting that a physiological slow–fast continuum may also exist (Hille and Cooper, 2015). Therefore, we envisage a fully integrated view of life history variation including a covariation of axes of variation defined not only by times, volumes, and physiology as currently understood in the concept of pace of life syndrome (Hille and Cooper, 2015), but also by behavior and molecular evolution.

See also: Life History: Pike. Life History, What is?. *r*- and *K*-Selection in Fluctuating Environments, Theory of

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Life History: Age and Stage Structure

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Introduction

In biology, the principal use of demographic theory is to show how individual life histories (vital rates) determine fitness. As discussed in previous articles, when age alone determines survival and fertility, fitness is the stable population growth rate, r, that solves Lotka's equation. Broad understanding relies on the approximation $r \simeq (\log R_0/T_c)$ (Dublin and Lotka, 1925). Here R_0, the expected lifetime reproduction of an individual, measures the level of reproduction in a life history, whereas T_c, the average age at which an individual reproduces, measures the timing of reproduction. However, numerous traits other than age can influence survival and reproduction, for example, size in plants (Hoffmann, 1999), developmental/reproductive stages in insects or birds (Hu and Tessier, 1995; Steiner *et al.*, 2010), body mass in mammals (Coulson *et al.*, 2010), and body-mass index in humans (Preston *et al.*, 2012). Hence the need for, and importance of, a generalized demographic theory that incorporates stage in addition to age which we describe here.

In this generalization, the term 'stage' indicates virtually every kind of trait that can affect the components of a life history – quantitative or qualitative, for example, behavioral, physiological, developmental, morphological, (epi-)genetical, or geographical. For an individual, the theory tracks how stage changes with age (deterministically or stochastically) using functions that describe growth or development or plasticity; survival probability as a function of stage and age; and the total number of offspring as a function of stage and age. Offspring can be born in many stages, for example, different sizes, or different kinds as in seeds versus clones, or in different locations, so we need a transmission function that tells us what kinds of offspring are produced by parents in different stages and ages. When time is measured in discrete intervals, these elements (growth, survival, fertility, and transmission) can be combined to produce discrete-stage (matrix) or continuous-stage (integral) population models (Cochran and Ellner, 1992; Caswell, 2001; Ellner and Rees, 2007; Steiner *et al.*, 2010; Tuljapurkar *et al.*, 2012). These models provide a rich description of population dynamics and an especially valuable feature is that matrix methods are easily extended to compute the moments of age-at-death (lifetime), lifetime reproduction, and r and their sensitivity to model parameters (Coulson *et al.*, 2010; Steiner *et al.*, 2012; Steiner and Tuljapurkar, 2012).

Here we summarize the structure of age–stage models, their dynamics, and the macroscopic (summary) parameters that generalize quantities that are familiar in the age-only demography. These macroscopic parameters are useful, for instance, in showing how detailed life history components (stage-and-age dependent growth and development, survival, reproduction, and parent–offspring transmission) determine the speed of a life history, the level of reproduction, or both. Our description of vital rates and population growth follows mainly Coulson *et al.* (2010) but also depends on important earlier works (Le Bras, 1971; Rogers, 1975; Lebreton, 1996; Childs *et al.*, 2004; Ellner and Rees, 2006). Further results and mathematical details can be found in the cited references.

Individual Vital Rates

We work with discrete time, age, stage, and so with matrices; but note that these results apply to integral operators for continuous stages under the conditions given by Ellner and Rees (2006). At time t a census enumerates newborns (ages 0–1) in age-class 1, and individuals in all higher age-classes $a = 2, 3, \ldots$ (a terminal age is easily accommodated). We use 'age' and 'age-class' interchangeably: a newborn arrives at age $a = 1$, moves to age 2 after one time interval, and so on. An individual (we follow only females) of a given age a is in one of many discrete stages z (with integral models, our discrete intervals are bins on a continuous stage space). A population census at time t yields numbers $n_a(z,t)$ for all ages a and stages z. For example, the number of newborns in stage z at time t is $n_1(z,t)$. Stages are indicated by the symbols γ, z.

Stage–age structured vital rates comprise information about one or multiple traits and their influence on survival, reproduction, trait change (dynamic across life), and the transmission of the trait from parent to offspring which can be due to processes including genetic inheritance, epigenetics, and maternal effects. All of these influences can change with age, and are captured by four relationships (summarized in **Table 1**) that can be estimated directly from data, as explained by Coulson *et al.* (2010). To help fix ideas, think about that case where stage is just size. The theory works with two summary matrices for each age.

1. Fertility $\mathbf{F}_a$ at age a is the number of stage γ offspring of age 1 produced by a stage z parent of age a. This matrix (see **Table 1**) is determined by (1) the total number of offspring $M_a(z)$ produced by a stage z individual at age a, and (2) the stage-distribution of offspring: for a stage z individual at age a, $D_a(\gamma,z)$ is the fraction of this individual offspring that is born into newborn stage γ (clearly $\sum_\gamma D_a(\gamma,z) = 1$).
2. Survivorship $\mathbf{L}_a$ is a matrix of cumulative survival probabilities from birth (at age 1) to age a. The matrix element $L_a(\gamma,z)$ is the probability that an individual born in stage z is alive in stage γ at age a. An individual moves from stage z at age a to stage γ at age $(a+1)$ if (1) it survives with probability $S_a(z)$, and (2) changes stage from z to γ with probability $G_a(\gamma,z)$ (so that $\sum_\gamma G_a(\gamma,z) = 1$). The combined probabilities $P_a(\gamma,z) = G_a(\gamma,z)S_a(z)$ of surviving and moving from stage z at age a to stage γ at age $(a+1)$ are elements of a survival matrix $\mathbf{P}_a$. In the convention used here, individuals are born into age-class 1, which means that $\mathbf{L}_1$ is just the identity matrix (see **Table 1**). Then $\mathbf{L}_2 = \mathbf{P}_1\mathbf{L}_1$ and so on.

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Table 1 Stage and age: vital rates

Quantity	*Equation*	*Notes*
Age	$a=1, 2,\ldots$ Newborns are in age-class 1	
Stage	y or $z=1, 2, \ldots$ An individual has both stage and age. Stage-and-age composition counted at a census	Stages may include several dimensions (e.g., size and developmental state)
Total recruitment	$M_a(z)$, number of offspring of age a, stage z individual that recruit as newborns in next census	
Inheritance	$D_a(y,z)$, fraction of $M_a(z)$ offspring that is born into newborn stage y	Parent–offspring transmission function $\sum_y D_a(yz)=1$
Fertility	$F_a(y,z)=D_a(y,z)M_a(z)$. Elements of matrix $\mathbf{F}_a$	Number of stage y recruits produced by a stage z parent of age a
Survival probability	$S_a(z)$, probability that individual in stage z at age a survives to $a+1$.	
Growth	$G_a(y,z)$, probability that individual in stage z at age a changes stage to y at $a+1$.	Conditional on survival, so $\sum_y G_a(yz)=1$
Stage transition matrix	$P_a(y,z)=G_a(y,z)S_a(z)$, probability that individual in stage z at age a is alive in stage y at $a+1$. Elements of matrix $\mathbf{P}_a$	Includes survival and stage change (e.g., growth).
Survivorship	$L_a(y,z)$, probability that newborn arriving in stage z is alive in stage y at age a. Elements of matrix $\mathbf{L}_a$. Here $L_1(y,z)=1$ if y=z and zero else	$\mathbf{L}_1=\mathbf{I}$=the identity matrix. $\mathbf{L_2}=\mathbf{P}_1\mathbf{L}_1$, $\mathbf{L}_3=\mathbf{P}_2\mathbf{L}_2$, etc.

Stable Cohorts

If age is all that matters, individuals born together are considered identical and make a birth cohort whose lifetime reproduction is readily described. But when stage also matters, individuals in the same birth cohort but different birth stages can differ in their future survival, growth, and reproduction. In other words, two cohorts with different stage compositions but equal total number will produce different numbers of offspring. What happens to such differences as we look forward in time?

Newborns in the first offspring generation go on to produce offspring over their lives; the latter is the second offspring generation, grand-offspring of our original cohort. The number of individuals in successive offspring is determined by a 'generation' matrix

$$\mathbf{A}_0=\sum_{a\geq 1}\mathbf{F}_a\mathbf{L}_a \qquad [1]$$

This matrix $\mathbf{A}_0$ has a dominant eigenvalue R_0. The total number of individuals in successive offspring generations will eventually change at the geometric growth rate R_0. There is a corresponding right eigenvector $\mathbf{c}$ that describes the stage-structure of offspring generations. In the long run, this unique stage-structure defines a 'stable cohort.' Such a cohort and only such a cohort produces offspring generations that grow at exactly the rate R_0. A corresponding left eigenvector $\mathbf{d}$ that describes the generational reproductive value. For an age-only model, $\mathbf{A_0}$ reduces to the number $\sum_a f_a l_a = R_0$, and the vectors $\mathbf{c,d}$ are each replaced by the single number unity.

At age a a stable cohort produces a fraction ϕ_a of its total reproduction:

$$\phi_a=\frac{(\mathbf{d}^T\mathbf{F}_a\mathbf{L}_a\mathbf{c})}{R_0} \qquad [2]$$

Here the superscript T indicates a transpose. The fractions ϕ_a sum to 1. This equation generalizes the simple product of survivorship and fertility for an age-only life history.

Using ϕ_a, we compute the average age of reproduction

$$T_c=\sum_a a\phi_a$$

The quantity

$$\mu_2=\sum_a a^2\phi_a$$

is the mean-square age of reproduction, and the age-dispersion $V_a=\left(\mu_2-T_c^2\right)$ describes how much reproduction occurs earlier or later than the mean age T_c.

Population Structure and Growth

In stage-and-age demography, the population at age a has components $n(a,t,z)$ at each discrete stage z. Letting $\mathbf{n}(t)$ be a vector with component $n(a=1,t,z)$, $n(a=2,t,z),\ldots$, the population's dynamics can be written in matrix form as

$$\mathbf{n}(t+1)=\mathbf{X}\mathbf{n}(t) \qquad [3]$$

where

$$\mathbf{X}=\begin{pmatrix}\mathbf{F}_1 & \mathbf{F}_2 & \cdots & \mathbf{F}_A\\ \mathbf{P}_1 & 0 & \cdots & 0\\ \vdots & \vdots & \vdots & \vdots\\ 0 & \cdots & \mathbf{P}_{A-1} & 0\end{pmatrix} \qquad [4]$$

An equivalent renewal equation can also be written for newborns (Le Bras, 1971; Rogers, 1975; Lebreton, 1996)

$$\mathbf{n}(1,t)=\sum_a \mathbf{F}_a\mathbf{L}_a\mathbf{n}(1,t-a)$$

Stable Population

Assuming demographic ergodicity (Keyfitz and Caswell, 2005), the dominant eigenvalue of $\mathbf{X}$ is $\lambda=e^r$ and is the

Table 2 Stage and age: cohorts to fitness

Struture of stable cohort	Normalized so that $(\mathbf{d}, \mathbf{c})=1$	Generational reproductive value of stages in a cohort
Level of reproduction by stable cohort	R_0	Net reproductive rate
Age-distribution of stable cohort reproduction	$\phi_a=(\mathbf{d}^T \mathbf{F}_a \mathbf{L}_a \mathbf{c})/R_0$, superscript indicates transposed vector	Fraction of stable cohort's reproduction that occurs at age a.
Mean age of reproduction by a cohort	$T_c=\sum_a a\phi_a$	Also called cohort generation time
Mean square age of reproduction	$\mu_2=\sum_a a^2\phi_a$	
Age-dispersion of reproduction	$V_a=(\mu_2-T_c^2)$	
Stage-dispersion of reproduction	$V_s=\xi_1-T_c^2$ $\mathbf{B}_1=(1/R_0)\sum_a a\mathbf{F}_a\mathbf{L}_a$ $\mathbf{Z}=\mathbf{c}\mathbf{d}^T$ $\mathbf{H}=\{\mathbf{I}-([\mathbf{A}_0/R_0]-\mathbf{Z})\}^{-1}$ $\xi_1=(\mathbf{d}^T\mathbf{B}_1\mathbf{H}\mathbf{B}_1\mathbf{c})$	
Simple stage–age structured approximation	$r_1=(\log R_0)/T_c$	R_0 and T_c differ compared to Dublin–Lotka approximation
Better stage–age structured approximation	$r_2=r_1+[V_a+2V_s](\log R_0)^2/2T_c^3$	Takes age- and stage-dispersion of reproduction into account

long-run growth rate in a constant environment. When the population becomes stable, write the stable stage-structure of newborns as $\mathbf{u}=\mathbf{U}_1$. For every other age a (from eqn [4]), the stable structure

$$\mathbf{U}_a=e^{-r(a-1)}\mathbf{L}_a\mathbf{u},\ \text{for}\ a\geq 1 \qquad [5]$$

Similarly, write $\mathbf{v}=\mathbf{V}_1$ for the reproductive value of newborns. Then for all ages a

$$\mathbf{V}_a^T=\mathbf{v}^T\sum_{b=a}^{b=A}e^{-r(b-a+1)}\mathbf{F}_b\mathbf{L}_{b|a},\ \text{for}\ a\geq 1 \qquad [6]$$

The always alert reader will note the strong similarity between these equations and the usual scalar versions of these that apply for age-structured demography.

The renewal equation shows that

$$\mathbf{u}_1=\left\{\sum_a \mathbf{F}_a\mathbf{L}_a m e^{-ra}\right\}\mathbf{u}_1=\mathbf{A}(r)\mathbf{u}_1 \qquad [7]$$

Here $\mathbf{A}(r)$ is the population 'renewal matrix'; its dominant eigenvalue is 1; the corresponding right eigenvector is the vector $\mathbf{u}$; and the corresponding left eigenvector is $\mathbf{v}$. The elements $v(i)$ are the relative reproductive values of individuals born in stage i. The stage-distribution of newborns and the stage dependence of reproductive value are fundamental distinctions between stage–age models and age-only models. The elements $u(i)$ are the fractions of newborns entering a stable population in stage i.

From Vital Rates to r: Levels and Timing

For general stage–age models, the stable population growth rate is given (Steiner *et al.*, 2014) by the approximation,

$$r=\frac{\log R_0}{T_c}+\frac{[V_a+2V_s](\log R_0)^2}{2T_c^3}+O\left(\frac{(\log R_0)^3}{T_c^3}\right) \qquad [8]$$

This precise mathematical relationship identifies measures that can be computed directly from the stage–age structured life history and predict r (see Table 2). The two explicit terms in eqn [8] predict r well so long as R_0 is close to 1. This equation is a direct generalization of the corresponding result in age-only structured demography, and can be extended to higher order in $\log R_0$ if desired.

The first two parameters describe the amount and average timing of a stable cohort's reproduction, as described above. The second term on the right of eqn [8] describes two kinds of reproductive dispersion.

1. Age-dispersion. Individuals of age a produce (a weighted) total of ϕ_a offspring, and the variance $V_a=(\mu_2-T_c^2)$ measures the age-dispersion of reproduction around the mean age of reproduction T_c. Larger age-dispersion of reproduction increases r, because early (relative to T_c) reproduction increases growth rate more than later reproduction reduces it.
2. Stage-dispersion. Suppose now that reproductive individuals produce the same stage-distribution of offspring at every age; in this case, there can only be age-dispersion of reproduction. But what if parents of different ages produce distinct distributions of offspring? Then the stage-distribution of the total number of offspring changes with age, producing a new kind of reproductive dispersion that can only happen in stage–age structured populations.

Discussion

We have summarized the demographic theory of stage-and-age structured population models. We have not presented here results on the mean and variance (and higher moments) of age-at-death and lifetime reproductive success; these and related work on dynamic heterogeneity are given by Tuljapurkar *et al.* (2009), Tuljapurkar and Steiner (2010), and Steiner and Tuljapurkar (2012). What about density-dependence? At a density-regulated equilibrium, the population's growth rate is zero, which means that the net reproductive rate R_0 must be 1, but T_c can vary. Our definitions of R_0 and T_c still apply of course, which means that we can examine how specific kinds

of density-dependence (e.g., predation, cannibalism, and resource competition) constrain different components (growth, survival, recruitment, parent–offspring correlation, or dispersal among locations of a meta population) of a life history at equilibrium. We can examine variation in T_c which still measures the speed of a life history and its response to environmental signals.

The results described here contribute to a powerful framework of stage–age structured models that is being applied in evolutionary and ecological population biology. In many populations, demographic dynamics, life histories, and quantitative variation can only be accurately predicted by these kinds of models. In particular, such models are needed to understand the consequences of variation in development, physiology, migration, behavior, or plasticity.

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See also: Life History: Pike. Life History Theory: Basics

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Life-History Evolution, Human

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Glossary

Demographic transition The change from high birth and death rates to low ones as a country develops from a preindustrial to an industrialized economic system.
Genome-wide association study (GWAS) A study determining the genomic regions underlying traits of interest by assessing statistical associations between traits and the genotypes of many individuals at many thousands of loci across the genome.
Heritability The additive genetic variance in a trait, divided by the total phenotypic variance in that trait: $h^2 = V_A/V_P$. The additive genetic variance is the variance accounted for by additive effects on that trait that is due to the alleles an individual possesses.
Life-history theory A branch of evolutionary theory that explains aspects of anatomy and behavior by determining how patterns of development, growth, reproductive schedule, and lifespan have been shaped by natural selection.
Pleistocene period The geological epoch spanning approximately 2.5 million to 11 700 years before the present, when modern humans evolved.
Quantitative genetics The branch of genetics which studies how genes contribute to variation in complex traits determined by many genetic loci, such as size or fecundity.
Quantitative trait loci (QTLs) Regions of the genome that are linked to, or contain, genes which explain a part of the genetic variation in a complex trait.
Single nucleotide polymorphism (SNP) A DNA sequence in which a single nucleotide (A, C, T, G) differs between members of a species or between paired chromosomes.

Then and Now: The Unusual Life History of Humans

The human life history is characterized by several puzzling aspects. Compared to our closest relatives, the other great apes, humans have a delayed onset of reproduction; a short birth interval between each offspring; a complete cessation of reproductive capacity in females (menopause); and a long post-reproductive lifespan (Mace, 2000; Hawkes and Paine, 2006). Evolutionary biologists use 'life-history theory' to determine how these unusual patterns have evolved. Traditionally, studies on human life-history evolution have focused on the past, attempting to determine what genetic and environmental factors favored the evolution of these unusual life-history characteristics. Consequently, most studies on human life-histories have focused on contemporary 'traditional' societies, such as extant hunter-gatherer groups (see Hawkes *et al.*, 1997), whose lifestyle is thought to resemble that during the 'Pleistocene period' when many key human characteristics evolved.

This focus on a limited subset of human societies may hinder our attempts to determine how human life-histories evolved, since there is remarkable variation across cultural and ecological contexts. For example, average age at first reproduction can differ by 10 years even between populations with no access to medical care and contraception (Walker *et al.*, 2006); average family size ranges from little over one in contemporary Europe to around ten in the Hutterites, an Anabaptist group who practice communal living and shun birth control (Tietze, 1957); and the age when women have their last child differs by several years between populations. An increasing number of researchers have therefore begun to determine how this variation arises, including asking to what extent human life-history traits are still evolving in contemporary populations. This is particularly significant in the wake of the recent transition of many contemporary populations to dramatically reduced mortality and fertility rates (the 'demographic transition'), associated with industrialization and the introduction of effective contraception and medicine ('industrialized populations'). Determining how such rapid environmental changes may have altered selection on life-history traits and associations between them will be important for predicting how human life-histories may evolve in the future and the epidemiology of health and disease.

The question of whether or not contemporary human populations are undergoing evolutionary change has been a subject of debate in many disciplines. The major argument against the continuing evolution of human life-histories has been that, thanks to modern medicine and improved nutrition, nearly all children survive to adulthood in industrialized countries, which starkly contrasts with child mortality rates in preindustrial populations (Gagnon *et al.*, 2009; Rickard *et al.*, 2010; Gillespie *et al.*, 2013). Thus, natural selection through survival is weak in industrialized populations, but survival alone does not guarantee descendants in future generations. Only individuals that reproduce gain fitness, and while survival is a prerequisite to reproduce, natural selection ultimately acts on variation in reproductive success. Therefore, even if a set of individuals all survive to reproductive age, they may vary substantially in their ability to find a mate and produce and raise children, and this indeed is the case in industrialized populations (Byars *et al.*, 2010; Stearns *et al.*, 2010). This observation is crucial to the study of human life-history evolution, since any trait associated with variation in lifetime fitness can be under natural selection.

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Phenotypic Correlations between Human Life-History Traits and Fitness

The traditional approach to studying life-history evolution has been to quantify phenotypic correlations between life-history traits and fitness. This approach has revealed that both the opportunity for selection (variation between individuals in fitness) and strength of selection on life-history traits, such as age at first and last reproduction, can be substantial in industrialized populations (Stearns *et al.*, 2010) and that selection pressures can change rapidly over time (Moorad, 2013). For example, in a contemporary Gambian population going through the 'demographic transition,' selection reversed from favoring decreased height and increased BMI before the transition to favoring increased height and decreased BMI after the transition, over a period of less than 60 years (Courtiol *et al.*, 2013). This change probably arose at least partially from improvements in the predictability of food supplies, which led to reduced need to carry energy reserves that allowed girls and women to survive past the annual hungry season.

A Crash Course in Quantitative Genetics

Although studies of phenotypic selection on human life-history traits are informative, selection on phenotype alone does not lead to evolution. The 'breeder's equation' (**Box 1**) shows that only traits that have 'heritable' genetic variation can show an evolutionary response to selection (Falconer and Mackay, 1996). Only recently have studies on humans followed the lead of studies of natural populations of animals (Kruuk, 2004; Wilson *et al.*, 2010; Charmantier *et al.*, 2014) in applying methods that estimate selection, heritability, and evolutionary responses. Applying these methods would allow us to predict how traits under selection can evolve over time, which is entirely feasible, given recent evidence for phenotypic selection operating in modern societies (Byars *et al.*, 2010; Stearns *et al.*, 2010; Courtiol *et al.*, 2013; Moorad, 2013), and evidence that many of these traits are heritable (de Bruin *et al.*, 2001; Kirk *et al.*, 2001; Pettay *et al.*, 2005; Milot *et al.*, 2011; Vink *et al.*, 2012; Bolund *et al.*, 2015). Life-history traits show considerable among-individual variation, which may be driven in part by social (e.g., age at marriage) or cultural factors (e.g., contraception), but each trait also has a complex genetic basis and is influenced by many genetic loci. For example, age at first birth depends on social practices, but has a substantial genetic basis in modern populations, with an individual's genes explaining ~11% of the total phenotypic variation (Stearns *et al.*, 2010). These genes may influence characteristics underpinning an individual's age at first reproduction, such as behavior, personality, appearance, and reproductive physiology. The study of such complex quantitative traits is the subject of quantitative genetics.

Quantitative genetics is based on estimating the genetic contribution to phenotypic similarities between relatives. By measuring the phenotypes of many individuals and coupling this with their relatedness information, the phenotypic variation in traits can be divided into contributions from genetic and environmental factors (**Figure 1**). A traditional approach has been to compare sets of relatives, such as parents and their offspring, or different types of siblings. Based on information about the 'average' relatedness of these sets of relatives, the proportion of variation in a trait due to genetics can be

Box 1 Predicting Evolution: The Breeder's Equation

$$R = h^2 S$$

This simple equation states that the evolutionary response (R) to selection of a trait is a product of the selection differential S (the difference in mean trait value between individuals that reproduce and the mean trait value of the population), and the heritability h^2 of the trait. This univariate Breeder's equation was developed for predicting a response to artificial selection by animal breeders. However, in wild populations, where selection acts on many correlated traits simultaneously, the multivariate form of this equation is more effective:

$$\Delta \mathbf{z} = \mathbf{G}\beta$$

The multivariate breeder's equation calculates the expected change in several traits at once: the vector of changes of mean trait values Δz is the product of the genetic variance-covariance matrix G (the diagonal elements $\sigma^2_{1,1}$ and $\sigma^2_{2,2}$ are the genetic variances for traits 1 and 2, and the off-diagonal element $\sigma_{1,2}$ is the genetic covariance between the traits) and the vector of selection gradients β:

$$\begin{pmatrix} \Delta z_1 \\ \Delta z_2 \end{pmatrix} = \begin{pmatrix} \sigma^2_{11} & \sigma_{12} \\ \sigma_{12} & \sigma^2_{11} \end{pmatrix} x \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix}$$

Here, the response of traits 1 and 2 to selection additionally depends on the genetic covariance between the traits and the strength of selection on the other trait:

$$\Delta z_1 = \sigma^2_{11}\beta_1 + \sigma_{12}\beta_2$$
$$\Delta z_2 = \sigma^2_{22}\beta_2 + \sigma^2_{12}\beta_1$$

Thus, the response to selection on one trait depends on the degree to which genes associated with that trait are also associated with related traits under selection. However, the Breeder's equation assumes that any association between a trait and fitness are causal, and that there are no unmeasured traits. This assumption may be unlikely to be met (Hadfield, 2008; Morrissey *et al.*, 2010), and so an alternative model has recently been advocated for the study of natural populations (Morrissey *et al.*, 2012). This model is known as the Robertson–Price identity, or Robertson's secondary theorem of natural selection (Robertson, 1966; Price, 1970), and states that evolutionary change can be calculated as the additive genetic covariance between a trait and fitness:

$$\Delta \bar{z} = \sigma_a(z, w)$$

This equation does not assume causality between trait and fitness: evolution is predicted by directly estimating the genetic aspect of the trait–fitness association. This change may be due to selection on an unmeasured, genetically correlated trait and so the equation does not identify the true form of selection, but unlike the breeder's equation it does provide a direct prediction of evolutionary change. These methods are vital for determining how life-history traits have evolved and how they may respond to selection in the future.

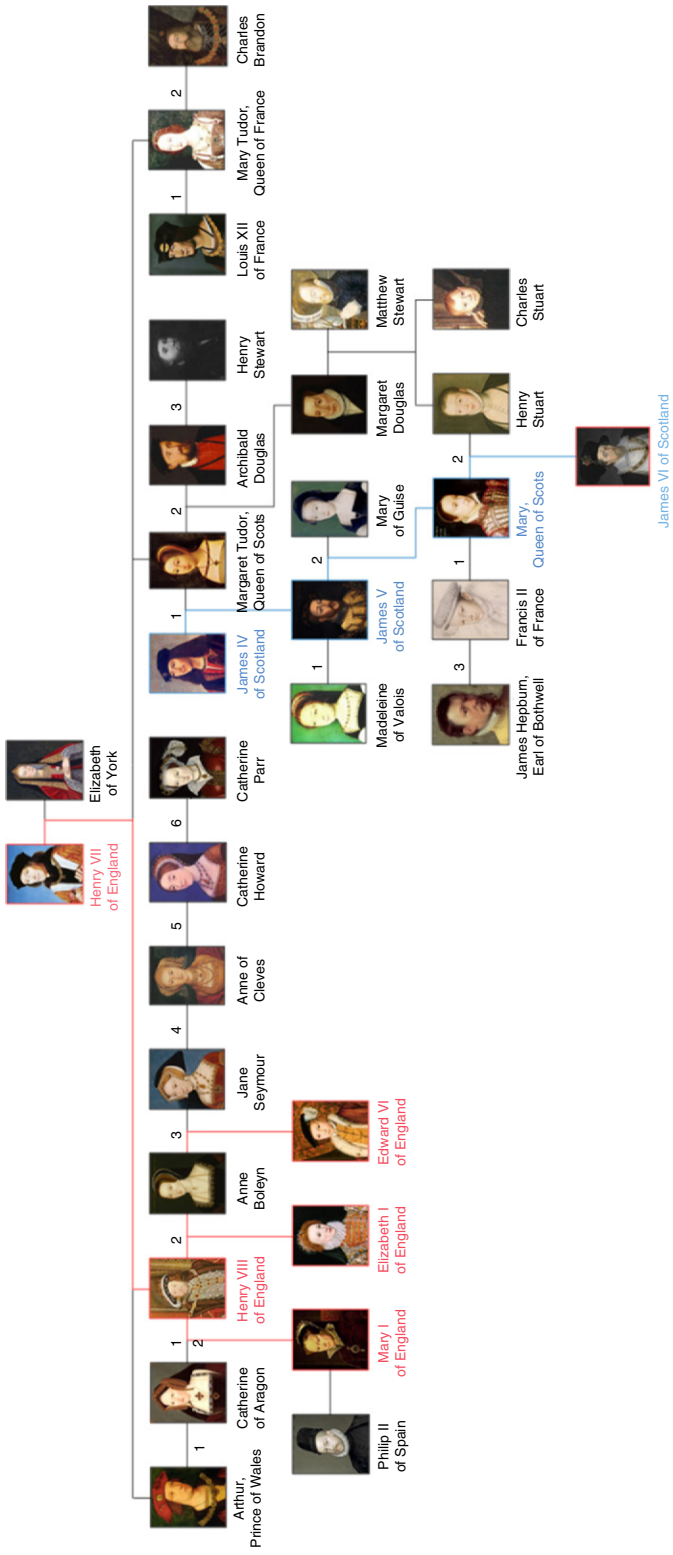

Figure 1 A pedigree showing the Tudor family tree, the dynasty of English monarchs, 1485–1603. Simple quantitative genetic analyses might calculate genetic parameters using trait values from parents and offspring (e.g., Henry VII and Henry VIII), or half-siblings (e.g., Mary I and Elizabeth I). The 'animal model' (see text) accounts for phenotypic information from all relatives: for example, James VI has 18 relatives in the pedigree depicted.

calculated: for example, monozygotic twins, full siblings and half-siblings share 100%, 50%, and 25% of their genes on average.

A drawback of this approach is that relatives share environments as well as genes: an individual's early puberty may arise because of genes inherited from their parents, or because they received a similarly good diet as their parents. In laboratory studies, this can be addressed by using breeding designs to accurately quantify genetic and environmental effects on specific traits (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Such manipulations are usually not possible in studies of wild populations, which must rely on the often incomplete and unbalanced data that are available. The same is true of human populations: close relatives commonly share environments and many traits are affected by cultural transmission, which upwardly biases heritability estimates calculated using traditional methods.

Another nonexperimental situation is in populations of domesticated animals, which include many different levels of relatives, some of which share genes but not environments. A statistical approach to estimate heritability of traits in such situations, the 'animal model,' was developed over 50 years ago (Henderson, 1950, 1975). It estimates the heritability of quantitative traits by using pedigrees (**Figure 1**), and has been successfully applied to answer a wide range of evolutionary questions in wild animal populations (Kruuk, 2004; Kruuk and Hill, 2008; Wilson *et al.*, 2010; Charmantier *et al.*, 2014). The animal model is able to adjust for confounding factors, and therefore can account for aspects of cultural transmission measured in pedigreed humans, such as socioeconomic status, in the model structure. The heritability estimate from such a model quantifies the genetic contribution to phenotypic variance, accounting for the fact that individuals within one socioeconomic group are likely to be more similar to each other than to individuals from other socioeconomic groups.

Several multigenerational pedigree datasets, collected for clinical, medical, or demographic reasons, but also suitable for quantitative genetic analyses are now available. Historic pedigree data on humans use social information to determine parentage, though this should not be a limitation given the low extra-pair paternity (EPP) rates in humans (Anderson, 2006) and the robustness of quantitative genetic models to low EPP rates (Charmantier and Reale, 2005; Firth *et al.*, 2015). The availability of such pedigree datasets and statistical methods means that an evolutionary perspective on human life-histories is gaining momentum.

Case Studies on Using Quantitative Genetics to Understand Life-History Evolution in Humans

Life-History Traits are Heritable

A system of data collection originally designed to enforce stringent tax collection may seem an unlikely source of data on human life-history evolution, yet a system of Finnish church records dating back to the seventeenth century has increased our understanding of human life-history evolution (**Box 2**). This dataset and others like it, comprising information on relatedness, survival, reproduction, and socioeconomic standing, have been used to determine the genetic basis of key life-history traits and how selection acts upon them. A landmark study using the Finnish dataset estimated heritabilities and genetic correlations of several important life-history traits (Pettay *et al.*, 2005). Heritabilities of female life-history traits were largely significant (estimates ranged 0.18–0.47 for number of children, number of surviving children, the time interval between births, age at last reproduction, and lifespan). Interestingly, bivariate models also revealed evidence for genetic trade-offs: a genetic correlation between inter-birth interval and lifespan suggested that genes associated with producing children rapidly were associated with a shorter lifespan. These results showed that selection could have shifted the average birth interval over time, due to the additive genetic basis of this trait, but that a response to selection could have been constrained because of genetic correlations between traits (Pettay *et al.*, 2005). In this case, selection for faster birth rate

Box 2 The Finnish tax system during the eighteenth century, and how it unexpectedly aided the study of human life-history evolution

The King of Sweden, of which Finland was formerly a province, decreed in 1749 that the Lutheran church was obliged to document all births, marriages, deaths, and movements in the whole population for tax purposes. At the time, the Finns mostly depended on farming for their livelihood, supplemented with fishing and hunting. The standard of living was low with famine and disease being common, and with the main causes of death being infectious diseases associated with malnourishment (Turpeinen, 1978; Hayward *et al.*, 2012). Genealogists have used such records to compile multigenerational datasets with accurate information on individual survival, reproduction, family configuration, and dispersal for up to 15 generations. Industrialization began relatively late in Finland, and fertility and mortality rates were high until this occurred in the early twentieth century (Liu *et al.*, 2012). This dataset offers rare opportunities to follow the same genetic lineages from 'natural' mortality and fertility periods through the different phases of the demographic transition until the modern day. Finnish church records also provide information on occupations of adult men, allowing classification of individuals into different socioeconomic groups. Distinguishing different levels of resource availability is important as it is often associated with survival, reproductive success, and selection on different life-history traits (Pettay *et al.*, 2007; Courtiol *et al.*, 2012). Similar datasets on historical human populations are also available for many other countries, and they are increasingly used in evolutionary research.

An example of a church record page (left) detailing life-events of historical Finnish families (on the right).

would also lead to indirect selection for shorter lifespan, with the likely overall outcome of selection being a compromise between such alternative outcomes. Such trade-offs are one of the forces maintaining genetic variation in key life-history traits (Stearns, 1992).

Genetics of Life-History Traits in the Two Sexes – an Arena for Conflict

Genetic correlations between traits can constrain independent evolution in the two sexes because, while the sexes share much of their genome, their evolutionary interests are often not aligned (Parker, 1979). If the sexes have different phenotypic optima for a trait, different selection pressures act in the two sexes. Coupled with a shared genetic basis of the trait (a high cross-sex genetic correlation, r_{MF}), this leads to conflict over optimal trait expression, or intra-locus sexual conflict (Lande, 1980; Bonduriansky and Chenoweth, 2009). Studies on the Finnish population have revealed that age at first and last reproduction, reproductive timing and reproductive rate were all strongly genetically correlated with fitness in both sexes, and thus under selection (Bolund *et al.*, 2013). Further, there were no differences between the sexes in these genetic correlations between life-history traits and fitness. The genetic correlations between the sexes (r_{MF}) were also high, suggesting that, for example, genes associated with an early age at first reproduction in women are also associated with an early age at first reproduction in men. This indicates that the sexes cannot reach their sex-specific optima of trait expression even if selection pressures are different between males and females, as was indeed the case. These results likely reflect the strictly monogamous mating system in preindustrial Finland, which meant that an individual's reproduction was severely constrained by that of their partner. Thus, the sexes constrained each other's independent evolution. Given that humans exhibit many different mating systems (monogamy, polygyny, polyandry, polygynadry), they offer the opportunity to investigate how changes in mating systems affect selection pressures and genetic correlations. For example, over the reproductive lifetimes of Utahans born between 1830 and 1894, socially induced reductions in the rate and degree of polygamy corresponded to a 58% reduction in the strength of sexual selection (Moorad *et al.*, 2011). This illustrates the potency of sexual selection in polygynous human populations and the dramatic influence that societal changes can have on evolutionary processes. Intra-locus sexual conflict can also have important implications for health in modern populations, as shown by recent work using the ongoing Framingham Heart Study in the USA, which showed that evolution of a contemporary population may be constrained by genetic conflict between the sexes (Stearns *et al.*, 2012). In this population, selection favored shorter women and taller men, but female height was negatively genetically correlated with male cholesterol. Thus, selection for shorter women could lead to higher cholesterol levels in men. The possibility that complex traits associated with diseases can have a sexually dimorphic genetic basis which is maintained by disparate selection pressures in the sexes is an exciting emerging area of study.

Genetics of Life-History Traits over the Lifetime

As well as differing between the sexes, the expression of additive genetic variance is often observed to increase with age in diverse taxa (Réale *et al.*, 1999; Charmantier *et al.*, 2006; Von Hardenberg *et al.*, 2007; Wilson *et al.*, 2007, but see Brommer *et al.*, 2007). Evolutionary theories of senescence predict this is due to the accumulation of late-acting deleterious mutations, which are not selected against due to the declining force of selection with age (the mutation accumulation theory of ageing; Medawar, 1952). Senescence may also have evolved through antagonistic pleiotropy (Williams, 1957), or the effects of genes promoting fitness in early life at the expense of fitness in later life, a scenario which would lead to antagonistic genetic correlations between early- and later-life fitness. Quantifying age-specific changes in additive genetic variance is thus crucial if we are to determine the evolutionary mechanisms for senescence (Wilson *et al.*, 2008). In the preindustrial Finnish population, the additive genetic variance for female fecundity increased with age (Pettay *et al.*, 2008), due to changes in maternal and genetic variance for fecundity. Before age 31, maternal identity explained 25% of the variance in fecundity, and the additive genetic effect only 13%; after the age of 31, the maternal effect accounted for only 2% of the variance in fecundity, and the additive genetic effect increased to 25%. This suggests that early in reproductive life, mothers may have played a substantial role in enhancing their daughters' fecundity, perhaps by proving childcare through a 'grandmother effect' (Hawkes and Coxworth, 2013; Lahdenperä *et al.*, 2004). However, the Finnish study found no evidence for a negative genetic correlation between fecundity at early and later ages, and thus no evidence for the antagonistic pleiotropy theory of ageing. Further research using these approaches could determine whether the trade-off between fecundity and survival changes with age, testing the prediction that the menopause could have evolved as a result of diminishing benefits and increasing costs of reproduction with increasing female age (Williams, 1957; Hawkes and Coxworth, 2013).

Genetics of Life-History Traits Over Time and Across Environments

A major goal of evolutionary quantitative genetics is to predict evolutionary change over several generations: given current selection pressures and patterns of genetic (co)variance, how will traits respond to selection and be expressed in the future? To this end, the Framingham heart study measured the strength of selection, estimated genetic (co)variation, and predicted gradual evolutionary change in several life-history and health traits in the contemporary USA. The descendants of the study women were predicted to become on average slightly shorter and stouter; have lower total cholesterol levels and systolic blood pressure; have their first child earlier and reach menopause later than they would in the absence of selection (Byars *et al.*, 2010). Two important caveats to these predictions are that both the selection pressures and the genetic (co)variance may change rapidly. Changes in climate have been shown to affect selection and patterns of genetic (co)variation in wild animals (Garant *et al.*, 2008; Björklund *et al.*, 2013) and could

have profound effects on human populations, particularly with regard to changing patterns of disease spread (Lafferty, 2009; McMichael, 2014). Generally, the genetic (co)variance of traits (conceptualized as **G**, **Box 1**) are predicted to change over time and across environments (Roff, 2000) but little is known about how rapidly this happens (Steppan *et al.*, 2002; Arnold *et al.*, 2008). This has important implications for predicting evolutionary change, because such predictions rely on stability of **G** and β (**Box 1**). Thus, it is important to reveal how rapid changes in culture and technology are changing human biology. A study on the Finnish dataset found that the **G**-matrix of four key life-history traits (age at first and last reproduction, lifespan and lifetime reproductive success) remained largely stable over the demographic transition in Finland, but showed a trend for increased additive genetic variance (increased evolutionary potential of the population) after the demographic transition (Bolund *et al.*, 2015). Similarly, recent studies of other populations found changes in the additive genetic variance of age at first reproduction over a 140-year period (Milot *et al.*, 2011) and female fecundity over a 100-year period (Kohler *et al.*, 2002). Such changes in patterns of genetic (co)variance mean that projections of evolutionary change over more than a few generations are likely to be unreliable.

Studying Human Life-History Evolution in the Genomics Era

The last 20 years have seen rapid progress in genomic technologies. A major aim of medical genetics is to identify 'quantitative trait loci' (QTLs) associated with risk of developing diseases including cancer, heart disease, and mental illness (Plomin *et al.*, 2009; Visscher *et al.*, 2012). Sequencing the human genome has enabled the development of 'genome-wide association studies (GWAS),' which assess an individual's genotype at many thousands of 'single nucleotide polymorphisms (SNPs)' across the genome (Donnelly, 2008; Hindorff *et al.*, 2009). GWAS can also provide insight into the genetics underpinning life-history traits, as has been demonstrated in wild populations (Slate *et al.*, 2010; Jensen *et al.*, 2014). Combining genome sequence data with pedigree and life-history data could determine how individual genetic loci underlie important associations between life-history and health traits. A recent study found a negative genetic correlation between the lifetime number of children born to women and their lifespan, and then identified five SNPs that were associated with this relationship (Wang *et al.*, 2013). However, this result was not robust to changes in the sample, illustrating the difficulty in identifying QTLs that underlie variation in quantitative traits that are likely to be influenced by hundreds or even thousands of loci.

In summary, application of quantitative genetic techniques can help us to determine how cultural and environmental changes have led to new selection pressures and, importantly, what the evolutionary consequences of these changes could be. The observation that our biological nature continues to evolve has important implications for public health and demographic forecasts because it will allow us to predict human evolutionary responses to a rapidly changing world.

See also: Aging: Why Do We Age?. Evolutionary Genetics, History of. Human Life Histories, Evolution and. Life History Trade-offs. Schools of Classification

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Life-History Evolution, Human Impacts on

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Glossary

Adaptive landscape Relationship between fitness and trait(s) under selection (= fitness landscape).

Fitness Individual contribution to the genetic pool of the next generation (= relative reproductive success).

Response to selection Change in mean trait value(s) in a population due to selection. Response to selection operates through the transmission of heritable information (nucleic acids, hormones, knowledge...) from one generation to the next.

Selection The process through which environmental conditions generate fitness variation among individuals. Selection operates on different units of selection corresponding to the different levels of biological organization (genes, cells, individuals…).

Introduction

The expansion of agriculture about 10 000 years ago allowed the settlement and growth of human populations, and from there the development of cities and of a social organization (civilization) that promoted technological innovations. Together, the human demographic expansion and technological improvements are the main drivers of so-called 'anthropogenic changes,' the perturbations brought about by human activities on natural systems.

Fundamentally, anthropogenic perturbations on natural systems simply arise from resource consumption by humans. However, its manifestations are multiple and interconnected, among which are often reported landscape alterations, soil deterioration, disruption of geochemical cycles, climate warming, or over-harvesting. All of these perturbations almost inevitably alter, directly or indirectly, the selective pressures acting on organisms.

Impacted populations must respond to these anthropogenic selective pressures by evolving life histories that are more fit to the newly selected, composite adaptive landscape that results from the combination of natural and anthropogenic selection. This response is the subject of the present article. If the response is too slow to track changes in the selective pressures, the population goes instinct. This failure to adapt is the underlying cause of the global biodiversity loss currently experienced by the earth ecosystems.

First, in this article, basic theory of selection and response to selection is recalled, then a specific focus on three major sources of anthropogenic selection (harvesting, habitat fragmentation, and temperature increase) is provided, and finally it is examined how rapid life-history evolution may change natural selection acting back on life histories, the so-called eco-evolutionary feedback loop.

Selection and Response to Selection

Natural and Anthropogenic Sources of Selection

In this article only selection acting on individuals transmitting genes to their offspring will be considered, because the individual is the level of selection having received the most thorough treatment in the literature (for a broader perspective see Wilson and Sober, 1994), and because genes represent the most widespread 70 support of heredity (for non-genetic inheritance, see Danchin, 2013). Selection of individuals may be 'natural' or 'artificial' depending on whether it results from nonhuman or from anthropogenic factors, respectively. To illustrate this, let me consider the case of pike *(Esox lucius)* in Windermere, the largest natural lake in England.

In Windermere, scientists have conducted long-term mark-recapture experiments on pike (Le Cren, 2001). The resultant data has allowed the computation of a component of natural selection, namely the form of the relationship between survival and body size (Haugen *et al.*, 2007; Carlson *et al.*, 2007; Vindenes *et al.*, 2014). This relationship shows that natural selection favors large-sized pike up to a body length of about 60 cm (blue solid line in **Figure 1**), after which survival is likely to remain constant (Vindenes *et al.*, 2014). A larger size likely provides pike with access to more resources, increased protection against cannibals and other predators, and increased energy stores to survive to winter fasting (Edeline *et al.*, 2007; Vindenes *et al.*, 2014). From this relationship, we may predict that there is a *directional natural selection* for rapidly reaching a body length of about 60 cm in Windermere pike.

In parallel with mark-recapture experiments, scientists have also submitted pike to gillnet fishing since the early 1940s (Le Cren, 2001). All pike captured in the gillnet fishery are killed, measured for several biological variables, and their opercular bones are removed. These bones record the age of each fish in the form of annuli (like in a tree trunk), and the radius of these annuli are proportional to individual body length, thus allowing the back calculation of length-at-age. From this information, it was possible to model the link between body length and the probability for a fish to be captured in the fishery. This relationship is depicted in **Figure 1** (red dashed line), which shows that the probability for a pike to be caught in the gillnet fishery suddenly increases after a body length of 50 cm. Hence, we predict that there is a *directional anthropogenic selection* for growing slowly so as to remain smaller than about 50 cm in Windermere pike.

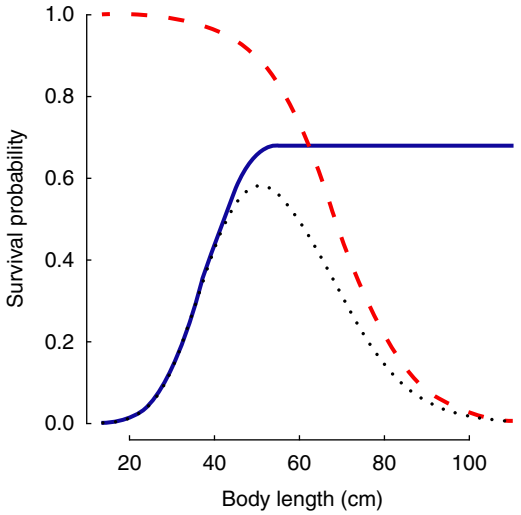

Figure 1 Size-dependent survival probability of Windermere pike (*Esox lucius*) through the directional components of natural selection (blue solid line) and the gillnet fishery (red dashed line). The resultant composite survival curve (the product of the two functions) is represented with the black dotted line and shows stabilizing selection around an adaptive peak at about 50 cm. The natural survival function was computed following Vindenes *et al.* (2014).

Combining these two (natural and anthropogenic) sources of selection acting on the body size of Windermere pike reveals the composite adaptive landscape, which reflects the truly experienced selective regime (black dotted line in **Figure 1**). We see that this composite landscape is very different from the naturally selected landscape. Anthropogenic selection decreases the overall area below the curves, i.e., it decreased overall survival probability of pike. This is predicted to induce an evolutionary response toward faster life histories in the pike population (see section Non-phenotypically Selective Harvesting of this article).

However, this increase in mortality is nonrandom, but instead biased toward large-sized pike, thus further reinforcing directional selection toward smaller sizes and faster life histories (see section Phenotypically selective harvesting of this article). This biased mortality is visible in that anthropogenic selection generates an adaptive peak (i.e., stabilizing selection) around a body length of 50 cm. Let us now more closely examine how response to these sources of selection may be qualitatively predicted.

Response to Directional Selection

Predicting response to selection is the central purpose of quantitative genetics theory (Lynch and Walsh, 1998). This theory predicts that dynamic response to selection of a single trait having negligible genetic covariance with other fitness traits obeys the equation:

$$\overline{\chi}_{t+1} = \overline{\chi}_t + R_\chi \qquad [1a]$$

where $\overline{\chi}_{t+1}$ = mean value of trait x in the population at generation $t+1$, $\overline{\chi}_t$ = mean value of trait x in the population at generation t, and R_χ = response to selection in trait χ, that may be recast as:

$$R_\chi = H_\chi S_\chi = \frac{Va_\chi}{Vp_\chi} S_\chi = Va_\chi \frac{\partial w_\chi}{\partial_\chi} \qquad [1b]$$

where $H_\chi = Va_\chi/Vp_\chi$ = narrow-sense heritability for the trait where Va_χ = additive genetic variance and Vp_χ = phenotypic variance (with $Vp_\chi \geq Va_\chi$), and $S_\chi = \overline{\chi}_a - \overline{\chi}_b = net$ directional selection differential where $\overline{\chi}_a$ = mean trait value in the population after selection and $\overline{\chi}_b$ = mean trait value in the population before selection. Here, following Lande and Arnold (1983) the equation is rearranged to obtain the directional selection gradient $(\overline{\chi}_a - \overline{\chi}_b)/Vp_\chi = \partial w_\chi/\partial_\chi$, where w_χ = relative fitness of an individual with trait x.

Note that eqn [1b] makes the assumption that Va_χ is independent of S_χ, which is not true if directional selection drives allelic frequencies toward 0 or 1 in the population (see section Evolvability of this article). In such a case, eqn [1b] can be used only to predict short-term response to selection. Additionally, eqn [1b] is often used by considering S_χ as a constant, which may be true when one considers artificial selection only, but which is wrong when trait change alters natural selection acting on that trait – the so-called eco-evolutionary feedback loop (see section Change in the naturally selected adaptive landscape of this article).

However, despite these shortcomings, eqn [1b] remains conceptually very useful in that it clearly shows that evolution of a life-history trait requires that both $Va_\chi > 0$ and $\delta w_\chi/\delta_\chi \neq 0$, and that the speed of evolution is proportional to the values of these two components. Generally, $Va_\chi > 0$ for life-history and morphological traits (Mousseau and Roff, 1987), which are thus highly susceptible to respond to anthropogenic directional selection. Let us now concentrate on reviewing some of the current knowledge on such responses.

Evolutionary Response to Harvesting

Humans have harvested wild plant and animal species since they appeared on earth. Hunting by human populations is thought to have played a role in the late Pleistocene (15 000 years ago) extinctions of large terrestrial mammals in Australia, North America, and South America (Barnosky *et al.*, 2004). The development of agriculture has made humans less dependent upon wild plant and animal resources, but the parallel rise in the human population and progress in harvesting technologies has also tremendously increased the pressure on those populations that remain exploited. The overexploitation problem is mostly documented in fish and mammals, but it may arise in any animal or plant population. Here, we will consider separately the effects of non-phenotypically selective and phenotypically selective harvesting.

Non-Phenotypically Selective Harvesting

Non-phenotypically selective harvesting adds a mortality component to the natural mortality background uniformly in all age or size classes, and thus provides a gain in relative fitness to early-maturing individuals which have a higher

probability to reproduce before being killed (Abrams and Rowe, 1996).

If maturation (and reproductive allocation) conflicts with somatic growth due to physiological or ecological trade-offs, then harvesting also induces a decrease in somatic growth rates. Similarly, trade-offs may occur between reproduction and somatic maintenance, so that we may expect to also observe increased senescence in exploited populations. Accordingly, a number of studies have demonstrated that reproduction incurs a cost in terms of somatic growth (Roff, 1992), predation risk (Magnhagen, 1991), or immune capacity (Zuk and Stoehr, 2002). A cost of reproduction in terms of senescence (Williams, 1957) has received more contrasted support (Reznick *et al.*, 2004).

Phenotypically Selective Harvesting

Harvesting is almost always phenotypically selective. Harvesters often target larger and older individuals due to their higher market or trophy values, and/or due to regulatory rules that impose a minimum size limit to the catch. Compared to non-size-selective harvesting, this biased mortality further strengthens the relative advantage for an early maturation, and also directly selects for slower somatic growth because larger individuals are often faster growers (Biro and Post, 2008). In line with this theoretical prediction, earlier maturation and/or smaller body size in response to harvesting have been documented in a variety of wild, exploited populations.

Size-related trait change in exploited populations are on average 3 times faster than in unexploited populations, and results in 20% smaller traits on average (Darimont *et al.*, 2009). For instance, in the snow lotus *(Saussurea laniceps)*, plant cropping for medicine has induced a decrease in mean plant size from about 30 cm in 1900 to about 20 cm in the 2000s (Law and Salick, 2005). In the bighorn sheep (*Ovis canadensis*), mean body weight of rams has declined from 85 to 65 kg from 1975 to 2005 due to trophy hunting, while mean horn sizes have decreased from about 70 to 50 cm (Coltman *et al.*, 2003). In the pink salmon (*Oncorhynchus gorbuscha*), weight at spawning (age 2) has decreased by up to 34% from the 1950s to the 1990s (McAllister *et al.*, 1992). In the Canadian Altlantic cod, female length at 50% maturation probability dwindled from about 65 cm (and 7 years of age) in the early 1950s to about 40 cm (and 4.5 years of age) in the early 1990s, and remained at this low level up to the early 2000s (Olsen *et al.*, 2004; Swain, 2011). On longer time scales, decrease in mean body size may be even larger. Archeological records of skeletal parts suggest that mean body size of cod in the Gulf of Maine (USA) has decreased from 100 to 30 cm due to overfishing (Jackson *et al.*, 2001).

Harvest-induced evolution is sometimes (maybe abusively) termed 'maladaptive,' because it may drive the population far from its naturally selected adaptive peak (i.e., evolution is maladaptive relative to natural selection acting alone). This 'maladaptive' evolution simply reflects a severe reduction in fitness due to anthropogenic selection. For instance, in Atlantic cod (*Gadus morhua*), earlier maturation at a smaller size in response to fishing is expected to increase the mortality cost of reproduction. A reduction of age at maturity from 6 to 4 years projects into a 25–30% decrease in population growth rate (Hutchings, 2005). This decrease in fitness may be linked to increased reproductive mortality (Hutchings, 2005; Swain, 2011), as well as changes in traits correlated with body size. For instance, selection against a large body size in Atlantic silverside (*Menidia menidia*) depresses fecundity, egg volume, larval size at hatch, larval viability, larval growth rates, food consumption rate, and conversion efficiency (Walsh *et al.*, 2006).

Evolutionary Response to Habitat Loss and Fragmentation

Habitat loss and fragmentation often result from 'modern' agricultural practices, road building or, more dramatically, from urbanization. Habitat loss is a primary cause of species extinction, indicating that the generated selective pressures are so strong that many populations can simply not adapt. Habitat fragmentation, in contrast, may allow species to persist, but tend to change large homogeneous populations into metapopulations, i.e., a population of populations between which individuals may disperse (Levins, 1969; Pulliam, 1988). Here, I consider how anthropogenic habitat fragmentation may affect life histories through selection acting on body size and on dispersal ability.

Size-Dependent Selection

Although many studies have linked a large body size with an increased extinction risk in response to anthropogenic perturbations (Purvis *et al.*, 2000; Cardillo *et al.*, 2005; Van Allen *et al.*, 2012), the link between body size and sensitivity to habitat perturbation is not clear (Henle *et al.*, 2004). This lack of evidence for size-dependent selection might come from conflicting selective pressures.

Large animals have large home ranges (Peters, 1983; Jetz *et al.*, 2004), and are thus more likely to be impacted by the fragmentation and loss of their vital habitat. For instance, carnivores in protected areas primarily die from 'edge effects,' i.e., due to conflicts with humans on reserve borders (Woodroffe and Ginsberg, 1998). In the Amazonian forest, fragmentation-induced edge effects magnify the negative impact of hunting on medium-sized and large vertebrates (Peres, 2001). Additionally, a large body size is associated with small population sizes (Woodward *et al.*, 2005). Hence, in a context of reduced habitat availability, large-bodied species may rapidly reach population sizes at which demographic stochasticity elevates extinction risk. Finally, because habitat perturbation imposes so strong selective pressures, population persistence critically depends on the capacity of the population to adapt (or disperse, see below). However, a large body size requires a long development time (i.e., long generation time), and thus imposes slow life histories and slow rates of adaptive evolution. In parallel with selection against a large body size, habitat perturbation may also select against a *small* body size. A small size is associated with a narrower range of prey sizes, reduced trophic generalism (Woodward *et al.*, 2005), and possibly also with an increased habitat specialization.

Accordingly, this link between a small body size, habitat specialization, and increased sensitivity to habitat loss was found in birds (Owens and Bennett, 2000) and freshwater fish (Olden *et al.*, 2007), but the underlying drivers remain unclear. Therefore, the net overall effect of habitat fragmentation might be to select against both small and large body sizes, ultimately favoring medium-sized individuals and species. More research is needed to test this hypothesis.

Dispersal-Dependent Selection

Dispersal generally brings a selective advantage in heterogeneous environments, where the environment is understood in terms of the ecological niche (Hutchinson, 1957). Hence, anthropogenic selection for increased dispersal propensity occurs when habitat fragmentation increases habitat heterogeneity in space and time. In particular, smaller patch sizes are likely to amplify population fluctuations due to increased demographic stochasticity (from smaller population sizes), elevate the temporal heterogeneity of the environment, and ultimately result in increased selection for dispersal (Parvinen *et al.*, 2004).

In turn, dispersal-dependent selection may induce the evolution of life-history traits correlated with dispersal (Roff and Fairbairn, 2001). For instance, the sand cricket (*Gryllus firmus*) shows a genetically based trade off between flight allocation and reproductive allocation (King *et al.*, 2011), indicating that selection for dispersal will also induce evolution towards reduced reproductive investment. In insects and fish, an increased ability for long-distance dispersal is often positively associated with a large body size, because a larger size increases the energetic efficiency of movement (Roff, 1991). Hence, anthropogenic selection for increased long-distance dispersal may indirectly select for a large body size, and is thus expected to interact with direct size-dependent selection (see previous section).

However, anthropogenic habitat fragmentation may also increase dispersal mortality and select against dispersal traits. Hence, the counteracting effects of landscape fragmentation on the evolution of dispersal and life histories may give rise to complex evolutionary dynamics when realistic ecological complexity is accounted for (Parvinen *et al.*, 2004). This complexity might be responsible for an often reported absence of a link between dispersal-dependent selection and life-history evolution (Ronce and Clobert, 2012).

Evolutionary Response to Climate Warming

Temperature determines the rate of metabolic reactions and is a key driver of individual physiology (Brown *et al.*, 2004). Warmer ambient temperatures have been repeatedly reported to favor smaller body sizes and faster life histories in both endotherms and ectotherms (Angilletta, 2009), a phenomenon dubbed 'Bergmann's Rule' (BR) in endotherms and temperature-size rule (TSR) in ectotherms (Atkinson, 1994).

BR posits that heat loss of an endotherm organism is proportional to its body surface-to-volume ratio. Because body volume increases faster than body surface area with increasing body size, there is a selective advantage to a small body size (higher body surface-to-volume ratio and easy heat loss) in warm areas, and conversely to a large body size in colder climates (lower body surface-to-volume ratio and reduced heat loss).

The thermodynamic argument proposed by Bergmann for endotherms does not hold for ectotherms, and the underlying drivers of the TSR remain far from clear (Angilletta, 2009). Proximate mechanisms so far proposed to explain the TSR include a higher thermal sensitivity of gonad growth rate than body growth rate, resulting in an increased fraction of energy allocated to gonads under warmer temperatures (Van der Have and de Jong, 1996; Zuo *et al.*, 2011), and/or smaller sizes of cells and genome at higher temperature (Partridge *et al.*, 1994; Hessen *et al.*, 2013), and/or limitation of oxygen path length at higher body sizes (Pörtner and Knust, 2007; Forster *et al.*, 2012). Ultimate (adaptive) mechanisms include increased mortality and/or higher population rate of increase at higher temperatures (Sibly and Atkinson, 1994).

However, none of these mechanisms has yet been shown to be universal. Recently, however, there has emerged the idea that different thermal sensitivities of ingestion and maintenance rates may drive a competition-mediated selective advantage for small-bodied individuals under warm conditions (Ohlberger *et al.*, 2011; Reuman *et al.*, 2014; Edeline *et al.*, 2013). To understand the arguments it is necessary to start from the R^* theory of exploitative competition (Tilman, 1982), adapted to account for the size-dependency of resource consumption and metabolic rates (Persson *et al.*, 1998; De Roos *et al.*, 2003).

In this framework, resource-dependent variations in individual body mass dB/dt are given by:

$$\frac{dB}{dt} = Fr(R) - m = aR - m \qquad [2]$$

where dB/dt is also equivalent to individual fitness since mass gain can be invested in reproduction, a = attack or intake rate in a Holling's type I functional response, and m = maintenance metabolic rate. Equilibrium, i.e., null fitness, occurs at $R = R^*$ such that $R^* = m/a$, i.e., where resource density makes aR^* equal to m (**Figure 2**). Just as in Tilman's (1982) theory, the individual having the lowest R^* wins the competition and excludes the others that starve to death (Persson *et al.*, 1998).

Biological rates usually follow power allometric exponents (Peters, 1983; Brown *et al.*, 2004), such that the relationship between R^* and body size can be written:

$$R^* = \frac{m}{a} = \frac{\mu B^{\gamma}}{\alpha B^{\delta}} = \frac{\mu}{\alpha} B^{\gamma-\delta} \qquad [3]$$

where μ, α, γ, and δ are positive constants. Equation [3] shows that whether R^* increases or decreases with an increasing body mass, B depends on the sign of the $\gamma - \delta$ difference. If maintenance metabolic rate increases faster with increasing body mass than intake rate, i.e., if $\gamma > \delta$, then R^* increases with increasing body mass, small individuals dominate large individuals in exploitative competition (Persson *et al.*, 1998; De Roos *et al.*, 2003; Persson and De Roos, 2006), and low resources select for smaller body sizes. Resource-poor environments select for reduced somatic growth rates (i.e., select for smaller body sizes) in a variety of taxa (Arendt, 1997), suggesting that an increase of R^* with body size is common.

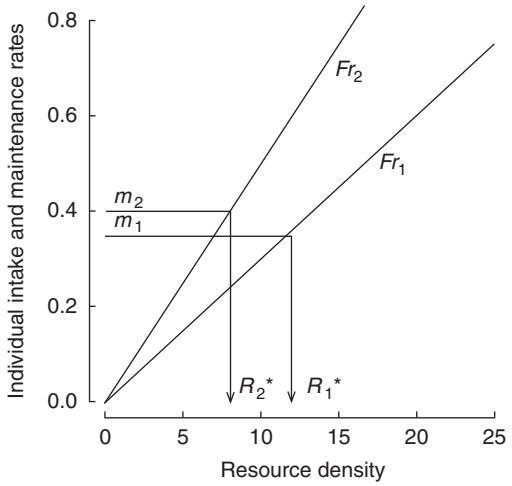

Figure 2 The R^* rule for two individual consumers competing for the same resource R. Resource intake rate is represented by a Holling's type I functional response Fr, while maintenance rate m is resource-independent. Zero growth (equivalent to null fitness in eqn 2) occurs where $R=R^*$. The individual having the lowest R^* (here individual 2) wins the competition. Reproduced from Tilman, D., 1982. Resource Competition and Community Structure. Princeton, NJ: Princeton University Press.

However, R^* *decreases* with body size in waterflies *Daphnia* sp. (Gliwicz, 1990), suggesting that filter-feeding organisms – and maybe generally organisms having a Holling's type I functional response – might constitute an exception. More research is needed to clarify this question.

Biological rates are in fact not just size- but also temperature-dependent (Gillooly *et al.*, 2001). The effects of temperature on intake and maintenance rates included in eqn [3], and thus on and its size dependency, are poorly understood. It was shown in a physiologically-structured model tailored for Eurasian perch Perca fluviatilis that higher temperatures may increase the advantage of small against large individuals in exploitative competition (Ohlberger *et al.*, 2011). Hence, climate warming seems to increase in large individuals relative to small individuals. Reuman *et al.* (2014) and Edeline *et al.* (2013) came to a similar conclusion in unicellular phytoplankton and river fish communities (52 species), respectively. Finally, Vindenes *et al.* (2014) showed in Windermere pike that this temperature-dependent R^* selection translated into accelerated juvenile growth and decreased survival of large individuals, such that the proportion of medium-sized individuals increased in the population. More details about warming-induced selective pressures acting on life-history traits may be found in the chapter An Age- and Length-Structured Life History Model for Pike, a Top Predator in Freshwater Ecosystems (00094) by Yngvild Vindenes. Taken together, these results call for an increased focus on size-dependent exploitative competition in climate-warming research.

Reversibility of Trait Changes and Population Recovery

A natural management response to human-induced ecological problems is to relax the source of perturbation, so that the ecosystem may return to its initial state. However, not all sources of perturbation are easily relaxed. In case of habitat loss and fragmentation, backward re-naturalization of urbanized or agricultural habitats requires will power and investments that often go beyond the capacities of local managers. This problem is even more insurmountable when it comes to reversing the global trend toward increased production of greenhouse gas and the parallel global warming.

Hence, most examples of relaxed anthropogenic forcing on life histories come from harvested systems. In particular, fisheries provide us with reassuring examples of systems that return to their initial state (rebound) after fishery relaxation or closure, but also with the worrying cases of systems that do not recover (stasis). Let us here concentrate on the possible causes of stasis (for rebounds, see Edeline *et al.*, 2007; Edeline *et al.*, 2009; Conover *et al.*, 2009 and Frank *et al.*, 2011). Evolutionary stasis has two possible sources: a loss of evolvability for life-history traits and/or a change in the natural selective pressures acting on life histories.

Evolvability

Equation [1b] assumes for simplicity that additive genetic variance (evolvability) and selection differentials are independent, such that the trait can endlessly evolve. However, in theory evolvability and selection are not independent because intense directional selection should exhaust additive genetic variance and impair reversibility of trait changes, unless genetic variation is constantly renewed (Crow, 2008). This gave rise to concern about a potential loss of evolvability in heavily exploited populations (Hutchings and Fraser, 2008), and might explain why some fish stocks have problems to recover even after the relaxation of exploitation rates (Hutchings and Reynolds, 2004; Frank *et al.*, 2005; Swain, 2011). In these stocks, relaxation of fishing would reestablish the naturally selected phenotypic optimum (i.e., $\delta w_x/\delta_x \neq 0$ in eqn [1b]), but traits could not follow due to lack of genetic variability (i.e., $Va_x \to 0$ in eqn [1b]). Is this a credible hypothesis?

The tremendous phenotypic diversity of formerly wild, now domestic animals (e.g., dogs, pigeons, chicken, …) or plants (roses, cereals, …) suggests that genetic diversity hardly limits the range of possible phenotypes under captive conditions. For instance, maize (*Zea mays*) still responds to strong bidirectional selection on oil and protein concentration in grains since more than 100 generations (Moose *et al.*, 2004). Similarly, white Plymouth Rock chickens continued to respond to bidirectional selection on body weight at 8 weeks of age since 54 generations (Dunnington *et al.*, 2013). At generation 54, males weighted on average 2 kg in the high-selected lines versus 0.173 kg in the low-selected lines, respectively (1.380 vs. 0.129 kg in females). These examples from domestic populations suggest that additive genetic variance often persists, even under severe directional selection (Crow, 2008). But what about wild populations?

A fundamental difference between captive and wild conditions is natural selection. If anthropogenic and natural selection act in opposition (**Figure 1**), then overall selective pressures are no longer directional but stabilizing, and stabilizing selection may erode trait genetic variance even more

rapidly than directional selection. However, in Windermere pike, the overall form of fishing-induced selection was in fact not directional (as presented for simplicity in **Figure 1**) but disruptive (Carlson *et al.*, 2007), which is typical for gillnets (Lagler, 1968). Consequently, scientific gillnet fishing for pike in Windermere, whose intensity was mild (mean exploitation rate was 6.5% per year $\pm$4.7 SD (Langangen *et al.*, 2011)), was associated with an increased variability for length-at-age in the population, and thus presumably increased genetic variance for body size in the pike population (Edeline *et al.*, 2009).

In contrast with scientific fishing, commercial fishing may impose very high exploitation rates (Hutchings (2000) reports reductions of 45–99% in reproductive biomass), in which case medium-sized individuals probably have no chance to reach a large size, even if harvest-induced selection is disruptive. Hence, in many situations anthropogenic selection likely generates strong directional selection acting in opposition with natural selection (**Figure 1**), and resulting in magnified stabilizing selection with a great potential for eroded genetic variance in body size and associated traits. To date, no empirical study has specifically explored the effects of commercial harvest on the genetic variability of fitness-related traits (for neutral genetic markers see for instance Allendorf *et al.*, 2008; Pinsky and Palumbi, 2014), and this obviously represents an important gap in our knowledge.

Change in the Naturally Selected Adaptive Landscape

If a loss of adaptive genetic variability for body size and correlated traits is not involved (i.e., $Va_x > 0$ in eqn [1b]), impediment to the recovery of exploited populations necessarily results from a change in natural selection (i.e., $\delta w_x/\delta_x \to 0$ in eqn [1b]). It has been argued that natural selection toward the phenotypic optimum is likely to be much weaker than harvest-induced selection away from it (Law, 2000; Enberg *et al.*, 2009). However, weak natural selection is incompatible with the fact that harvest-induced evolution incurs severe fitness costs (see above). Instead, as a population phenotype evolves away from the naturally selected optimum, the strength of natural selection toward the optimum should increase. A more likely hypothesis to explain impediment to recovery involves a fishing-induced change in the naturally selected optimum itself.

This idea is well illustrated by a study by Douglas Swain on cod (*Gadus Morhua*) in the southern Gulf of Saint Lawrence (Swain, 2011). This cod stock, like several other Canadian cod stocks, has collapsed during the early 1990s in parallel with a rapid decrease in age and size at maturity, which presumably represented a response to fishing-induced selection (Olsen *et al.*, 2004). However, despite severe reductions in the fishing pressure, age and size at maturity remained low in this stock and apparently did so because of an increase in natural mortality (Swain, 2011). Hence, the fishery seems to have changed the ecological conditions, shifting the naturally selected adaptive landscape of cod such that a faster life history is now the new adaptive optimum. This important result requires that we now examine what ecological changes may cause this shift in the naturally selected adaptive landscape.

Swain (2011) points to potential candidate mechanisms for increased natural mortality in southern Gulf of St. Lawrence cod. First, increased reproductive investment (as initially favoured by a high fishing mortality), together with low food conditions (Shelton *et al.*, 2006), might have caused an increase in reproductive mortality. This hypothesis is supported by the fact that post-fishery increase in natural mortality mainly affected cod older than 5 years, i.e., reproductively active cod (Swain, 2011). Second, cod collapse was paralleled by an increase in the number of gray seals (*Halichoerus grypus*), which are predators for cod. However, seals tend to prey on juvenile cod, while the increased natural mortality effectively affected mainly adult cod (Chouinard *et al.*, 2005), suggesting that predation from seals might not be a major cause of the now high natural mortality in adult cod.

We may propose additional mechanisms for increased mortality in adult cod. Cod and seals might in fact interact through intraguild predation (IGP), a type of interaction in which predators and prey also compete for common food sources (Holt and Polis, 1997). Hence, increased seal numbers might thus participate in depleting food resources for adult cod, and thus favor starvation during energy-demanding reproductive periods. Other species probably interact with cod through IGP. For instance, in the North Sea, herring (*Clupea harengus*) – a prey for cod – inhibits cod recruitment through competition with juvenile cod for zooplankton, but also through predation on cod eggs and larvae (Fauchald, 2010). In the southern Gulf of St. Lawrence cod, increased adult cod mortality possibly operated through exploitative competition from smaller-sized (i.e., lower *R*-starred) fish species that may also be prey for cod. Hence fishing for cod might have pushed an initially cod-abundant system to a new, stable but cod-sparse system in which selection favors smaller-sized cod (Abrams, 2011).

The cod example highlights the potential for eco-evolutionary feedback loops in human-perturbed ecosystems (**Figure 3**). These feedback loops act through density- or frequency-dependent natural selection, and are of crucial importance if one wants to correctly manage natural populations

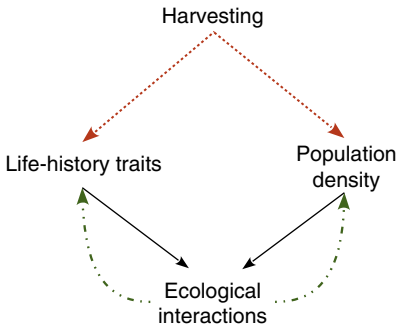

Figure 3 Density-dependent feedback loops arising from harvesting wild populations. Harvest-induced selection (dashed red arrows) favor in parallel faster life histories and reduced population density, which both potentially alter the strength or even nature of intra- and interspecific interactions (solid black arrows). Ultimately, natural selection acting through these ecological interactions (dotted and dashed green arrows) may feedback on life histories and population densities to either oppose (stabilizing eco-evolutionary feedback loop) or reinforce (runaway eco-evolutionary feedback loop) the effects of anthropogenic selection.

(Dieckmann and Ferriere, 2004). A natural selection opposing the effects of anthropogenic selection results in a stabilizing feedback loop, helps in maintaining the system under control, and fosters recovery when the perturbation has ceased. However, in some cases (e.g., cod) natural selection may instead reinforce anthropogenic selection, accelerate change to a new population or ecosystem state, and impede reversal to the initial state. Hence, eco-evolutionary feedback loops stress the need to fully integrate food–web interactions and life-history theory in the next generation conservation plans.

See also: Evolvability, Quantitative Genetics of. Life History: Pike. Natural Selection, Measuring. Responses to Climate Change, Evolution and

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Life History Evolution in Guppies, Experimental Studies of

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Glossary

Costs of reproduction Costs refer to any loss of fitness, either in the form of increased risk of mortality or decreased potential for future reproduction, associated with current investment in reproduction. Examples of costs include the risk that parents take when caring for young, the increased susceptibility of females to any mortality risk when pregnant, or any consequences of the resources parents invest in their offspring. For example, the energy and nutrients that a mother invests in producing offspring restrict their availability for other functions, such as growth, storage, or maintenance.

Demographic theory This is a diverse body of theory that models the evolution of populations with age structure, including nonreproducing juveniles and reproducing adults. Each age class can be characterized by its probability of survival to the next age class and the average number of offspring each individual produces. This body of theory models how the life history is predicted to evolve as a function of the risk of mortality of the different age groups. The predicted outcome can vary, for example, if mortality rates are higher in juveniles, adults, or are uniform across all age classes. The predicted outcome can also be modified by whether or not the populations experience density regulation. The sorts of evolution that are predicted are the timing of reproduction (when to begin, how often to reproduce) and the quantity of resources that are devoted to reproduction, as opposed to growth, maintenance, or storage.

Eco–evo dynamics Such dynamics occur when an organism evolves in response to some type of selection and, as a consequence, alters its environment and in turn alters the kind of selection it experiences, causing an ongoing feedback between ecology and evolution. Density regulation represents one possible forum for such dynamics. As an organism increases in abundance, its impact on its natural community may also increase, causing it to experience some change in the selection it experiences. For example, increased abundance of a predator could cause a decline in abundance of some categories of prey which in turn can select for changes in the predator's feeding strategies or perhaps its metabolism and life history if food is less abundant.

Integral projection model (IPM) IPMs produce an estimate of the growth rate of a population in a given set of circumstances. In our case, each of our artificial stream experiments gave us an estimate of the growth rate and risk of mortality for each size class of fish. We were able to preserve and dissect all females at the end of the experiment to get an estimate of size-specific fecundity. These data are then combined to yield an estimate of the population growth rate. Since there were different populations represented and different experimental treatments, the population growth rate represents a point estimate of relative fitness – faster population growth rates mean that a population/treatment group has higher fitness in the context of the experiment.

Mark-release-recapture A powerful tool for studying evolution in action is to mark individuals so that they can be recognized when seen again. If marking is associated with regular formal censuses of the population, then it is possible to estimate mortality rates from the probability of recapture of marked individuals. If such studies are combined with pedigrees, such as by marking juveniles when they are still under the care of their parents or by determining who the parents are from tissue samples collected from each individual when it is marked, then it is possible to determine how many offspring each individual contributes to the next generation. If we also characterize the phenotype of each individual then it is possible to estimate the genetic contribution to the phenotype from phenotypic similarities among relatives. It is also possible to determine whether or not differences among individuals in phenotype contribute to reproductive success.

Purifying selection Selection that 'weeds out' deleterious mutations. This type of selection is also often referred to as 'negative Darwinian selection.' The alternative of 'positive Darwinian selection' refers to natural selection, or selection that results in increased fitness via improved adaptation to the organism's environment. Note that 'environment' refers to the physical environment and biotic interactions, or interactions with other organisms.

Senescence Age-related decline in performance or fitness, with the latter being estimated from the mortality rate and rate of reproduction.

William's generalization of Lack's Principle David Lack postulated that the number of eggs that a bird laid would evolve to be that number that resulted in the maximum number of offspring. This became known as the Lack Optimal clutch size, but practice showed that the most common clutch size tended to be smaller than the optimal clutch size. George Williams postulated that this was because of a trade off between reproductive value, the return on current investment in reproduction, and residual reproductive value, or the expected return from future reproductive investment. Birds, and other organisms, invest less now to save for the future.

The Evolution of Life Histories in Guppies

My associates and I have been studying the evolution of life histories in guppies since 1978. A consequence of persisting for so long is that there has been a maturation of the way we think about the evolution of life histories and the methods we use to study them. I will present key results of this research program in a chronological fashion so that I can simultaneously highlight the main accomplishments of the research program alongside the growth of the discipline.

Life History Evolution in the 1970s

At the outset of my work, the theory of life history evolution was dominated by the concept of r- and K-selection (MacArthur and Wilson, 1967; Pianka, 1970), George Williams' generalization of Lack's principle (Williams, 1966), and evolution in age-structured populations (Charlesworth, 1980; Charlesworth and Leon, 1976; Law, 1979; Michod, 1979). Empirical research had progressed to the point of producing some synthetic overviews of life history variation, such as on the evolution of clutch size or nestling development rate in birds (Cody, 1966; Ricklefs, 1969, 1970), and comparative analyses of a broader array of life history traits in reptiles (Tinkle, 1969; Tinkle *et al.*, 1970; Vitt and Congdon, 1978). The field had some diversity in both theory and empirical research, but was small enough to be covered well in two review articles (Stearns, 1976, 1977).

I chose to study differences in life histories among populations within a species because I wanted to couple life history variation with other features of the environment. Such correlations help establish the causes of life history evolution. I was also most impressed with demographic theory because it explicitly incorporated the details of age-specific survival and reproduction and generated testable predictions. I wanted to add some form of genetic analysis of any interpopulation differences in life history that I discovered. Prior work on diverse organisms often revealed that species or populations had different life history phenotypes but did not give us a measure of the extent to which these differences were attributable to genes or the environment.

Guppies and Trinidad

Guppies are small (1.5–3 cm as adults), have rapid development (as little as 10 weeks between the birth of a female and the birth to her first litter of babies) and are easy to maintain and breed in the laboratory. The diversity of habitats guppies occupy in the Northern Range Mountains of Trinidad was already known to be associated with significant differences among populations in local adaptation (Endler, 1978).

The Northern Range Mountains are a seasonal, tropical rainforest that receive from 3–4 m of rain per year. The slopes have many small streams that flow through steep ravines before emptying into larger streams in the more level lowlands. The streams are punctuated by waterfalls that often serve as barriers to the upstream dispersal of some fish species. Guppies range from the mouths of the largest rivers up into the smallest headwater streams. They co-occur with a diversity of larger fish species in the lower elevation streams; some of these larger fish frequently prey on guppies. Above barrier waterfalls, guppies can often be found in streams where the killifish *Rivulus hartii* is the only other resident fish species. *Rivulus* will occasionally eat guppies, but is more inclined to eat insects. These contrasting communities are found in close proximity to one another, above and below a barrier waterfall on the same stream that excludes the larger predatory fish but not guppies and *Rivulus*.

John Endler established that guppies that lived with predators downstream were less brightly colored than those that lived upstream with *Rivulus* (Endler, 1978). Females prefer to mate with brightly colored males, but brightly colored males also attract the unwanted attention of predators. When predators are absent, females are free to choose to mate with more brightly colored males. When predators are present, brightly colored males suffer higher mortality, causing the average male to be less brightly colored than when predators are absent. Endler then did two remarkable things (Endler, 1980). He found a tributary to the Aripo River that had a dramatic waterfall that stopped all species of fish save *Rivulus*. He transplanted guppies from a high predation locality downstream into this previously guppy-free low predation locality. This new population became more brightly colored than their ancestors after only 2 years. He also built artificial streams in a greenhouse and populated them with either *Rivulus* or the pike cichlid *Crenicichla alta*, one of the guppy predators from downstream communities. He introduced a genetically diverse population of guppies. He found that the guppies kept with *Rivulus* became significantly more brightly colored than those with *Crenicichla*, again within around 2 years.

Comparative Life Histories

Endler's results provide an important clue about how *Rivulus* and *Crenicichla* differ as predators. *Crenicichla*, and other predators that co-occur with *Crenicichla*, must eat adult male guppies much more frequently than do *Rivulus* for them to be able to shape the evolution of male coloration. This pattern suggests that adult guppies have a higher risk of mortality in the downstream fish communities (hereafter referred to as high predation, or HP, localities) than they do in communities where *Rivulus* is the only other fish species present (hereafter referred to as low predation, or LP, localities). The demographic theory of the 1970s predicted that this higher adult mortality risk should select for an earlier age at maturity and a greater investment in reproduction than seen in fish that live in low predation environments (Law, 1979; Michod, 1979; Charlesworth, 1994; Gadgil and Bossert, 1970). I set out to test these predictions.

I quantified guppy life histories first by comparing the phenotypes of wild-caught fish. I preserved guppies from a series of high and low predation localities then performed dissections to characterize the size at maturity, number of offspring, size of offspring, and reproductive allocation (RA) (Reznick and Endler, 1982). RA is the proportion of female body weight that consisted of developing offspring. I then performed laboratory experiments in which I quantified the life histories of the grandchildren of wild-caught fish (Reznick, 1982). Phenotypic

differences in life histories of HP and LP fish that persisted after two generations in a common laboratory environment are likely to have a genetic basis. I was also able to quantify additional aspects of the life history, including age at maturity, frequency of reproduction and the proportion of consumed food that was devoted to reproduction versus growth and maintenance.

The field and laboratory work produced consistent results. Guppies from HP environments were smaller and younger at sexual maturity than their counterparts from LP environments. They also devoted more resources to reproduction. They did so by beginning to reproduce when they were younger, reproducing more frequently and, in most comparisons, by devoting more resources to each litter of offspring (Figure 1). All of these results were as predicted by theory. In addition, the HP guppies produced more offspring in each litter and each baby was smaller, on average, than the babies produced by LP guppies.

This work presented two forms of progress in the study of life history evolution. First, the association between predation and life histories was replicated in different rivers that represent independent instances in which guppies had adapted to life with and without predators, so life history evolution is

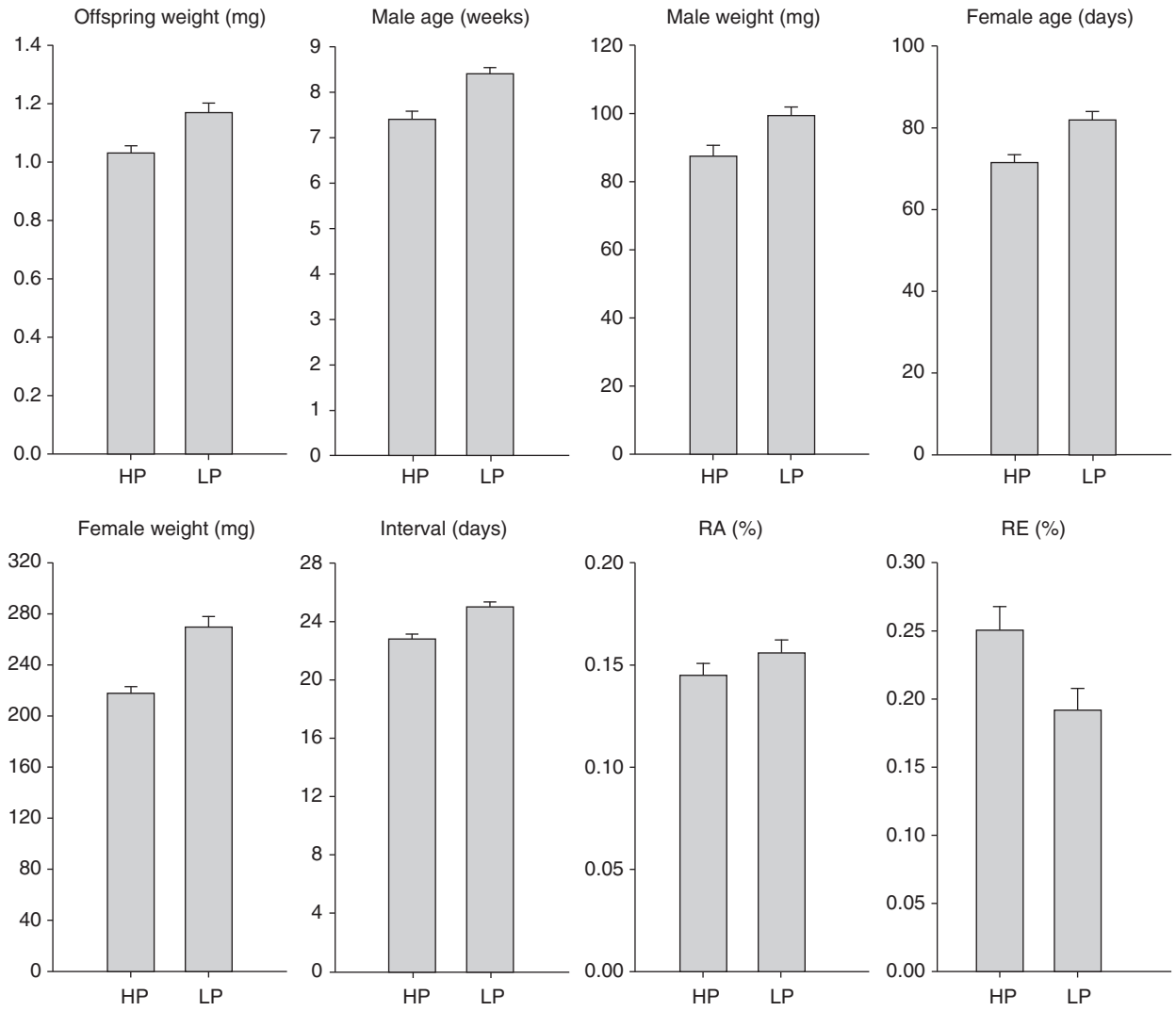

Figure 1 Results of the laboratory assay of life histories of guppies from two high and two low predation environments. Reproduced from Reznick, D.N., 1982. The impact of predation on life history evolution in *Trinidadian guppies*: The genetic components of observed life history differences. Evolution **36**, 1236–1250. Reported here are means and one standard error. 'Offspring weight (mg)'=the mean dry mass of offspring at birth. In wild-caught fish, the difference in mean mass is much larger. 'Male age (weeks)'=mean male age at maturity. 'Male weight (mg)'=mean male wet mass at maturity. 'Female age (days)'=mean female age at first parturition. 'Female weight (mg)'=female mean wet mass at first parturition. 'Interval (days)'=the mean interval between the birth of successive litters. 'RA (%)'=mean reproductive allocation, which is the percent of total female dry mass that consists of developing embryos. RA is higher for HP guppies in all large-scale field comparisons and all other lab studies. 'RE (%)'=reproductive effort, or the cumulative percent consumed resources devoted to the production of offspring in the first three litters. RE is higher in high predation guppies because they began to produce offspring at an earlier age and reproduced more often. Redrawn from Reznick, D.N., 1982. The impact of predation on life history evolution in *Trinidadian guppies*: The genetic components of observed life history differences. Evolution 36, 1236–1250.

repeatable. Second, there is a genetic basis to the differences in life histories among guppies from high and low predation environments.

Experimental Evolution

We followed John Endler's lead of treating streams as if they were giant test tubes. We transplanted guppies from high predation sites below a barrier waterfall into previously guppy-free low predation sites above a waterfall. We also added predators to a section of stream above a fall that excluded predators but not guppies. These two manipulations caused either a decrease or increase in guppy mortality risk, respectively. The two guppy introduction experiments included the one initiated by John Endler in 1976 and a second initiated by me in 1981. In both we observed significant life history evolution in as little as 4 years (Reznick *et al.*, 1990, 1997; Reznick and Bryga, 1987). The life histories of the introduced HP guppies evolved to become more similar to what is typical of LP environments; they were older at maturity, produced fewer, larger babies and invested less in reproduction in comparison to the ancestral HP guppies found below the barrier waterfall (Figure 2). All of these conclusions are derived from the same combination of field and laboratory results as the original comparative studies. Our introduction of predators to what had been a low predation locality caused the resident guppies to evolve smaller sizes and earlier ages at maturity in comparison to guppies found above a barrier waterfall that excluded the predators (Reznick, 1997).

Life History Evolution in the 1990s – Awkward Adolescence

Mortality Rate Estimates

Progress in science can be driven as much by methods as ideas. Theory predicts how organisms would evolve in response to age-specific mortality risk, so one must quantify mortality risk. I had assumed that guppies that lived with predators had higher adult mortality risks, but I did not actually know that this was true. I developed a way to mark individual guppies in the late 1980s and was able to measure comparative mortality risk by the early 1990s. My colleagues and I also discovered that it was easy to apply mark-recapture methods in smaller

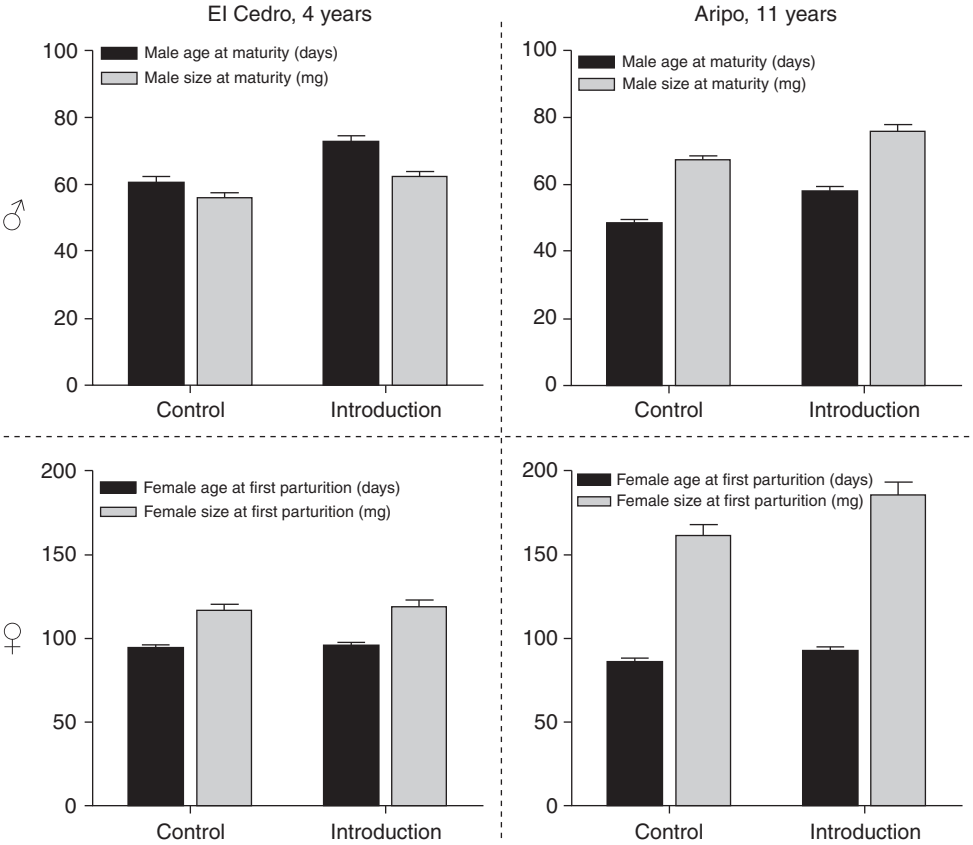

Figure 2 Mean (1 standard error) age and size at maturity in males and females from two experiments in which guppies were transplanted from a high predation site downstream (the control) into a previously guppy-free low predation site upstream (the introduction). Data are derived from a laboratory study of the grandchildren of wild-caught fish. Male age and size at maturity were significantly later/larger in the introduction site only 4 years after the introduction on the El Cedro River and 11 years after the introduction on the Aripo River (we first looked for evolution after 11 years). Females showed no response after 4 years on the El Cedro, but were significantly older and larger at maturity in the introduction site after 11 years in the Aripo. Redrawn from Reznick, D.A., Bryga, H., Endler, J.A., 1990. Experimentally induced life-history evolution in a natural population. Nature 346, 357–359.

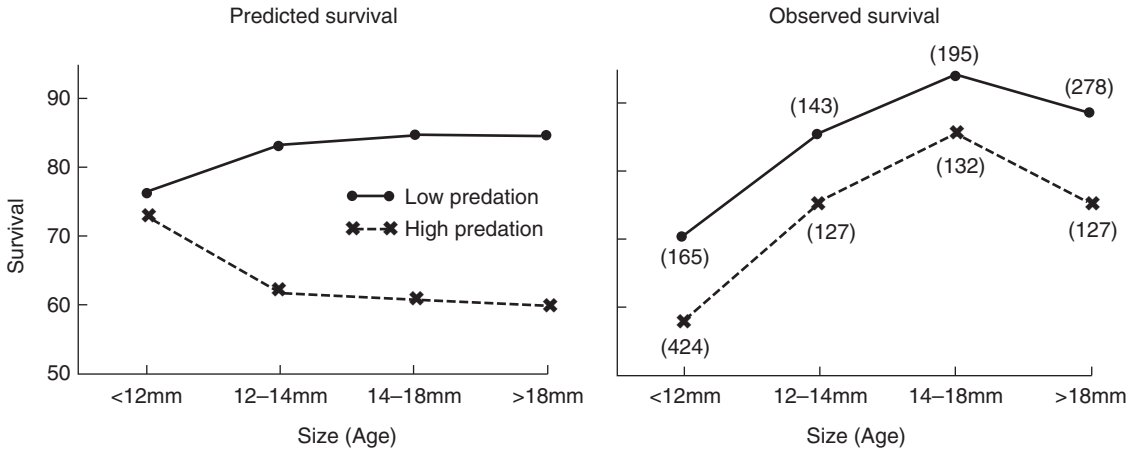

Figure 3 Predicted and observed differences in mortality risk in guppies from high and low predation environments. Data are derived from 14 mark-recapture studies, seven each in high and low predation environments. The observed data represent recapture probability after 12 days. *X*-axis = size class of fish, with <12 and 12–14 mm fish being immature. 14–18 mm fish includes mature males and females that have just matured and are producing their first litters of babies. >18 mm are adult females only. The theory that provided the initial predictions for how guppies should evolve in response to predator-induced mortality also assumed that there would be a proportionately higher risk of mortality in adult fish, hence the nonparallel lines in the curves that predict the relationship between size and survival. Redrawn from Reznick, D.N., Butler, M.J., Helen Rodd, F., Ross, P.,1996. Life history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. Evolution 50, 1651–1660.

streams that had a pool-riffle structure. Such streams are like a string of beads, where each bead is a pool. Each pool is separated from its neighbor by a steeper, shallower portion of stream, a riffle, where the water flowed more rapidly to the next pool. We found that guppies cluster in pools and are disinclined to migrate among pools.

Two things had to be true about the comparative mortality risks of guppies from high and low predation environments for there to be a good match with demographic theory. First, HP guppies must have higher mortality risks. Second, these higher mortality rates must fall more heavily on the adult age classes (Figure 3). Guppies from HP localities clearly experienced higher risks of mortality, but the difference is not magnified in adults. We instead found that there was an approximately equal increase in risk across all age/size classes (Reznick *et al.*, 1996). This may seem like a small difference, but it creates a mismatch between theory and reality. The theory I had been using had shown that an increase in mortality risk spread equally across all age classes would result in no evolution, yet I had already seen guppies evolve in the context of introduction experiments and had comparative data that suggested evolution.

This result became an invitation to think more carefully about how the theory was constructed and about all of the alternative models that had been proposed. I had derived predictions from a model that only included the effects of predators on guppy mortality rates, but otherwise allowed the guppy populations to grow without limit. An alternative model that is perhaps more realistic is to assume that guppies are also resource limited so that, even in the absence of predators, their populations will be regulated because of limited resource availability (Figure 4). There are actually diverse models that include such added ecological complexity. With such added complexity it becomes possible for the sort of mortality differences we found between high and low predation environments to cause the sort of life history evolution we had observed. Our result thus suggested that such added ecological complexity might be playing an important role in shaping guppy life history evolution.

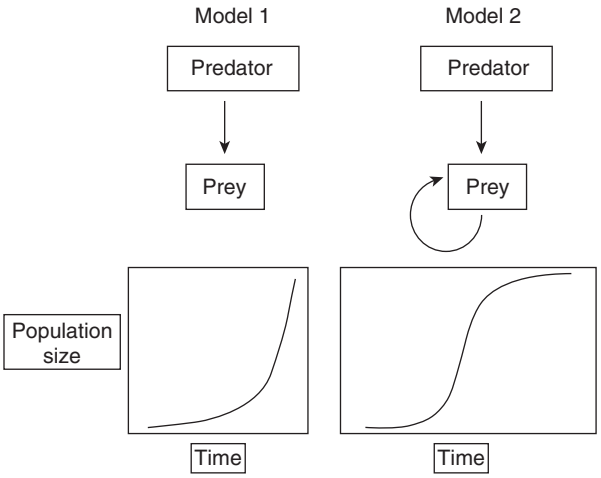

Figure 4 Two schematic models of life history evolution: Model 1 = density independent (exponential population growth in the lower panel). Here, all that is modeled is the increased risk of mortality caused by predators. Model 2 = density dependent (logistic population growth in lower panel). Here, the prey population is regulated by both predation and by its own density.

Comparative Ecology

We quantified features of the ecology of HP and LP environments at the same time that we estimated mortality risk. We wanted to know if there were any predictable ecological differences in HP and LP environments.

LP environments have higher population densities of guppies, lower light level and lower primary productivity. Individual guppies have slower growth rates. The lower growth rates of LP guppies could be a simple consequence of lower light levels, but they could also be an indirect consequence of predation. When predators are present, they trim guppy populations and the survivors have more food resources. Conversely, when they are absent, guppy populations expand and per capita resource availability declines (Reznick *et al.*, 2001).

Evolution of Senescence

A corollary of the sort of life history theory we were testing pertains to the evolution of the aging process. Guppies adapted to life with predators are predicted to age more quickly and hence have shorter life spans than those from low predation environments, for two reasons. One is a by-product of costs of reproduction. HP guppies begin to reproduce at an earlier age and devote more resources to reproduction. It is predicted that there is a price to be paid for this because it means having less to devote to growth and maintenance. The second reason is a by-product of 'purifying' or 'negative Darwinian' selection. Mutations of diverse sorts are a predictable occurrence of cell division. Most are deleterious. These are either removed or kept rare by purifying selection. Some of these mutations are age-specific in when their damaging effects occur. If their deleterious effects are felt early in life, they are more likely to be eliminated by selection because they will have a large impact on an individual's ability to contribute offspring to the next generation. If the mutations instead affect only older individuals, their impact on fitness will be smaller and they will be subject to weaker selection. Because guppies from HP environments are much less likely to live to advanced ages, they are more likely to accumulate deleterious mutations that only affect older individuals. It is the accumulation of these late-acting mutations that is proposed to cause accelerated aging.

We tested these predictions by quantifying lifetime reproductive success and lifespan in a laboratory experiment on the grandchildren of wild-caught guppies (Reznick *et al.*, 2004). When guppies are reared in the laboratory they are free of extrinsic sources of mortality, like predation. We can then ask if there are differences in intrinsic mortality rates. We performed this experiment on guppies from two rivers, each represented by fish derived from a high and low predation site. The guppies in these rivers (the Yarra and the Oropuche) are genetically distinct and represent independent instances in which guppies have adapted to life with and without predators. Our results were the opposite of predictions. HP guppies had lower probabilities of mortality throughout their lives and longer life spans in spite of their investing more in reproduction early in life. Furthermore, they continued to produce offspring at a higher rate throughout their lives and were substantially older when they ceased reproduction.

These results raise two issues. First, was the underlying theory appropriate? Our estimates of mortality rates provided indirect evidence that ecological interactions were playing an important role in shaping the evolution of the early life history. There has been the parallel development of theory for the evolution of senescence that also argues that such ecological interactions (e.g., density regulation) can play a role in shaping the evolution of senescence. In some cases, this added complexity could cause the sorts of results we had observed. Second, if these data alone define the relative fitness of HP and LP guppies in nature, then they tell us that HP guppies are unconditionally superior to LP guppies and that the LP life history should never evolve. But the LP phenotype does evolve and does so repeatedly which tells us that it is likely to be adaptive in some circumstances. The key is figuring out what it is about the natural HP and LP environments that could cause the LP phenotype to have superior fitness. If this second explanation applies, then it says that the apparent superiority of HP guppies is only realized in some environments.

Summary

The results of our mortality rate estimations suggest that resource limitation and density regulation are likely to play a critical role in shaping the way guppies adapt to life without predators. The results of the comparative study of senescence might also be explained by density and resource limitation. They at least suggest that ecological context should affect the relative fitness of HP and LP guppies. The comparative ecology study suggests that the indirect effects of predators may also play a role in shaping guppy evolution. When predators are present, guppy population densities are low and food is more abundant. When they are absent guppy population densities increase and food resources are limiting.

Life History Evolution in the Twenty-First Century

The way we study guppy life histories has been refined by the advent of a different theoretical framework, new methods, and new statistical tools. The new theoretical framework is eco–evo dynamics, or the idea that ecology and evolution are contemporary processes that feedback on one another in real time. Eco–evo dynamics emerged as a distinct facet of evolutionary ecology in the 1960s but has now gained prominence (Pimentel, 1961, 1968; Yoshida *et al.*, 2003). The connection between this concept and guppies lies in the implied importance of density regulation and indirect effects of predators. Both processes suggest that the high density of guppies in LP environments plays a role in shaping the evolution of the LP phenotype. If so, then this means that the way guppies are evolving is determined at least in part by how a high density of guppies reshapes their ecosystem. We are characterizing this aspect of guppy life history evolution by integrating studies of the feedback between guppies and their ecosystem into our life history work.

We also have new methods for studying guppy evolution. We can now individually mark and track thousands of guppies in experimental populations. We can also genotype all of the marked fish. When we mark them, we save two or three scales from each individual and use them as a source of DNA.

We have initiated four replicate introduction experiments in which guppies from a single HP population were introduced into four small, guppy-free headwater streams. Each

founder was marked, photographed, and scales were saved for DNA. We exhaustively census each population once per month, mark all new recruits and collect scales from them. At the same time, we are assessing how components of the ecosystem are changing in response to guppy occupancy. We have also created replicate artificial streams in which we can conduct short-term, multi-factor experiments to quantify how guppies affect their environment and how the fitness of HP and LP guppies depends upon the environment.

Our new methods are matched by new statistical approaches for quantifying evolution in action. We can now reconstruct the pedigrees of our evolving populations based on the genetic fingerprints of the founders and all of the new recruits.

This enables us to study evolution in the traditional way, as a change in the average attributes of a population over time, but also as variation in individual reproductive success, which is the root of changes in population means. We can quantify how attributes of individuals contribute to this variation. We have deployed the newly developed integral projection models to estimate relative fitness of HP and LP guppies in our experimental streams to gain a better understanding of the factors that have shaped the evolution of these alternative phenotypes.

Here we offer some examples of recent progress that illustrate how we have combined the new theoretical framework, methods, and statistics to address some of the problems suggested by the results of the '1990s.'

Density Regulation

We have now proven that guppies are density regulated in LP environments and that LP guppies have adapted to living at high population densities (Reznick *et al.*, 2012). We performed field experiments in natural LP streams that had a pool-riffle structure. We either increased or decreased density in natural pools while leaving other pools unmanipulated to serve as controls. Increased density causes a decline in population growth rate and reduced density causes an increase in population growth rate relative to the controls. This combination of responses tells us that resources are limiting at ambient population densities.

We then performed experiments in our artificial streams in which we stocked streams with either HP or LP guppies at either high or low population densities (**Figure 5**; Bassar *et al.*, 2013). HP guppies had higher population growth rates and hence higher fitness than LP guppies (as estimated with the application of IPMs) at low population densities. HP and LP guppies had equal fitness at high population densities, suggesting that adaptation to density at least partially explains how the LP phenotype has evolved. However, this result also says that adaptation to density alone is not a complete explanation for the evolution of the LP phenotype. LP guppies must have higher fitness than HP guppies for the LP phenotype to evolve.

Guppy–*Rivulus* Interactions and the Demise of the Superguppy

In a separate series of experiments in the artificial streams, we reared HP and LP guppies in the presence of *Rivulus*. Since guppies and *Rivulus* live together at high densities in LP streams, we reasoned that coadaptation between guppies and *Rivulus* could also play a role in shaping the evolution of the LP phenotype. LP guppies have higher fitness than HP guppies when both are kept with *Rivulus* (**Figure 6**). Furthermore, the fitness advantage of LP guppies is even greater when they are kept with *Rivulus* derived from a site where they co-occur with guppies than if they are derived from a site where *Rivulus* is the only fish species present. This result suggests that there has been some form of coadaptation between guppies and *Rivulus*. While this result resolves the dilemma posed by our

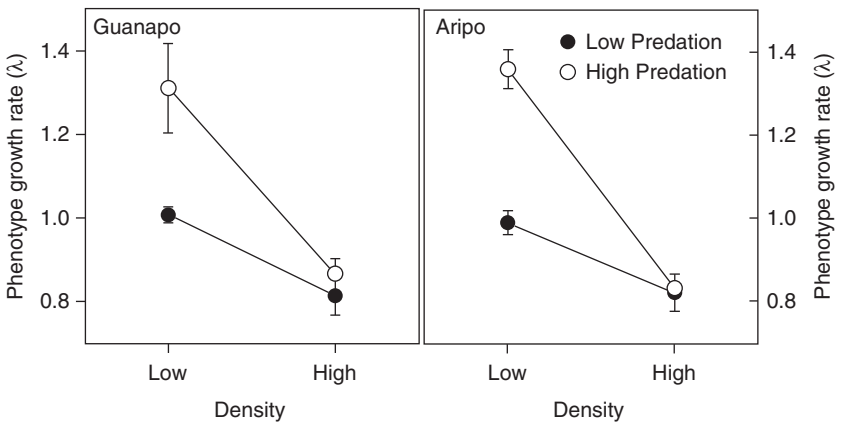

Figure 5 Replicate experiments in artificial streams that document the potential role of density-dependent evolution in shaping guppy life histories. This experiment was done twice. We compared the response of guppies from high and low predation environments, first with fish derived from such sites in the Guanapo River, then with fish derived from such localities in the Aripo River. The high and low density treatments were parameterized from our comparative studies of natural populations in low and high predation communities, respectively. *Y*-axis (phenotypic growth rate) is the estimated population growth rate of the guppies in each of the four treatment groups derived from an integral projection model. These models combine the survival, growth and reproduction data from each treatment group into a life-table estimate of population growth rate. Redrawn from Bassar, R.D., López-Sepulcre, A., Reznick, D.N. Travis, J., 2013. Experimental evidence for density-dependent regulation and selection on Trinidadian guppy life histories. American Naturalist 181(1), 25–38.

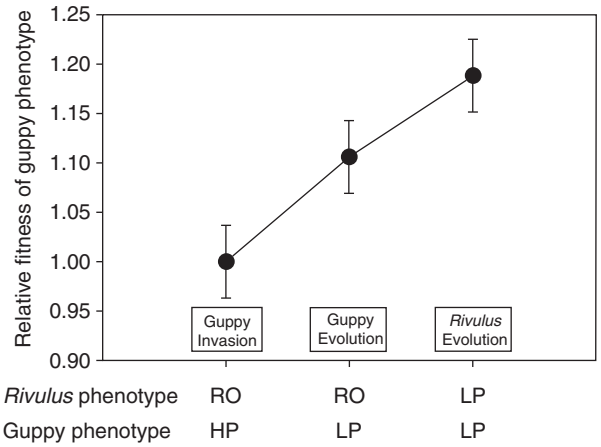

Figure 6 Estimated population growth rate of guppies when interacting with *Rivulus* in experiments conducted in artificial streams. *Y*-axis = estimated population growth rate in each treatment group, derived from an integral projection model. 'Guppy Invasion' = a recreation of the invasion of a previously guppy-free stream, with *Rivulus* only (RO), by guppies from a high predation site below the barrier. 'Guppy Evolution' = a combination of locally adapted, low predation guppies with *Rivulus* from a natural RO site. '*Rivulus* evolution' = pairing of guppies from a LP locality with *Rivulus* from an LP locality, or *Rivulus* that have coadapted to life with guppies.

senescence results, which suggested that HP guppies are unconditionally superior to LP guppies, it also begs a question. Why do LP guppies have higher fitness than HP guppies in these headwater streams? We are now addressing this question. We know that one big factor is that LP guppies have higher growth rates than HP guppies when kept with *Rivulus*. Faster growth affects age at maturity and how many babies they produce. It can also be a factor of traits other than the life history, like foraging behavior or foraging efficiency.

Experimental Evolution II

Our new series of four introduction experiments is yielding a picture of the dynamics of guppy evolution and the associated dynamics of ecosystem change. We have seen an impact of the introduced guppies on the resident *Rivulus* populations in all four streams. Up to this point, we had thought of guppies as the victims who evolve in response to predation. Now we know that they are also the aggressors because adult guppies prey on newborn *Rivulus* and compete with adolescent *Rivulus*. When guppies invade, the abundance of *Rivulus* declines and their size distributions shift to larger sizes because guppies are choking off the recruitment of young *Rivulus*. Guppies also sometimes cause a reduction in the abundance of invertebrates. In artificial streams, guppies clearly reduce the abundance of algae, but that signal is less apparent in the natural streams. We have also begun to apply our pedigrees to the estimation of individual reproductive success and the evolution of male size at maturity and male coloration. The pieces are in place to study the feedback between ecology and evolution.

Our research began with the narrow focus of life history evolution. This approach was practical but it also made sense because the different components of the life history (age at maturity, age-specific allocation of resources to reproduction) could represent an integrated package of attributes that could be treated as an independent feature of the phenotype. One consequence of our new perspective is that the life history ceases to be the sole focus of research. The life history is instead absorbed into the background of other features of the organism that are evolving. We knew at the outset that the HP and LP phenotypes differed in male coloration, courtship behavior, and schooling behavior. Now we know that they differ in shape, jaw morphology, diet, and other aspects of behavior. Studying the life history in isolation of other traits and thinking about how the different components of the life history traded off with one another was a logical place to start. What we know now suggests that we need to think more broadly and to integrate life history evolution with these other components of the phenotype.

See also: Life History Trade-offs. *r*- and *K*-Selection in Fluctuating Environments, Theory of

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Life-History Evolution in Island Populations of Birds

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Islands are unique ecosystems sharing a distinctive set of characteristics that make them particularly interesting to study adaptation by organisms. The isolation and reduced area of islands result in simplified ecosystems with an impoverished fauna – including reduced number of predators and parasites. In addition, being surrounded by ocean, islands benefit from a generally milder climate, with less extreme fluctuations than similar mainland areas at the same latitude (Weigelt *et al.*, 2013). All these biotic and abiotic features have predictable effects on several characteristics of organisms and are expected to lead to convergent patterns of adaptation, the oft-called 'insularity syndrome' (Blondel, 2000; Clegg and Owens, 2002; Covas, 2012; Grant, 1998; Losos and Ricklefs, 2009; McNab, 1994; Whittaker and Fernández-Palacios, 2007).

The effects of the island environment on life histories provide an excellent illustration of the factors affecting life-history evolution. Island populations have long been thought to have reduced fecundity, increased survival, and increased investment in young. These ideas on island evolution were triggered by observations of extreme island forms, and birds played a prominent role in building the stereotype of what island forms tend to become. Well-known examples of morphological evolution such as the gigantism and loss of flight in species like the dodo *Raphus cucullatus* or moas (Dinornithiformes) and reproductive adaptations such as the extremely large eggs and slow development of kiwis (*Apteryx* spp) promoted a general view of a slow pace of life on islands. In brief, the absence or low numbers of predators on islands and a generally benign climate would promote loss, or reduction, of flight capacity and dispersal and a generally slower rhythm of life, with increased longevity, reduced fecundity, and slower development, but greater investment per young (reviewed in Whittaker and Fernández-Palacios, 2007; Blondel, 2000). Some of these ideas gained theoretical support through the works of Cody (1966) and MacArthur and Wilson (1967) and the model became a popular paradigm. However, it lacked formal testing until recently (Blondel, 2000; Clegg and Owens, 2002; Covas, 2012). Whether there is or not a set of convergent adaptations on island, and hence whether there is or not an 'island syndrome' can only be established if it is shown that there is repeated convergent evolution by distinct island animals living on different islands. Additionally, several factors need to be taken into account when attempting to make generalizations about adaptations on islands. In particular, closely related species share a common evolutionary past that will constrain their evolutionary trajectories after colonizing an island. Furthermore, life-history characteristics are known to be influenced by several ecological factors, in addition to those that might or not change on islands. A particularly prominent one is the positive effect of latitude on body size (Olson *et al.*, 2009) and fecundity, while adult survival decreases with increasing latitude (Lack, 1968). Establishing whether there are patterns of life-history evolution specific to islands therefore requires carefully controlling for latitude. In recent years, several studies have tackled these tasks and a more precise picture of patterns of evolution in island birds (and other groups) is now starting to emerge. These studies confirm several previous expectations which concur with life-history theory, but also reveal some unexpected trends that offer interesting new insights.

Body Size and Shape

Efforts to establish patterns and processes of morphological evolution in island animals are hindered by the number of extinctions among island forms (Steadman, 2006). Islands experience extremely high levels of extinction compared to mainland areas (e.g., ca. 90% of bird species known to have become extinct in historical times were island species; Johnson and Stattersfield, 1990). Among the most severely affected are large flightless species which forage and breed on the ground and hence are interesting targets for humans and easy prey for human-introduced predators (rats, cats, dogs, etc.). These species may also suffer disproportionately from the rapid habitat changes that follow island colonization by humans. Recent analyses have therefore focused mostly on extant faunas (but see Boyer and Jetz, 2010; Steadman, 2006). These analyses have nonetheless confirmed evolutionary trends on islands which set them apart from their mainland counterparts.

Morphological evolution of island organisms is thought to follow the 'island rule' according to which small-bodied organisms evolve toward a larger size and large bodied organisms evolve toward a smaller size (Lomolino, 1985, 2005; Price and Phillimore, 2007; Van Valen, 1973). In birds, the most well-known examples are the large flightless birds from several Pacific and Indian Ocean islands, most of which are now extinct (e.g., Boyer and Jetz, 2010; Steadman, 2006) and the insular ducks, which are usually smaller than mainland ducks (Lack, 1970). Whether or not the island rule is valid has been strongly debated, given the several exceptions that have been found for different taxonomic groups (e.g., Meiri *et al.*, 2008; Meiri *et al.*, 2006). In addition, an important difficulty is to determine that the giants or dwarfs that appear on islands are the results of evolution taking place in response to insularity and not simply a bias in colonization success (e.g., Raia, 2009). However, it has been argued that there is some general support in favor of the island rule (Price and Phillimore, 2007). Specifically for birds, support for the island rule has been found for extant faunas from islands around the world (Clegg and Owens, 2002). This pattern would be most likely stronger if extinct large flightless forms were included in the analyses, as illustrated by a recent analysis of body size trends in relation to island area in Pacific birds (Boyer and Jetz, 2010). In this study, which included both extant and extinct faunas, support for the island rule was stronger when extinct and flightless species were included (**Figure 1**).

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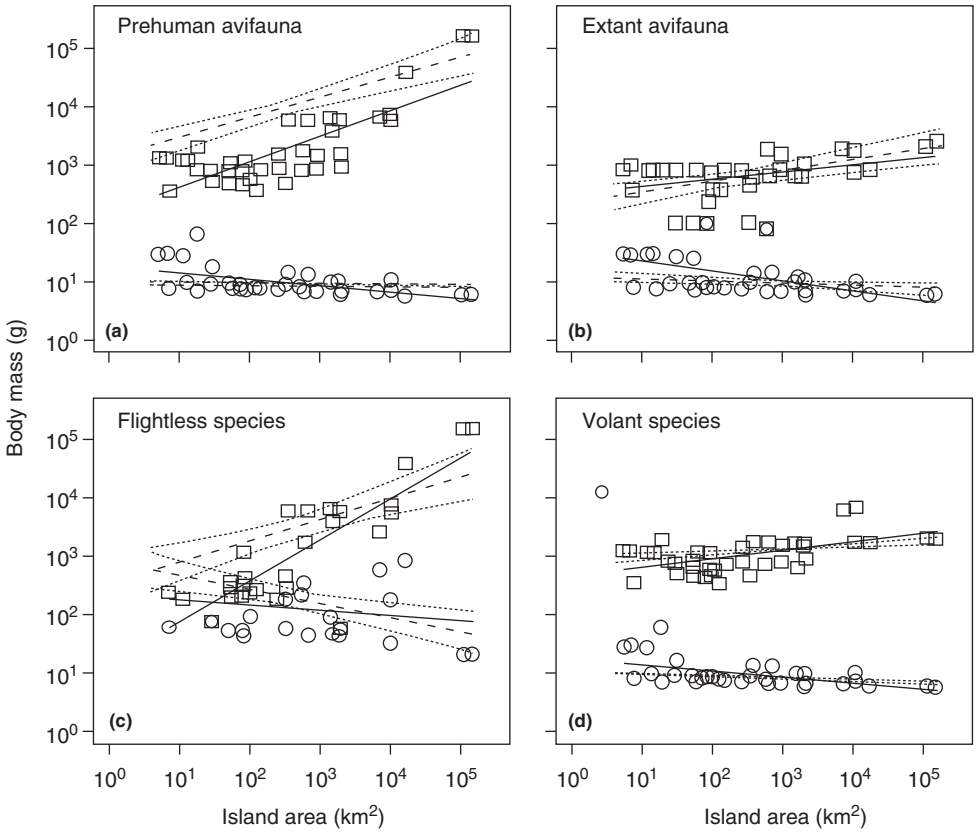

Figure 1 Boyer and Jetz (2010) investigated the relationship between island area and body size for non-predatory birds in 48 Pacific islands. The figure illustrates the scaling of body size extremes with island area for non-predatory birds. The squares indicate maximum and the circles minimum body mass values. Solid lines are the result of a linear regression and dashed lines indicate the predictions of a null model. The four graphs illustrate these relationships separately for: (a) the prehuman avifauna; (b) extant birds; (c) flightless; and (d) volant species on each island.

However, it remains unclear which factors may underlie these changes in body size on islands. Lomolino (2005) suggested that under a release from predators and competitors, island forms evolve toward a size that optimizes their use of the available food resources, i.e., as opposed to a body size that is affected by a combination of energetic, predator avoidance, and inter-specific competition constraints. However, both dwarfism and gigantism are likely to be influenced by a combination of other factors associated with island life and no consensus has emerged on which factors play a predominant role (see also reviews in Meiri and Raia, 2009; Raia, 2009; Meiri, 2009).

First, as a general pattern, large animals may become smaller on islands since the limited island size may not be able to provide the large amount of food required for large species. This mechanism may explain several striking cases, such as the dwarf extinct faunas of elephants and hippopotamuses that are known from several Mediterranean and Indonesian islands (e.g., Lomolino, 2005; but see Meiri and Raia, 2009). In addition, however, several other mechanisms may favor dwarfism on islands, although these mechanisms are likely to arise from a combination of specific characteristics of the island and former mainland habitat experienced. For example, a decrease in size may be beneficial if it allows specialization for a niche which was not available on the mainland (see also review in Whittaker and Fernández-Palacios, 2007). A remarkable example is the Inaccessible rail *Atlantisia rogersi*, the smallest flightless bird in the world (**Figure 2**), which is confined to the tiny and remote Inaccessible island in the south Atlantic where it appears to occupy the ecological niche of a mouse (Del Hoyo *et al.*, 1996). In addition, a release from predators may also favor a smaller size if, in the ancestral mainland form, a larger body allowed escaping from local predators, an advantage that becomes unimportant in the island environment (e.g., Heaney, 1978). Analyses in birds suggested additionally that thermal ecology may play a role as evolution toward a smaller size should facilitate heat dissipation at lower latitudes (Clegg and Owens, 2002). However, this mechanism may be equally important in mainland areas (Olson *et al.*, 2009). Climatic variables have been repeatedly associated with changes in size in island birds and mammals (Boyer and Jetz, 2010; Clegg and Owens, 2002; McClain *et al.*, 2013), but given the correlation between variables such as precipitation, temperature, and productivity it remains unclear how they influence body size evolution and further study is currently needed.

Second, the benefits of evolving toward a larger body size after island colonization are also presumably linked to energy use optimization under a release from predation pressure and inter-specific competition, but acting in the opposite direction for smaller species. Specifically, given the reduced number of species on islands, some niches will be vacant, and island

Figure 2 The Inaccessible rail *Atlantisia rogersi* – here showing its stubby wings while sunning – is the smallest flightless bird in the world. It is confined to the tiny and remote Inaccessible Island (Tristan da Cunha archipelago) in the south Atlantic, where it appears to occupy the ecological niche of a mouse (Photo Peter G Ryan).

Figure 3 Two sunbirds (Nectarinidae) endemic to the island of São Tomé (São Tomé and Principe, Gulf of Guinea, West Africa). The São Tomé sunbird *Dreptes thomensis* (right) is the largest of all sunbirds. It lives in primary and secondary forest and in addition to nectar it feeds also on fruit pulp and on arthropods that it obtains from leaves and by probing the bark of trees in a way similar to the typical feeding habits of woodhoepoes or scimitarbills (Phoeniculidae), which it resembles in size. The co-occurring Newton's sunbird *Anabathmis newtonii* is one of the smallest species in the family.

species may take advantage of these situation and use an enlarged niche, thereby gaining access to more food (Heaney, 1978; Lomolino, 1985). However, this idea has been criticized since there is no solid association between body size and niche breadth (Palkovacs, 2003). In addition, the tendency to increase body size is likely to be favored by the crowded island environments, with higher population densities than what is usually found on the mainland (Lomolino, 2005). This peculiarity of islands is the result of ecological niche widening (in the absence of several species that are present on the mainland) and higher survival (a process known as 'density compensation'; see MacArthur *et al.*, 1972). Under these crowded conditions, intra-specific competition is stronger and there is selection to increase body sizes, as this increases competitive capacity; this was demonstrated by an impressive experiment on Caribbean *Anolis* lizards where island and predator densities were manipulated (Calsbeek and Cox, 2010). The same mechanism has been suggested to be particularly important to explain and increase in size in island birds. Clegg and Owens (2002) found a relationship between increased body size of small birds and intra-specific competition as indicated by territoriality or group foraging. Finally, as with the tendency for dwarfism discussed above, gigantism may be favored in specific situation where a larger size on the new island habitat allows to exploit a new niche. For example, the São Tomé sunbird *Dreptes thomensis* (**Figure 3**) is the largest of all sunbirds (Nectarinidae). It lives in the forests of São Tomé island (Gulf of Guinea, West Africa) feeding partly on nectar, as other members of its family, but also on fruit pulp and on arthropods that it obtains from leaves and by probing the bark of trees in a way similar to the typical feeding habits of woodhoepoes or scimitarbills (Phoeniculidae) (Christy and Clarke, 1998). Finally, and specifically for birds, gigantism may be associated with reduced tendency for dispersal on islands and associated loss of flight (e.g., Steadman, 2006; Boyer and Jetz, 2010). Given that flight is highly costly, reduced need of dispersal and escape from predators should promote the evolution of flightlessness and gigantism could appear as a side effect, since there would no longer be a strong constraint on maximum body size (Raia, 2009).

In addition to the island rule and loss of flight in birds, other patterns of morphological change have been observed, most prominently a tendency for increase in beak and tarsus size (Grant, 1965). Changes in beak size are interesting as they may reflect switches in ecology, and particularly a tendency for species to become ecological generalists on islands (Grant, 1965). However, this trend may be only valid for passerines; subsequent analyses based on a larger number of passerine and non-passerine birds found that instead of a general increase, bill length either followed the island rule (i.e., longer bills decreased an shorter bills increased on islands; (Clegg and Owens, 2002) or did not follow any specific trends. On the other hand, the trend for longer tarsus on islands seems to be a robust one (Grant, 1965) and likely reflects an adaptation to increased time spent walking, which is presumably made possible under decreased predator pressure on islands.

Reproductive Behavior and Life-History

Reproductive strategies in island animals are believed to follow a shift toward 'slower' (or 'K-selected') life histories (Cody, 1966; Blondel, 2000; Grant, 1998; MacArthur and Wilson, 1967). The specific characteristics of islands, with milder climates and less predators and parasites are expected to lead to improved adult survival on islands which, according to life-history theory, should favor reduced fecundity as part of a strategy to allocate more resources into self-maintenance, since maximizing survival is key to maximizing lifetime reproductive success (Barbraud and Weimerskirch, 2001; Charlesworth, 1994; Clutton-Brock, 1988; Goodman, 1974).

Furthermore, since island populations usually live at higher densities than their mainland counterparts (Blondel, 2000; Crowell, 1962; MacArthur and Wilson, 1967; MacArthur *et al.*, 1972), greater competition for food (at the intra-specific level) is expected, which may further favor reduced fecundity (Ashmole, 1963; McNab, 1994; Ricklefs, 1980). Broad-scale analyses in birds based on a large number of islands around the world have now confirmed this tendency for reduced fecundity on islands (Jetz *et al.*, 2008; Covas, 2012). A parallel analysis in lizards has produced a similar result (Novosolov *et al.*, 2013).

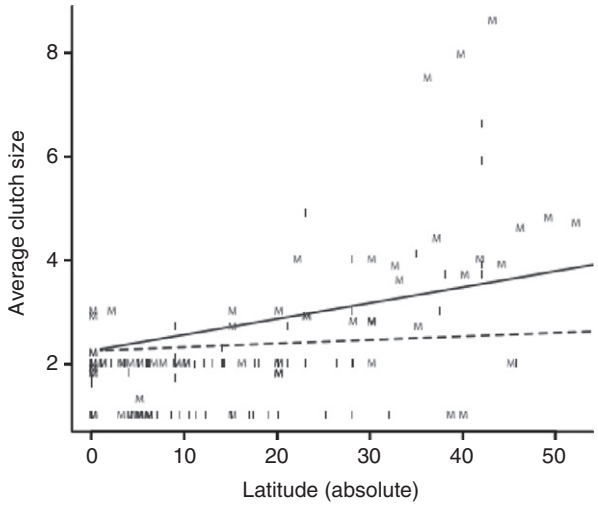

Figure 4 Island birds generally have lower fecundity than their mainland counterparts, but this effect of insularity is strongly influenced by latitude. In a study that compared clutch sizes of 148 island endemics with their close mainland relatives, clutch size increased with latitude at a pace that was ca. 4.5 times faster on the mainland (solid line) than on the island (dashed line). The letters 'M' and 'I' correspond to the data points from the mainland and island species (Covas, 2012).

Fecundity in birds is additionally influenced by an interesting interaction of insularity and latitude. Clutch size in birds is known to be strongly influenced by latitude, being generally lower in the tropics and increasing toward the high-latitude temperate regions (Lack, 1948). This effect of latitude strongly influences the response to insularity, making the differences in clutch size between the island and mainland species much stronger at higher latitudes (Covas, 2012; **Figure 4**). This mechanism probably has a stronger effect on temperate islands because climatic fluctuation is more markedly reduced when compared to the mainland (MacArthur and Wilson, 1967; Whittaker and Fernández-Palacios, 2007). In the tropical mainland regions, climatic conditions are already fairly benign and resource levels less fluctuating, which promotes survival, and hence island-mainland differences are less pronounced. This result thereby further supports that improved adult survival is one of the main factors driving life-history change and reduced fecundity on islands (Ashmole, 1963; Cody, 1966; MacArthur and Wilson, 1967; Blondel, 2000; Jetz *et al.*, 2008). Nonetheless, increased intra-specific competition on islands should favor reduced clutch size regardless of latitude (Ashmole, 1963; MacArthur *et al.*, 1972; Blondel *et al.*, 1988; Blondel, 2000; McNab and Ellis, 2006). However, mainland tropical species already have small clutches (often two and, occasionally, even a single egg, as is the case of some sunbirds; Del Hoyo *et al.*, 2008). Hence, it may be difficult for selection to reduce clutch size further after island colonization by these species.

While fecundity generally decreases on islands, investment per young increases. Specifically, and echoing the example of the kiwi, island birds usually lay larger eggs (**Figure 5**) and have extended developmental periods (Covas, 2012). Under

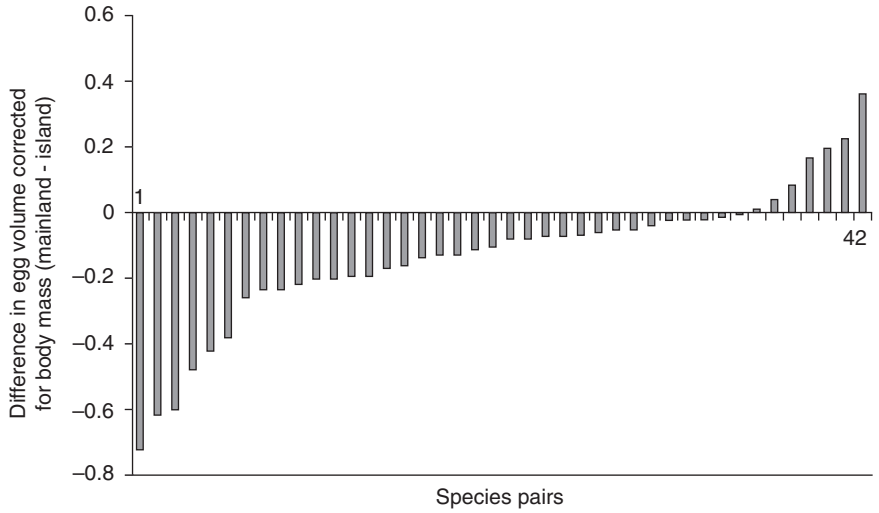

Figure 5 Islands species generally produce larger eggs, in relation to their body size, than do their close mainland relatives. This figure illustrates the difference between the mainland and island egg size for 84 species (an island endemic and its close mainland relative); negative values indicate larger egg volume on the island. The figure accounts for egg size by using the residuals of a regression of egg volume against body mass (Covas, 2012).

lower pressure from predators, juvenile survival should be higher and hence it should be advantageous for parents to increase their investment per young in order to improve offspring quality and competitiveness (Cody, 1966; MacArthur and Wilson, 1967).

In birds, given the external development of young, this can be achieved through larger eggs (see Styrsky *et al.*, 2000; Christians, 2002) and extended developmental periods (if the latter are beneficial for the development of young, which remains debated; Martin, 2002; Ricklefs, 2006; Martin *et al.*, 2007). In addition, extended developmental periods may be more common on islands since nest predation is reduced (and hence the pressure to quickly take offspring off the nest is released). This is advantageous for parents as it allows them to decrease their investment in incubation and nestling feeding in a short time period, allowing them to save energy through lower incubation effort and reduced feeding rates (reviewed in Dmitriew, 2011; see also Ricklefs, 1992; Martin *et al.*, 2011).

Other interesting, but poorly explored characteristics of reproductive strategies on islands relate to mating and parental care. First, the frequency of extra-pair paternity (EPP) appears to be lower on islands (Griffith, 2000). EPP is known to be common in birds and provides an indication of the strength of sexual selection in a given species or population (Petrie and Kempenaers, 1998). Females engaging in EPP are believed to be searching primarily 'good genes' for their offspring, though other direct benefits may be involved, for example, if females gain increasing foraging opportunities at other males' territories (Petrie and Kempenaers, 1998; Petrie *et al.*, 1998). On islands, populations are mostly derived from a small founding group. This leads both to lower levels of genetic diversity and higher relatedness levels (Frankham, 1998). Hence, the advantages brought by EPP are reduced in this genetic setting. Under this scenario, males have higher certainty of paternity, which is expected to increase reproductive investment by males, further reinforcing the shift toward greater investment in young as described above (see also Bennet and Owens, 2002).

The decrease in sexual selection implied in this hypothesis is further corroborated by yet another evolutionary pattern observed in birds: island populations would be less colorful than their mainland counterparts (Grant, 1968; Fitzpatrick, 1998; Figuerola and Green, 2000). This pattern was supported by a recent broad-scale study based on spectrophotometric measurements which found that island birds have a reduced diversity of colors and plumage brightness and well as reduced number of plumage patches (**Figure 6**). These changes, however, may be partly explained by a decrease in the need for species recognition on islands (Figuerola and Green, 2000; Grant, 1968). Plumage color and patches are used in mate choice, but also in species recognition and as such have important consequences for the fitness of individuals since attempting to mate with individuals from the wrong species would represent a significant cost. The impoverished diversity of species on islands translates in reduced number of closely related species living in sympatry, and hence in reduced risks of attempting to mate with heterospecifics. Under this condition, the complexity of signals (here plumage pattern and coloration) is expected to decrease, as observed for bird coloration islands (see also Morinay *et al.* (2013) for a similar result on bird song).

Figure 6 Island birds tend to have duller plumage, reduced number of colors and less colored patches than their mainland counterparts. This is illustrated here by specimens of fruit dove (*Ptilinopus* spp) held at the British Museum of Natural History. The species on the left is *Ptilinopus purpuratus* from Tahiti Island and on the right is *Ptilinopus regina* from mainland Australia (Photo C Doutrelant).

Finally, islands hold strikingly high numbers of cooperatively breeding species (Covas, 2012). Cooperative breeding is an interesting behavior where nonbreeding sexually mature birds assist with raising the offspring of others. These 'helpers' are often (though not always) offspring of the breeding pair that delay dispersal and stay in the parental territory forming family groups (Brown, 1987). While this type of parental care is known from ca. 9% of all birds species (Cockburn, 2006), on islands, one study found cooperative breeding to occur in 33% of the islands species surveyed (Covas, 2012). Why some species breed cooperatively and others do not remain actively debated (e.g., Andrew Cockburn and Russell, 2011)? One view that has received some support is based on the observations that cooperatively breeding species tend to have high survival (Arnold and Owens, 1998; Cockburn, 2003; Covas and Griesser, 2007). Increased survival may favor cooperative breeding since long-lived species often start to breed later in life (Covas and Griesser, 2007). Long-term occupancy of breeding territories by long-lived residents should decrease the opportunities for independent breeding by younger birds (Emlen, 1982; Arnold and Owens, 1998). Hence, the demography of these species may frequently lead to a situation where individuals do not attempt to breed in their early years but remain in the natal territory, where they are exposed to the subsequent breeding attempts, which they will assist. This route to cooperative breeding is consistent with the demographic shift observed on islands (Blondel, 2000; Covas, 2012; MacArthur *et al.*, 1972; see also Cockburn, 2003). However, additional more in-depth study is needed to test the links between island life and the different steps leading to cooperative breeding and hence to fully understand this change.

The islands around the world continue to fulfill the role of natural laboratories of ecology and evolution. The simplicity of island ecosystems serve as a magnifying glass to examine some of the most important drivers of evolution, while the number of islands around the world – with their varieties of faunas, geographic and climatic conditions – provide natural replicates which offer unique opportunities to test

evolutionary mechanisms. The examples above illustrate how islands have contributed to our understanding of several factors affecting life-history evolution, including body size and several reproductive and behavioral traits. Global patterns of adaptation on islands are beginning to emerge and some of the key factors underlying change are beginning to be understood. Reduced species diversity (including the reduced number of predators and parasites) and the milder climates appear to play a prominent role for life-history change on islands through ecological release and enhanced survival. However, even on simplified island environment several factors may interact and more detailed work remains to be done to conclusively assess several of the hypotheses advanced here.

See also: Biogeography of Islands, Lakes, and Mountaintops; Evolutionary. *r*- and *K*-Selection in Fluctuating Environments, Theory of

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Life History Evolution, Plants

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Glossary

Integral projection model (IPM) A demographic model that reflects size-dependent demographic rates (i.e., survival, fertility vary as a function of the size of the individual) and from which classic fitness measures can be extracted.

Monocarpic A plant for which reproduction is fatal.

Background

In previous articles we have seen how fitness can be defined as the asymptotic population growth rate in deterministic models, as the long-run stochastic growth rate in stochastic models and, for some density-dependent models, as carrying capacity. However, in many cases, the frequency or density of different classes of individuals can impact the fitness of other classes. In these cases, fitness cannot be universally estimated as a single quantity, and the researcher needs to rely upon simulation approaches where different strategies are competed against one another. These techniques, referred to as evolutionary game theory or adaptive dynamics, are a powerful approach to identify the eventual outcome of evolution. However, in order for the approach to be tractable, one or two key assumptions are required. The most important one is the way that strategies are passed from parents to their offspring; the approaches assume near perfect fidelity. A second requirement is that trade-offs exist between different components of the life history. Without a trade-off, Darwinian demons evolve. One trade-off that is often incorporated into approaches to identify Evolutionarily Stable Strategy (ESS) in frequency dependent environments is one between offspring number and offspring size. In this article, the author gives a brief overview of life history approaches and their application, primarily to plants that can live for a variable number of years, but which flower only once – monocarpic perennials.

Using Evolutionarily Stable Strategy Methods to Understand Evolution in Perennial Plants

An ESS is a life history strategy (i.e., a time of flowering, or a seed size) that cannot be invaded by any other strategy. A mutation that results in a phenotype that is not at the ESS (a smaller or larger flowering size, or a larger or smaller seed size) will not be able to invade a population where the ESS is present at high frequencies.

Interactions between individuals are central to the ESS concept. As well as its own strategy (genotype), the strategies (genotypes) of other individuals within the population affect a focal individual's survival or fertility, and thus fitness. The centrality of interactions between individuals implies that either density or frequency dependence is operating within the population. Density dependence might result from a restriction of the total number of seeds recruiting, or adults surviving, or reproducing due to resource limitation (e.g., microsites for recruitment, nutrients, or water for growth and survival). Positive frequency dependence occurs when a particular strategy is disproportionately represented in subsequent generations when it is rare – self incompatibility alleles are a classic example in plants. As a consequence of either density or frequency dependence, the success of a strategy will depend on what other individuals in the population are doing. Therefore, both an individual's strategy and the strategies of individuals around them will shape evolutionary outcomes.

This inherent nonlinearity can result in evolutionarily singular strategies that are not, in fact, convergent stable. Although such strategies can invade every other possible strategy (e.g., every possible flowering size), they are themselves invasible – their dominance within a population results in a density or frequency dependent environment that is more favorable to strategies other than themselves. However, once these strategies establish, the ESS will in turn invade their replacements successfully. Complex evolutionary cycles and branching points may result (Geritz *et al.*, 1999).

ESS theory has been successfully deployed to understand evolution of timing of flowering in monocarpic perennials. For these plants, reproduction is fatal, and therefore timing of flowering is the outcome of a relatively simple trade-off. Plants should wait as long as possible before flowering, since the longer they wait, the larger they grow, and the larger they grow, the more seeds they produce. However, if they wait too long, they risk dying without ever reproducing (Metcalf *et al.*, 2003). The ESS balances these two forces. Further, given the simplicity of the monocarpic life history (in particular, the lack of complex costs of reproduction requiring parameterization) the ESS can be predicted using integral projection models parametrized from field data (Rees and Rose, 2002; Rees *et al.*, 2006; Metcalf *et al.*, 2003). Since density dependence appears to act mostly on seedling establishment within these systems (Metcalf *et al.*, 2003), complex feedbacks are not expected (Mylius and Diekmann, 1995); and the result is a convergent stable strategy (CSS), which is often found to be a close match to observed flowering sizes. Stochasticity plays a major role in determining the convergent stable flowering size, as timing of flowering is inherently a bet-hedging trait. Longer delays before flowering act to distribute flowering over more years, allowing for a spread of seed recruitment across conditions. This spread acts to reduce variance in the population growth rate (Childs *et al.*, 2010), increasing fitness and invasion rates in a stochastic environment, even though the mean one time-step growth rate is likely to be reduced, as delayed reproduction allows more time for extra mortality. Similar

analyses have been used to predict optimal rates of germination from the seedbank (Rees *et al.*, 2006), again with strong matching of predictions with observations.

ESS theory has also been deployed to address the question of the evolution of seed size. This is usually framed in the context of a trade-off between seed size and seed number (plants may either produce many small seeds, or a few large ones, with larger seeds having higher competitive ability, or survival). A range of theoretical outcomes have been described (Geritz *et al.*, 1999). It has proved more difficult to test this theory in natural systems, given complexity in parametrizing the range of underlying trade-offs, particularly in the face of resource heterogeneity.

Summary

ESS methods are the appropriate method to identify the optimal life history strategy when frequency or density dependence is operating. They make the assumption that strategies are passed from parent to offspring with near perfect fidelity, consequently ignoring genetic mechanisms. They have been applied across a number of animal and plant systems, with a large body of work on monocarpic perennial plants.

See also: Inheritance: From Quantitative Genetics to Evolutionary Stable Strategies. Life Histories, Axes of Variation in. Life History Theory: Basics. Life History Trade-offs. Life History, What is?

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Life History Evolution: The Role of Mating Systems

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Why Mating and Life History Have to be Associated?

Evolution results in strategies that maximize fitness. A life history strategy is determined by life history traits, which include those listed in Table 1. Many of these traits are associated with, or are a consequence of, decisions regarding reproduction.

A part of reproduction is mating itself, a process that includes searching for a mate, mate choice, mating system, mating tactic, and behavior. Some mating decisions are made at the individual level, for example, the choice of whom to mate with. Others, like the mating system, are population-level descriptors. Finally others, including generation length, are genetically and phylogenetically constrained.

Life history traits can directly be aspects of mating. For instance, the tactics that individuals use to successfully mate can directly drive life history evolution. However, mating traits can also limit the evolution of alternative life history traits: for example, hermaphroditic (monoecious) plants are unlikely to evolve into dioecious plants. The next section discusses how mating influences life history as well as how mating is influenced by life history. A later section highlights sex allocation and sexual conflict. This article primarily focuses on the animal kingdom although it includes a discussion on plant mating systems. Finally, a population model that allows a whole range of questions on how life history and mating interact is presented.

Table 1 Non-exhaustive list of life history traits

Gestation length
Body weight at birth, seed size and mass
Developmental state after birth (altricial and precocial)
Length of growth period, growth pattern, germination
Optimal body size
Dormancy
Allometry
Age and weight at maturation
Distribution of resources to maintenance, growth, and reproduction
Reproductive strategy, reproductive morph
Reproductive timespan
Period between litters or reproductive events
Mating preference
Litter size, seed number
Pollen–ovule ratio
Sex ratio of offspring
Parental care, length of care
Sociality
Age of dispersal
Timing of sex reversal for hermaphrodites
Number of reproductive events (Semelparity and iteroparity)
Mortality rates, life expectancy

The Role of Mating on Life History Evolution

Timing, Behavior, and Mating Preference

Mate choice evolved because specific mate combinations have higher fertility and produce fitter offspring than alternative combinations. Mating preferences can consequently influence population growth rate by impacting the fertility rate, but also by impacting the distribution of fitness-related traits in the offspring generation (Schindler *et al.*, 2013).

The timing of mating can affect lifetime reproductive output via routes beyond an earlier age of sexual maturity being expected to result in a longer reproductively active life. Trade-offs between reproduction and mortality or between reproduction at different ages mean that reproducing at as young an age as possible is not the fittest life history strategy (Stearns, 1992). However, delaying mating can also carry a cost: in butterflies *(Bicyclus anyana)* late reproduction increases life span but reduces fertility at the first reproductive attempt (Zijlstra *et al.*, 2000).

Decisions on when to mate, and with whom, can consequently influence not only individual offspring number and offspring phenotype, but also population-level survival and reproductive rates.

Mating System

Mating system classification differs between plants and animals. In animals, the classification is based on how many mates can be monopolized, how mates are acquired, characteristics of a pair bond, and patterns of parental care (**Table 2**; Emlen and Oring, 1977). In plants, the classification of the mating system is based on the amount of selfing and inbreeding depression (**Table 3**; Sakai and Westneat, 2001).

Table 2 Animal mating systems

Parthenogenesis	Reproduction without fertilization
Monogamy	Pair bond between one female and one male for the reproductive season, often shared parental care
Polygyny	Several females mate exclusively with one male
Polyandry	Several males mate exclusively with one female
Polygynandry	Both sexes mate exclusively with a small set of members of the other sex
Promiscuity	Short-term association between any female and male
Polybrachygyny	Absence of pair bonds, no parental care by males, males are constantly seeking mating opportunities
Lekking	Males aggregate in a display area where they defend small territories, females arrive at display area to find a mate

For both plants and animals, ecology influences the evolution of the mating system (Lloyd, 1980; Emlen and Oring, 1977). A change in the mating system in turn drives life history adaptation. In plants, for example, the transition from outcrossing to selfing causes selection for a lower pollen–ovule ratio (Dudley *et al.*, 2007), a younger age at maturity (Dudley *et al.*, 2007), and from a perennial to an annual life cycle (Zhang, 2000). In animals the transition from monogamy to promiscuity has two implications. First, if females mate

Table 3 Plant mating systems

Selfing	Self-fertilization
Outcrossing	Fertilization with unrelated pollen
Apomixis	Vegetative propagation or seeds with complete maternal genome
Dichogamy	Flowers have female and male function at different times
Gender change	Change in sex-expression or sequential hermaphroditism
Heterostyly	Two to three different reproductive flower morphs, self-incompatibility, mechanism for outcrossing
Dioecy	Variance in sex-expression at individual level, for example, female or male flowers or plants, or at population level

polyandrously then increased longevity is selected for in males. For example, in two butterfly species from the genus *Yponomeuta* (Lepidoptera: Yponomeutidae), *Y. cagnagellus* and *Y. padellus*, male life span increases with the level of polyandry and becomes better matched to female reproductive life span (Bakker *et al.*, 2011). Second, the mate of a promiscuous individual that provides parental care will be uncertain as to whether it is the parent of an offspring or not. As the certainty of parentage declines, the lower the expected contribution of the sex that cannot determine whether it is a parent or not to offspring care. For example, when females provide care, male promiscuity evolves and male reproductive success increases with the number of mates he acquires (Sæther, 1986). However, the increase in male reproductive success is counterbalanced by a reduction in reproductive life span as male–male competition for mates increases (Lukas and Clutton-Brock, 2014). The higher female promiscuity, the lower the probability of finding the same paternal genes in litter mates and future siblings and the greater mother–offspring conflict over resource provisioning. Offspring of promiscuous mothers demand more resources, which in turn increases her costs of reproduction (Garratt *et al.*, 2014). Female promiscuity consequently selects for a reduced reproductive life span. In summary, while a female balances a shorter reproductive life span with increased genetic variation in her offspring, a male balances a shorter reproductive life span with the benefits of potentially producing more offspring. For both sexes, a change in the mating system toward higher promiscuity alters life history.

Ways Life History Can Influence Mating

Phylogenetic studies suggest that evolution from one mating system to another cannot always be reversed. For instance, selfing in plants has evolved several times from outcrossing, but not vice versa (Barrett *et al.*, 1997). Similarly, polygyny in animals has evolved several times from monogamy but there are no examples of evolution in the reverse direction (Lukas and Clutton-Brock, 2013). The lack of these reversals suggest that life history adaptations to the mating system (see Section Mating System) can generate evolutionary constraints.

Although life history constrains the evolution of the mating system, life history can select for a change in mating system because it determines optimal mating behavior. For example, studies of seed beetles (*Callosobruchus maculatu*) where lines are selected for either reproduction at young or old ages revealed that mating frequency at early ages can evolve quickly (Maklakov *et al.*, 2010).

In addition, juvenile mortality can impact the benefits of polygynous or monogamous mating in males. Under the assumption that monogamous males invest more into paternal care than polygynous ones, polygyny is expected to evolve when the fitness reduction for males due to reduced parental care for each of its offspring is compensated by more offspring resulting from more matings (Sæther, 1986). In other words, the benefits of monogamy increases with juvenile survival, or the ratio of juvenile survival to adult survival (Sæther, 1986). In plants, longevity correlates negatively with inbreeding depression independently of whether mating is random (outcrossing) or mixed (some self-fertilization) (Morgan, 2001), which further supports the hypothesis that the evolution of annual life cycles preceded the evolution of selfing (Barrett *et al.*, 1997).

In the following sections further examples of life history effects on mating focusing on survival rates, fertility rates, and reproductive morphs are given.

Survival

Survival rates usually – but not always – decrease in older age (Jones *et al.*, 2013). If survival rates decrease with increasing age beyond some age, then life history theory predicts that mate choice should decline and mating effort should increase with age. There are various lines of evidence in support of this. First, both female and male mate choice in the fruit fly (*Drosophila melanogaster*) decreases with age (Edward and Chapman, 2013). Second, young male scorpionflies give more valuable nuptial gifts to high-quality females than to low-quality ones. In contrast, old males are less discriminative (Engqvist and Sauer, 2002). Third, female mate choice in cockroaches declines with age as does her fecundity (Moore and Moore, 2001). Fourth, adult survival rate determines reproductive effort at early ages in many species including alpine ibex (*Capra ibex*), while male reproductive effort at young ages decreases with survival rates across species (Willisch *et al.*, 2012). Sex-differences in mortality also impact the operational sex ratio and consequently the variance in mating success (Partridge and Endler, 1987). In addition, sex-differences in survival and other demographic rates enhance the effect that mate preferences have on the population growth rate (Schindler *et al.*, 2013).

Finally, vital rates limit the extent of sexually selected traits (Partridge and Endler, 1987) as the prevalence of such traits correlates negatively with survival rates (e.g., red deer *Cervus elaphus*, Clutton-Brock *et al.*, 1985).

Fertility

Females and males can adjust their reproductive investment as a function of the quality or behavior of their mate. A meta-analysis of dragonflies, for example, has shown that tandem

species (males of tandem species remain in physical contact and guard females they have mated with until she oviposits) lay larger eggs and produce faster growing larvae (Koch and Suhling, 2005).

Males can adjust the amount and quality of sperm ejaculate as a function of the mating status and fecundity of their mate. For example, some male insects adjust sperm number on whether their mate has previously been mated (Wedell *et al.*, 2002) and male sailfin and Amazon mollies (*Poecilia latipinna* and *Poecilia formosa)* transmit less sperm to unisexual females than to those that are bisexuals (Riesch *et al.*, 2012). In addition, sperm quality can be adjusted to the attractiveness of females. For example, male fowl transmit faster sperm to mates with larger combs (Cornwallis and O'Connor, 2009). Females can adjust offspring number to the quality of the male. For example, in cichlid fish, females lay smaller clutch sizes to a male that has been more helpful in its juvenile state (Schürch and Heg, 2010).

Reproductive Morphs

Within a species there can be alternative life histories that are manifest in different morphs. This occurs primarily in males, with mating behavior often differing between morphs. A common set of alternative mating tactics is the sneaking (cuckoder) morph that either disguises itself using female mimicry or hides and tries to sneak matings and avoids aggression from the other morph that is usually bigger, has weapons, or provides paternal care (e.g., lampreys *Petromyzontiformes*, Hume *et al.* (2013); sand-bubbler crab *Scopimera globosa*, Koga and Murai (1997); Alpine ibex *Capra ibex*, Willisch *et al.* (2012)). Some crabs (*S. globosa*) that grow throughout their life apply a size-dependent mating strategy of either underground copulation or surface copulation which is less costly but involves lower reproductive success (Koga and Murai, 1997). Alternative life histories are maintained in a population via disruptive selection such that intermediate morphs produce less offspring than either sneaker or the big morph (e.g., coho salmon *Oncorhynchus kisutch*, Gross, 1985). The expression of one of two alternative life histories is almost always due to a conditional strategy and arises via frequency- and status-dependent selection (Gross, 1996). Examples for the status of an individual include quality, competitive ability, or condition (Gross, 1996).

Although alternative reproductive strategies are most common with two alternatives, there are species with three or more alternative reproductive strategies (e.g., side-blotched lizard *Uta stansburiana*, Sinervo and Lively (1996); Mediterranean wrasse *Symphodus ocellatus*, Alonzo *et al.* (2000)). The maintenance of more than two strategies is not yet fully understood and might be species-specific (Sinervo and Lively, 1996; Alonzo and Calsbeek, 2010).

Sex Allocation and Sexual Conflict

Sex Allocation

Life history affects sex allocation because mating patterns and parental care determine the optimal offspring sex (Stearns, 1992). Sex allocation can also be explained as a response to ecological or environmental conditions: for example, parents in some species can alter the amount of energy invested in sons and daughters, often investing more in the rarer sex or the frailer sex (Fisher, 1930), the sex that disperses more (local resource competition, West, 2009) or the sex that is likely to face the strongest competition later in life (local mate competition, Hamilton, 1967). Individual mating status and condition can also influence offspring sex ratio. For example, previously mated female spider mites (*Tetranychus urticae*) produce fewer offspring, but more daughters (Macke *et al.*, 2012), while sexually exhausted male rats *(Rattus norvegicus)* sire more female-biased litters (Bartos and Trojan, 1988; Hornig and McClintock, 1996). Trivers and Willard (1973) predicted that good-condition mothers of polygynous and sexually dimorphic species should produce the sex with highest variation in the reproductive value (Trivers and Willard, 1973; Leimar, 1996). In spite of more than 40 years of tests of the Trivers–Willard theory, empirical evidence is rather inconclusive (Hewison and Gaillard, 1999; Sheldon and West, 2004), which is due to a failure to account for sex-differences in demographic rates (Schindler *et al.*, 2015).

Sexual Conflict

Sexual conflict (the focus is on interlocus sexual conflict as opposed to intralocus conflict which arises via sex-specific gene expression (Arnqvist and Rowe, 2005)) arises when females and males have different life histories. Sexual conflict – like life history – shapes the mating system, and the mating system can lead to sexual conflict (King *et al.*, 2013). For example, male ungulates can induce estrus in females and reduced male presence can consequently increase female breeding age (Mysterud *et al.*, 2002). Additionally, young female seed beetles (*Canthoscelides obtectus*) can reduce their resistance to male mating attempts but pay a reduction in lifetime fecundity (Maklakov *et al.*, 2007).

Modeling Framework

Often it is not possible to identify the causal sequence of effects in the feedback loop between life history and mating in a real system. Models are therefore a useful tool to study the effect of mating on life history evolution and the effect of life history traits on mating probabilities. A recent model combines the major demographic rates – survival, ontological growth, and fertility rates which represent a specific life history strategy – with explicit age and phenotype-based mating preferences (Schindler *et al.*, 2013; Traill *et al.*, 2014) and thus allows the study of the link between life history and mating in detail. The model is a two-sex integral projection model (IPM) which projects into the future a population that is structured by age and a continuous trait, for example body size. The two-sex IPM has been proven useful to show how mating preferences affect population growth rate (Schindler *et al.*, 2013), how trophy hunting affects the demography of the trophy-bearing species when hunters target a sexually selected trait (Traill *et al.*, 2014), and how optimal sex allocation

depends on the interaction between the mating system and sex-differences in life history strategies (Schindler *et al.*, 2015). This model allows the study of a range of questions, for example, how changes in the demographic rates affect mating chances and reproduction, or how demographic rates adapt to the mating system.

Conclusion

This article provided a non-exhaustive overview about studies that investigated the link between mating traits and life history traits. It becomes clear that our understanding of this link is very limited. Besides having identified a handful of trade-offs between both sets of traits, the mechanism or precise nature of these trade-offs still have to be revealed. The use of models – such as the two-sex IPM described – can help to investigate hypothesis on the interface of mating dynamics and life history evolution.

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See also: Mating Systems, A Brief History of. Operational Sex Ratio. Sexual Conflict

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Life History Patterns

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Glossary

Determinate growth Growth that is terminated once a predetermined size or structure has been reached, often at around the age of sexual maturity in many animals.

Germ line The germ line is composed of the sex cells (giving rise to the gametes, sperm, and eggs) that pass on genetic information between generations in sexually reproducing organisms.

Indeterminate growth Growth that continues over an organism's life course is not terminated once a predetermined size or structure has been reached.

Senescence A decline in the age-specific probability of survival, or age-specific reproductive output with age.

Soma/somatic cells Somatic cells (collectively known as the 'soma') give rise to all cells of the body except reproductive cells (sperm and eggs). Mutations in somatic cells are not passed on to offspring.

Survivorship The proportion of a population cohort surviving to a given age.

Background

Survivorship is defined as the proportion of a cohort that survives to a given age, and survivorship curves represent this quantity over age. Three archetypes of these curves were described by Deevey (1947), after Pearl and Miner (1935): type I, II, and III. Naturally, these curves are intimately related to age-specific mortality trajectories, with constant mortality with age resulting in a type II curve, while increasing or decreasing mortality with age result in type I and type III curves, respectively (**Figure 1**). Thus, trajectories of survivorship are tightly bound together with life span, trajectories of mortality, and the evolutionary theories of senescence.

The classical evolutionary theories of aging, 'mutation accumulation' and 'antagonistic pleiotropy' (Medawar, 1952; Hamilton, 1966; Williams, 1957), rely on the observation that there is a decline in the force of natural selection with increasing age. A third theory (Kirkwood, 1977), now known as the 'disposable soma theory,' argues that senescence is the result of balancing trade-offs between maintenance of the precious and immortal germ line (i.e., sex cells) and maintenance of the body (soma), which is viewed as merely a disposable vessel for transmitting genes. Together these theories imply that senescence, an increase in age-specific mortality rate over the life course (a decline in age-specific survival) is inevitable in organisms that separate germ line and soma, 'even in the farthest reaches of almost any bizarre universe' (Hamilton, 1998). Hamilton (1966) noted that reproductive senescence is a more complex trait than mortality senescence, and much less theoretical work has been carried out on it, but it follows that one would expect a decline in fertility with age to be concurrent with the increase in mortality.

Senescence in Humans

From maturity, human mortality rates, like those of many other animals, approximate a Gompertz (positive exponential function) or Gompertz–Makeham function (Ricklefs, 1998; Finch and Pike, 1996; Finch *et al.*, 1990), at least until very advanced ages (Vaupel, 1997). Considering the entire life course though, mortality rates are usually J- or U-shaped. For example, in historic human populations, and in modern hunter-gatherers, mortality is characterized by a U-shaped trajectory where infant mortality rates are high, and decline with age. Mortality rates then remain low for a period before increasing rapidly at older ages. The initial decline, dubbed ontogenescence, is a common feature across a wide range of taxa (Levitis, 2011) and has variously been hypothesized to be an adaptive quality control mechanism to remove defective individuals and increase the viability of their more robust kin (Hamilton, 1966), a result of trade-offs among growth processes, maintenance, and reproduction (Chu *et al.*, 2008), a heterogeneity effect whereby the frailest die first causing robustness to increase with age at the population level (Vaupel and Yashin, 1985), and a result of genetic or developmental malfunctions during transition points during development, concentrated in early life, when mechanisms are first expressed and tested (Levitis, 2011).

With the advantages brought by medical and public health advances, infant mortality has been reduced in most modern human populations, while the onset of senescence has been delayed (**Figure 2**) (also see Burger *et al.*, 2012), resulting in a transition from a U- to a J-shaped mortality trajectory. In some populations, declines in the rate of increase in mortality and even mortality plateaus at very advanced ages have been recorded (Vaupel, 1997). This is a puzzling phenomenon that usually requires immense amounts of data to detect, but that has also been observed in non-human taxa (e.g., Curtsinger *et al.* (1992) and Carey *et al.* (1992) in fruit flies, and Chen *et al.* (2013) in nematodes). Fertility in humans is hump-shaped, and there is an extended period of post-reproductive survival in women (**Figure 2**). But how do these trajectories compare to other animals?

Senescence in Non-Human Vertebrates

Until recently it was often argued that senescence would not be an important feature of the demography of wild animal

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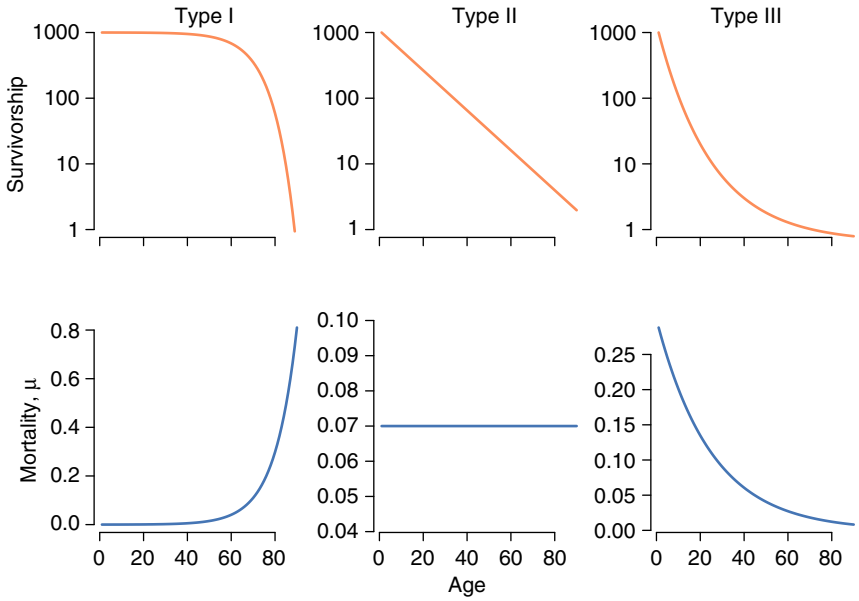

Figure 1 Type I, Type II, and Type III survivorship curves on a logarithmic scale (top row, orange lines) arise from increasing, constant, and declining mortality trajectories (bottom row, blue lines).

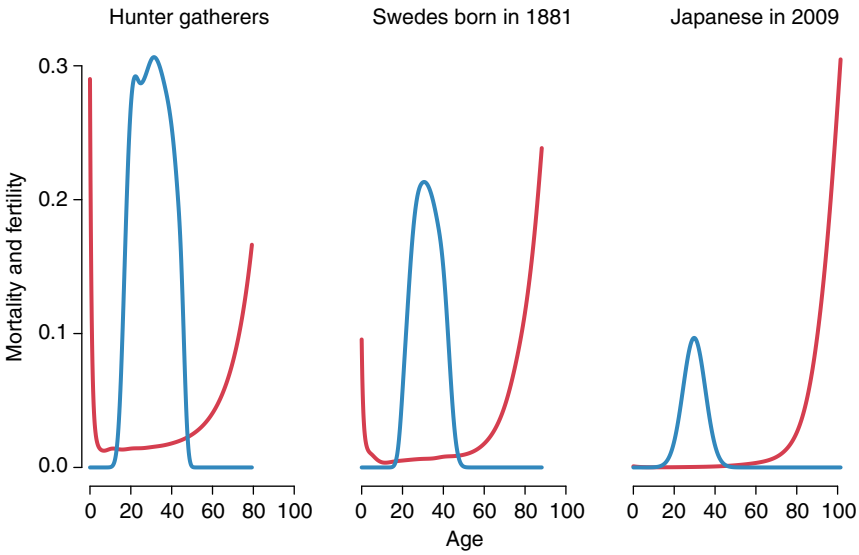

Figure 2 Mortality (red) and fertility (blue) trajectories in humans. From left to right, a hunter-gatherer population (the pre-contact Ache of Paraguay), a historic human population (Swedes from the nineteenth century), and modern humans, exemplified by the Japanese in 2009. Each curve ends as proportion surviving from a synthetic cohort reaches 0.05. Note how, with medical and public health advances, infant mortality has declined and the length of the life course has increased. At the same time, fertility has declined and occupies a smaller proportion of the life course. Data obtained from the Human Mortality Database (Human Mortality Database, 2013) and Hill and Hurtado (1996).

populations because individuals would 'die before they got old' from environmentally driven mortality like predation (Lack, 1943; Medawar, 1952; Comfort, 1956; Kirkwood and Austad, 2000). However, in recent decades there has been a growth in the availability of longitudinal datasets of intensely monitored mammal and bird populations (Nussey *et al.*, 2008, 2013), complemented by the development of sophisticated statistical methods such as capture-mark-recapture (Lebreton *et al.*, 1992; Gimenez *et al.*, 2008; Colchero *et al.*, 2012). Together these advances have brought ample evidence that actuarial senescence is a common feature of mammalian and avian life histories (e.g., Jones *et al.* (2008), Jones *et al.* (2014), Nussey *et al.* (2013) and references therein). Collectively the weight of evidence suggests that, in these groups, senescence is inevitable with U-shaped mortality trajectories, confirming the early comparative demographic work of Caughley (1966) on a small subset of mammals.

Evidence for reproductive senescence is a little more equivocal, perhaps because measuring fertility in the wild can be challenging and, although it is certainly detectable as a

hump-shaped fertility trajectory in many species (e.g., Jones *et al.* (2008), Jones *et al.* (2014), Nussey *et al.* (2013) and references therein), in others there is no hint of a decline after maturity with rates remaining constant well into advanced age (Jones *et al.*, 2008). Detailed studies of pregnancy rates in roe deer (Hewison and Gaillard, 2001) show that rates remain constant through most of the adult life course, but decline sharply at advanced age suggesting that in many cases the lack of detection could simply be an artifact of data quality and/or quantity for older individuals. In other species though rates decline with age directly from maturity (e.g., in olive baboons (*Papio anubis*) and lions (*Panthera leo*) (Packer *et al.*, 1998), and in red deer (*Cervus elaphus*) (Nussey *et al.*, 2009)). Senescence in the demographic traits of mortality and fertility are concurrent with changes in physiology (e.g., Catry *et al.*, 2006; MacNulty *et al.*, 2009), immune function (Palacios *et al.*, 2011), and body mass (e.g., Mysterud *et al.*, 2001; Nussey *et al.*, 2011), which represent more proximate causes for the observed population-level demographic trends.

There appears to be a degree of predictability in both the rate and timing of senescence. Closely related species tend to share similar life spans and qualitative trajectories (Healy *et al.*, 2014; Jones *et al.*, 2014). In birds and mammals, life spans increase and senescence rates decrease as the pace of life, measured by generation time, increases (Jones *et al.*, 2008; Peron *et al.*, 2010; Ricklefs, 2010; de Magalhaes *et al.*, 2007). In addition, protected populations – those that in some way avoid predation or other causes of mortality – tend to have lower rates of senescence and consequently longer life spans. This includes captive populations (Lemaître *et al.*, 2013), island populations (Austad, 1993), and species who's particular life history renders them less vulnerable to predation (e.g., bats (Healy *et al.*, 2014; Wilkinson and South, 2002; Munshi-South and Wilkinson, 2010)). Other factors that may shape senescence in these groups include the social system, with the expectation that the observation that males tend to have short lives compared to females being due to intra-sexual competition (Clutton-Brock and Isvaran, 2007). Studies that have examined the timing of senescence (e.g., Jones *et al.*, 2008; Loison *et al.*, 1999; Peron *et al.*, 2010) have demonstrated that, contrary to Hamilton's (1966) prediction that senescence should begin at maturity, senescence usually begins well after this point. But how do these patterns manifest outside the commonly studied groups of mammals and birds?

Beyond Mammals and Birds

Mammals and birds are all determinate growers, but there are many animal species that have very different architecture. For example, some species are clonal (e.g., coral), while others are indeterminate growers whose vulnerability to predation declines, and whose fertility increases with size and age (e.g., many fish, reptiles, and mollusks). In fact there are many species, especially indeterminate growers where size may be a more relevant predictor of demographic state than age (Sauer and Slade, 1987; Kirkpatrick,1984). Furthermore, since Vaupel *et al.* (2004) have shown mathematically that senescence may be avoided in indeterminate growers if small decreases in reproduction now can increase survival and reproduction later, it is of particular importance to study senescence in species with a wider range life histories – including indeterminate growth.

Jones *et al.* (2014) used data from a wide taxonomic scope of animals and plants and examined the shape of mortality and fertility trajectories (*sensu* Baudisch, 2011) over a typical life course from maturity. Their study revealed the existence of a great diversity of demographic trajectories: mortality trajectories can increase, decrease, or remain constant, while fertility trajectories can be hump-shaped, increase or decrease. Only one species, the nematode worm *Caenorhabditis elegans*, showed both mortality and fertility trajectories that conformed to the expectation of a consistent decline in fertility and an increase in mortality with age from maturity. Some species showed no, or negligible, increases in mortality. For example, the study confirmed the work of Martínez (1998) showing that the cnidarian *Hydra*, which reproduces clonally, has extremely low and unchanging mortality rates, and constant fertility rates. Other species like the desert tortoise (*Gopherus agassizii*), an indeterminate grower, exhibited declining mortality and increasing fertility with age – presumably related to increases in body size. Other chelonians are reported to show negligible changes in mortality (Hayflick, 1998; Congdon *et al.*, 2003). Studies on other reptiles have shown increasing mortality, despite their indeterminate growth patterns (Massot *et al.*, 2011; Bronikowski, 2008; Sparkman *et al.*, 2007), although at the same time fertility rates in some species increased with age (and size) (Sparkman *et al.*, 2007; Paitz *et al.*, 2007). Insects like *Drosophila* and medfly (*Ceratitis capitata*) have trajectories that are qualitatively similar to those of humans (Snoke and Promisiow, 2003; Vaupel *et al.*, 1998; Khazaeli and Curtsinger, 2013) – albeit with considerably shorter life spans.

Although the life spans of most insect species are undoubtedly short, the life spans (and presumably mortality trajectories) of some insects can be considerably longer. In eusocial insects (e.g., many Hymenoptera), although the worker castes have fairly short life spans, the adult queens, although genetically identical, enjoy life spans that are 100 times greater than those of solitary species (Keller and Genoud, 1997). This stark difference has been attributed as an evolutionary consequence of the extremely low mortality of the queens which reside in heavily defended nests and are usually virtually immune to predation (Keller and Genoud, 1997) – a hypothesis supported by the observation that species with larger colonies tend to have a larger life span differential between worker and queens than smaller ones (Kramer and Schaible, 2013).

The Plant Kingdom

The plant kingdom also shows great diversity in its demographic trajectories. This is reflected in the huge variation in maximum recorded life spans from short-lived annual species like corn, wheat, and rice, to incredibly long-lived tree species like the bristlecone pine (*Pinus longaeva*) with maximum recorded life spans of 4600 years, and the almost unbelievable life spans of clonal species like King's lomatia (*Lomatia tasmanica*) (>43 000 years) (Thomas, 2012).

The most important demographic factor that would help a plant attain a long life span is a low and flat, or even declining

mortality trajectory. Demographic trajectories can be derived from matrix population models (Caswell, 2001). The first major comparative study on plant senescence, by Silvertown *et al.* (2001), used this approach and showed that although many plants senesce, others have the declining rates of senescence necessary for extreme life spans. Additional work using a similar approach, but on a larger sample of species has confirmed that mortality rates in plants can increase, decrease, or remain approximately constant over the life course of a plant, but that the majority of Angiosperm species have declining mortality over most of their life course (Baudisch *et al.*, 2013; Jones *et al.*, 2014). Using individual-based data, other workers have shown both negative senescence (improvement with age) (Garcia *et al.*, 2011) and senescence (Roach, 1993). This great diversity of plant demographic trajectories likely stems from the great variety of architectures employed across the plant kingdom (Munné-Bosch, 2007; Thomas, 2012; Noodén, 2013).

All vascular plants have a modular architecture – they develop by the repetitive construction of similar units – and can replace and renew damaged modules. In addition, the totipotency of plant cells allows great flexibility in resource allocation in the face of changing environmental conditions. During times of hardship, many plant species can therefore allow tissue to die back (i.e., shrinkage – Salguero-Gomez and Casper, 2010), or undergo vegetative dormancy (Garcia *et al.*, 2011), and can then continue to grow when conditions improve. Finally, all plants grow indeterminately, and their increasing size is a key factor in reducing vulnerability to the ravages of the weather, and to herbivores. Thus, although the history of aging research on plants is shorter than that on animals, great strides have been made in understanding the driving factors, and there is much that animal-focused researchers can learn from it.

Conclusions

It is interesting to consider that the mechanisms of senescence escape noted above for plants are not restricted to the plant kingdom. There are representatives in the animal kingdom that exhibit clonal reproduction, modular architecture (e.g., corals), dormancy, and varying degrees of tissue regeneration. Other evolutionary innovations such as sociality, flight, and fossoriality are also likely to have strong influences on aging patterns but have, with some notable exceptions (e.g., Healy *et al.*, 2014; Buffenstein, 2007; Holmes and Austad, 1994), been somewhat neglected. The underlying framework that can explain these observations is life history theory, which in part studies the compromises made in resource allocation among processes of growth, reproduction, and maintenance, which vary among species and environment (Baudisch and Vaupel, 2012; Stearns, 1992). It is clear that across the tree of life, evolution has generated a dazzling array of life history strategies giving rise to a diversity of demographic trajectories, and resulting in life spans that range from days to millennia. Several taxonomic groups remain demographically unexplored and it is certain that aging research will benefit from widening its taxonomic scope.

See also: Aging: Why Do We Age?. Life History Trade-offs

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Life History: Pike

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Introduction

The Goal of This Section

A key step toward understanding life history evolution is to find out how survival and reproduction vary with age and size (Stearns, 2000), as these patterns create different selection pressures throughout the life cycle. As in many fish species, the vital rates of pike (*Esox lucius*) are largely size-dependent, but other state variables such as age, as well as external factors like temperature and resource availability, may also be important factors shaping the population dynamics and life history. To understand the joint effect of different vital rates in the life cycle on fitness (long-term population growth rate) and other life history quantities, we need a population model including the effects of individual state variables as well as external drivers. Integral projection models (IPM) fulfill these requirements, allowing both continuous and discrete state variables (Easterling *et al.*, 2000; Ellner and Rees, 2006; Coulson, 2012). Having discrete time steps, IPMs are especially suited to studies with empirical data from natural populations that are often gathered at discrete time intervals. Because continuous state variables like body size are often quantitative traits, IPMs represent a promising framework to study eco evolutionary dynamics, i.e., changes in both ecological and evolutionary quantities on a contemporary scale (Smallegange and Coulson, 2013; Vindenes and Langangen, 2015). This section presents an example of an age- and length-structured IPM for pike (*Esox lucius*) from Windermere, UK, demonstrating (1) how to estimate and derive the main vital rate functions defining the model, and (2) how to derive key life history quantities from analysis of the model (fitness, net reproductive rate, generation time).

Some Aspects of Pike Ecology and Life History

Pike is a top predator occurring in freshwaters around the Northern hemisphere and the most widespread species of the esocid taxa (Diana, 1996; Craig, 2008). They live under a wide range of climatic and ecological conditions, but generally prefer shallow waters and depend on vegetation for spawning and throughout early life (Bry, 1996; Craig, 2008). Pike hunt by visual cues and stalk their prey, remaining still for long periods of time. This is largely reflected in their morphology, with an elongated body shape and cryptic coloring providing camouflage in the vegetation. Pike are also highly plastic in choice of prey type, prey size, and prey behavior, and can easily adapt to new prey species (Craig, 2008). This opportunistic behavior, and the fact that pike is a cannibalistic species, may explain the rather low variation in recruitment (i.e., the number of offspring produced) found in pike compared to many other fish species (Craig, 1996b).

As for many species with indeterminate growth, body size is an important predictor of vital rates like survival, somatic growth rate, and fecundity in pike (Craig, 1996a). The vital rates are also influenced by other extrinsic and intrinsic factors, such as temperature and water levels (Craig, 1996b). Temperature is one of the most important environmental factors affecting several physiological and behavioral processes and thereby the vital rates (Casselman, 1996; Diana, 1996; Winfield *et al.*, 2008).

Depending on location, pike can mature at an early age and continue to grow after maturation. Maturation is largely determined by body size, and differences in age at maturity mainly arise because of differences in growth rate (Frost and Kipling, 1967; Billard, 1996). Females produce a large number of eggs, which increases with body size (Frost and Kipling, 1967; Vindenes *et al.*, 2014). Spawning occurs in spring, providing the offspring a long season to grow before next winter. The egg development in the female occurs throughout the autumn and winter preceding spawning (Frost and Kipling, 1967; Billard, 1996).

Pike survival depends on body size via different mechanisms, in particular predation (Craig, 1996b). Small individuals experience much higher risk of predation (also from cannibalism) than larger ones, and very few survive to maturity. In the early life stages individuals also undergo important processes of physiological development and are more vulnerable to environmental stresses such as food limitation or extreme temperatures (Kamler, 1992). One such critical period for fish is the phase where the larvae has absorbed the egg yolk and starts external feeding (Kamler, 1992). Large fish also have a higher capacity to store energy, allowing them to survive longer periods of low food availability (even though their overall energy requirements are higher). However, large fish are also more susceptible to fishing mortality (in areas where fishing occurs), which might represent an opposing selection pressure to the natural selection (Carlson *et al.*, 2007; Edeline *et al.*, 2007).

Pike can potentially grow to a large size and are relatively long-lived. The somatic growth rate is size- and age-dependent, and generally declines with size (individuals grow toward an asymptotic length over life). There is a high selection pressure for rapid early growth, as that increases the chances of survival during the first critical period in life (reducing the time spent in the size range of high mortality). As a top predator pike can play an important role in ecological communities (Craig, 2008). Changes in a pike population, for instance due to climate warming, can potentially cascade down to affect other species in the ecosystem (Estes *et al.*, 2011).

Methods

Study System and Data

The model presented here is based on data collected from the glacial lake of Windermere, UK (54°22′ N, 2°56′ W; altitude 39 m). Data on pike and other fish species as well as biotic and

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abiotic variables have been collected since the 1940s (see review of the history of the data collection by Le Cren, 2001). The annual mean air temperature of the study area is predicted to increase over the next century (Jenkins *et al.*, 2009), and the observed surface temperature of Windermere (**Figure 1**) has shown a marked increase over the recent decades (Winfield *et al.*, 2008).

The four data sets used in this model are available online (see URL for each data set in the references), and include information on (1) fecundity (Winfield *et al.*, 2013a), (2) somatic growth (Winfield *et al.*, 2013b), (3) survival (Winfield *et al.*, 2013c), and (4) the annual mean surface temperature (Winfield and Fletcher, 2013). These data sets were used by Vindenes *et al.* (2014) to construct and parameterize a length-based IPM to investigate population dynamical consequences of climate warming. The model presented here is largely building upon this model, but also includes age as a state variable affecting somatic growth and reproduction. In addition, a reaction norm for size at maturity is also included, instead of having a fixed size at maturation.

The pike data for growth and fecundity were collected in a winter sampling period (October–February) where pike were captured using 64 mm mesh gillnets. The survival data are derived from a separate capture mark recapture (CMR) study, where sampling was also done in spring. In the winter sampling captured pike were measured for body length (in centimeters, measured as fork length), weighed (in kilograms), and sexed, and opercular bones were removed for age and length back-calculation, following a method validated for Windermere pike by Frost and Kipling (1959). The growth data set (Winfield *et al.*, 2013b) contains measured and back-calculated lengths from 1944 to 1995 (7939 females). Since 1963, data on female reproductive investment (gonad weight, egg number, and egg weight) have also been collected (Frost and Kipling, 1967). The fecundity data set (Winfield *et al.*, 2013a) contains fecundity data (egg number) from the period 1963–2002 (3634 females).

The survival data set (Winfield *et al.*, 2013c) contains survival data from 1953 to 1990, for both males and females (3992 individuals), based on the CMR study (for details on this sampling see Kipling and Le Cren, 1984; Haugen *et al.*, 2007). There is no age information in these data, so survival probability is assumed to depend on length only. Analysis of this data set is complicated by the fact that for individuals who were never recaptured, the time of death and size at death are unknown (but Windermere is a closed system so individuals do not leave, see Carlson *et al.*, 2007). To improve the survival estimates Vindenes *et al.* (2014) used a Monte Carlo resampling method to correct for this bias, using the somatic growth model to predict size at death for the unobserved individuals. The survival function used in this model is the same as in that study.

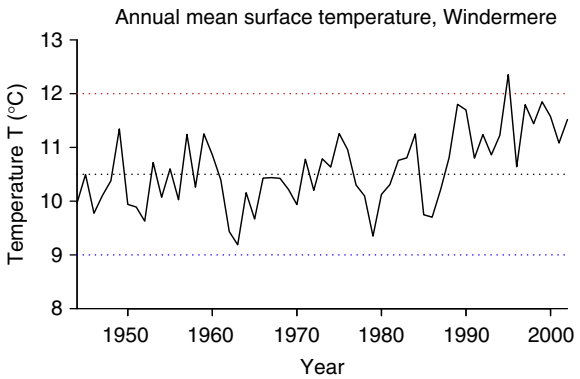

Figure 1 Observed annual mean surface temperatures in Windermere, 1944–2002. The dashed lines represent the three values of temperature used in other plots of this study (red corresponding to a warm conditions, 12 °C; blue corresponding to cold conditions, 9 °C; and black corresponding to 10.5 °C, close to the observed average over the time period.

Estimation of Vital Rates

Estimation of the vital rate functions was done using the software R (R Development Core Team, 2013), with the package 'nlme' (Pinheiro *et al.*, 2013). Mixed effects modeling is only one of many methods available, and the preferred method may depend on the vital rate function, the data, and the general questions of the study (Rees *et al.*, 2014). Vindenes *et al.* (2014) included year as a random effect in order to estimate environmental variance and covariance among the vital rates. This article focuses on the deterministic dynamics (dynamics of the expected population size), but given that the annual variance and covariance of vital rates are already estimated in the regressions, the model can easily be extended to also consider stochastic dynamics (Vindenes *et al.*, 2014).

Over the long study period changes have occurred in the abiotic and biotic environment, the level of fishing pressure, and also the sampling techniques (Paxton and Winfield, 2000; Le Cren, 2001). Such changes are reflected in the data as some of the vital rates show a trend (sometimes non-linear) over time, also when other effects are accounted for. To account for (and measure) the temporal trends due factors that are not of direct interest to the study, year effects were included in the regression models (Vindenes *et al.*, 2014).

There are often several candidate models for each of the vital rates. When the goal is to understand the effect of a particular variable (e.g., temperature), this variable was always included in the model (if it has small or insignificant effects the resulting effect on the model outputs will also be small). Interactions were only considered if they were expected for biological reasons. For instance, an interaction between temperature and body length on the vital rates is expected, because large and small individuals typically respond differently to temperature (Pörtner and Farrell, 2008). Model selection was based on the Akaike information criterion (AIC) (Zuur *et al.*, 2009). Here, the fixed effects structure was evaluated by fitting different candidate models with a procedure maximizing the log-likelihood (not the restricted log-likelihood). The final model was then refitted to maximize the restricted log-likelihood (Zuur *et al.*, 2009).

After the regression analysis is conducted, even though the estimated vital rate models may show good statistical fits, this does not necessarily mean they are biologically reasonable across the entire state space of the IPM (Merow *et al.*, 2014). For instance, it is common that data are scarce for parts of the state space (e.g., for the largest or smallest individuals).

Depending on the statistical regression model used, the data-heavy parts of the state space may have a large influence on the regression fit in other parts of the state space.

Even though back-calculated lengths are available for early ages in the Windermere pike, these are based on the subsample of individuals that have survived long enough to be captured. Pike are not susceptible to the sampling until they are large enough to be captured by the gillnets (usually around 3 years of age), so that for age 1 (and largely also age 2) only the back-calculated length estimates are available. The description of the growth model below includes an explanation of how this may lead to a bias in the estimated growth rate of small individuals, and how this can be adjusted in the final IPM. It is important to use knowledge of the species and system in question to evaluate whether the estimated vital rate functions are biologically reasonable, and perhaps make some adjustments before using them to construct the projection kernel (Merow *et al.*, 2014).

Model Description

Pike life cycle of the model

Figure 2 shows an overview of the life cycle for this model, with six age classes and length structure within each age class. The model is female-based and density-independent, assuming a pre-reproductive census so that offspring are counted as 1-year olds (offspring number includes offspring survival over the first year). The individual state variables are current length x (cm, $0<x<\infty$) and age class j ($j=1,\ldots,6$, where the last age class represents ages $\geq$ 6 years). The environmental state variable is the annual mean surface temperature T (for simplicity referred to as 'temperature'). For a year with temperature T, an individual of age j and length x survives to next year with a probability $s(x,T)$ (independent of age). If the individual survives, it grows to reach a new length y next year, according to a probability density function $g_j(y;x,T)$. It is assumed that no growth or mortality occurs over the winter before spawning. The individual can also produce a number of female offspring $b(x,T)$ that enter the population next year. The offspring length distribution $f(y,T)$ describes the initial lengths of offspring at age 1.

Vital rate functions

In this subsection, each of the four main vital rate functions that determine the IPM is presented in more detail including underlying functions. An overview of the model parameters and variables is given in Table 1, while values of the estimated effects and their standard errors are given in Table 2.

Offspring number

Offspring number is defined as the number of 1-year-old female offspring produced by a female of length x in age class j, at the temperature T, and is given by

$$b_j(x,T) = 0.5 p_j(x) m(x,T) s_O$$

where $p_j(x,T)$ is the probability of maturity given age j and size x, $m(x,T)$ is the fecundity (number of eggs produced) given size x, and $s_O(T)$ is the survival probability from egg to 1-year old. The factor 0.5 enters because only females are counted (assuming equal sex ratio among fertilized eggs). The function $m(x,T)$ describing egg numbers was estimated by Vindenes *et al.* (2014), and is given by

$$\sqrt{m(x,T)} = \beta_{m0} + \beta_{mx}x + \beta_{mT}T + \beta_{mxT}Tx$$

Offspring survival s_O (from egg to age 1) cannot be directly estimated from data, because the pike are not susceptible to the gillnet sampling until about 3 years of age. However, based on a model for the age-specific population densities estimated by Langangen *et al.* (2011), and using the estimated vital rate models for survival, fecundity, and growth, it is possible to obtain a prediction of the annual offspring survival probability over the study period (53 years, not shown). The mean offspring survival based on these predicted values is 0.00028, in line with estimates from other studies (on the order of 10^{-4}; Kipling and Frost, 1970; Wright, 1990; Craig and Kipling, 1983). Here, it is assumed that offspring survival is independent of maternal size or age, but a positive effect of temperature is included, as indicated by this analysis and other studies (Craig and Kipling, 1983; Paxton *et al.*, 2009), and which is also consistent with the estimated survival function with a positive effect of temperature for small pike (Vindenes *et al.*, 2014). Offspring survival is modeled on the logit scale, as

$$\text{logit}\, s_O(T) = \beta_{se0} + \beta_{seT}T$$

The probability of maturity, $p_j(x,T)$, could not be estimated from the winter sampling data because few pike are caught until they reach a length of $\sim$55 cm, at around 3 years of age (Frost and Kipling, 1967). Instead this function was determined to fit the results reported by Frost and Kipling (1967), based on data that also included smaller pike. According to this study most female pike in Windermere become mature at age 2, at a mean length of $\sim$41.5 cm (ranging from 31 cm to 49.8 cm). The study also noted that maturity is determined by size rather than age, and that differences in age at maturity largely arise from differences in somatic growth rate. Here, the probability of maturity, $p_j(x)$, is modeled on the logit scale,

$$\text{logit}\, p_j(x,T) = \begin{cases} \beta_{p0} + \beta_{px}x, & j \geq j_{\min} \\ -\infty, & j<2 \end{cases}$$

so that the minimum age of maturity is 2. The values of β_{po} and β_{px} are chosen to fit the results of Frost and Kipling (1967).

Figures 3(a)–3(d) show all the underlying functions determining fecundity, as well as the resulting total fecundity $b_j(x,T)$ (number of 1-year-old female offspring produced). The shape of the total fecundity is determined largely by the egg number function $m(x,T)$. Note that even though many females are mature at a length around 40 cm, they produce much fewer eggs compared to larger females.

Offspring length distribution

Vindenes *et al.* (2014) estimated the offspring length distribution $f(y;T)$ from the back-calculated data, and used a log-normal distribution where the mean $\mu_{L1}(T)$ depends on temperature but where the variance σ^2_{L1} is constant. The mean (on absolute scale) is given by

$$\mu_{L1}(T) = \beta_{L10} + \beta_{L1T}T$$

Figure 3(e) shows a histogram of the data together with the distribution $f(y;T)$ at different temperatures.

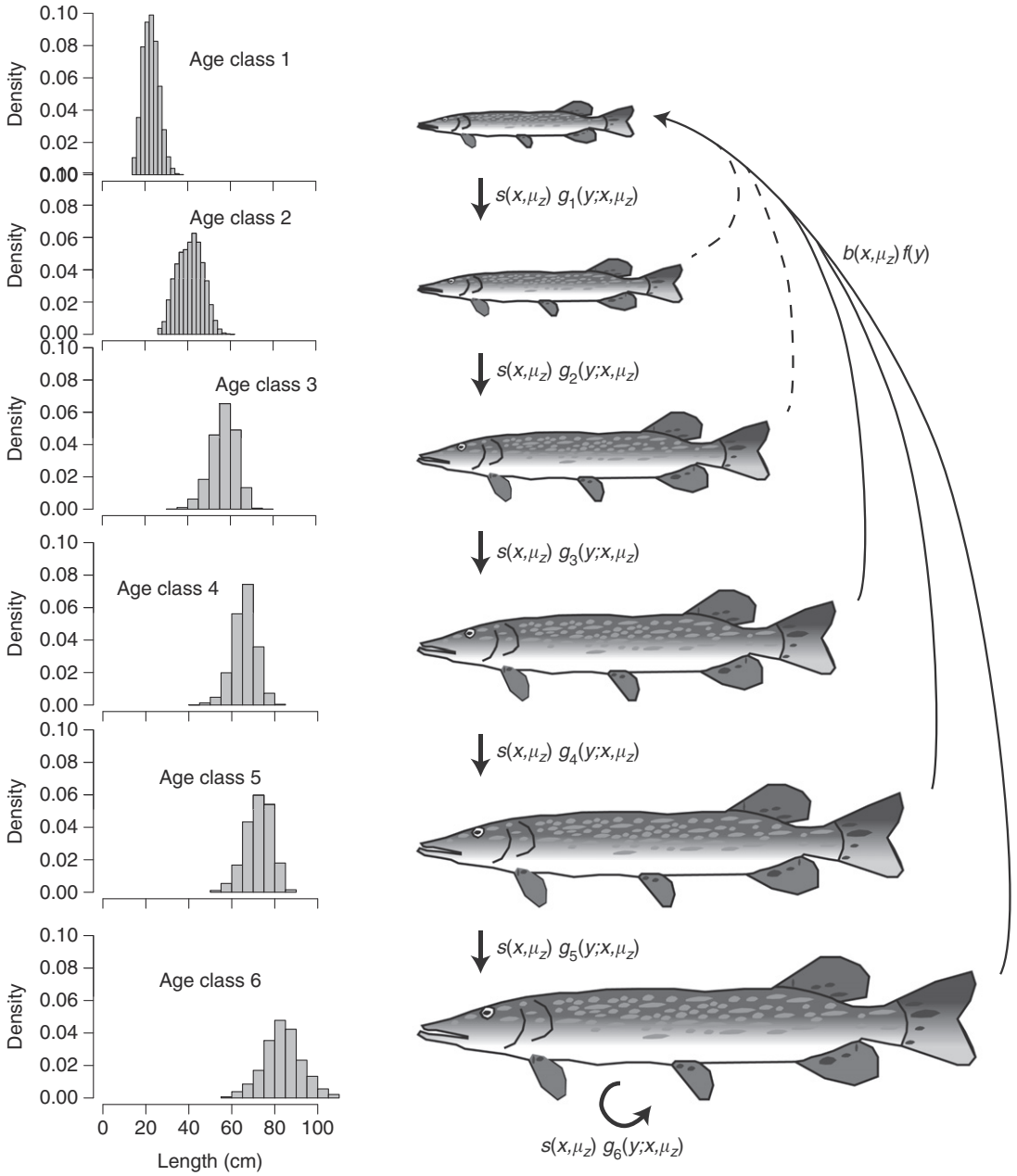

Figure 2 An illustration of the pike life cycle used in the model, with the observed size distributions at different ages. Age class 6 contains all individuals of age 6 and older. The model assumes a pre-reproductive census, and is defined by the vital rates describing survival probability $s(x,T)$, somatic growth $g_j(y;x,T)$, reproduction $bj(x,T)$ (number of age 1 offspring), and the offspring length distribution $f(y;T)$.

For this analysis, it is assumed that there is no correlation between parental and offspring size at age, because data on maternal identity are not available. Such a correlation could arise from genetic inheritance or from maternal effects. It could have been included in the model by letting the offspring size distribution depend on parental size x and age j.

Survival probability

The survival probability was estimated as a function of length and temperature by Vindenes *et al.* (2014). The survival data do not include age, but there are also good reasons to assume that survival is primarily determined by size rather than by age (as many mortality processes are closely related to size, e.g., predation risk). There could be some age effects as well, for instance through senescence at old ages. For population growth, however, senescence is probably not so important (at least in this particular case) because the older and larger individuals constitute a very small proportion of the population. Because data on the largest individuals are scarce, and because the capture probability may be lower for these, the estimated survival probability is uncertain for large lengths. Vindenes *et al.* (2014) therefore imposed a constant survival after the maximum is reached, although there is a possibility that it is declining with size for the largest individuals. This function is

Table 1 Overview of main variables and parameters in the IPM

Variable/parameter	*Signification*
x	Current body length (cm)
y	Next year's body length (cm)
j	Age index ($j = 1, ..., 6$, final class contains all ages >5 years)
T	Annual mean surface temperature (°C)
$s(x,T)$	Survival probability
$b_j(x,T)$	Offspring number (number of 1-year-old female offspring)
$g_j(y,x,T)$	Distribution of next year's length (growth)
$f(y,T)$	Offspring length distribution
$\mathbf{K}(T)$	Projection kernel at temperature T
$K_{ij}(x,y)$	Contribution from age j and length x to age i and length y (for $j\leq5$, $i=j+1$ and for $j>5$, $i=j$).
$\mathbf{S}(T)$	Survival/growth kernel at temperature T
$\mathbf{B}(T)$	Reproduction kernel at temperature T
$\mathbf{R}(T)$	Net reproduction kernel at temperature T
$n_j(x,T)$	Joint density distribution of age and length
$\lambda(T)$	Long-term population growth rate/expected fitness
$u_j(x,T)$	Joint stable distribution of age and length
$v_j(x,T)$	Reproductive value of individual with age j and length x
$R_0(T)$	Net reproductive rate at temperature T
T_0	Generation time defined as $\frac{\ln R_0}{\ln\lambda}$
T_M	Generation time defined as $\frac{\lambda}{\sqrt{Bu}}$

shown in Figure 3(f). The survival probability function is given by

$$\text{logit } s(x,T) = \beta_{0s} + \beta_{xs}x + \beta_{Ts}T + \beta_{Txs}Tx$$

restricted to its maximum for x above this size.

Somatic growth rate

Vindenes *et al.* (2014) estimated the somatic growth rate as a function of length and temperature, however the data indicate that there are clear age patterns as well. Because of a high correlation between age and body size in the data (0.91), including both in the same model would likely lead to problems of multicolinearity. Instead, a separate growth model is estimated for each age class. The cost is a reduced sample size, in particular for the older ages where there are fewer data points. Because of this, and because there is no evidence of large changes with age in the older age classes, all individuals of age 6 and above are lumped together in the final age class. The variance in growth rate was approximately constant within each age class. Thus, there are six age classes, and the final growth models are given by

$$\mu_{G1}(x,T) = \beta_{0G1} + \beta_{xG1}x + \beta_{x2G1}x^2 + \beta_{Tg1}T + \beta_{xTg1}xT,$$
$$\mu_{G2}(x,T) = \beta_{0g2} + \beta_{xg2}x + \beta_{x2g2}x^2 + \beta_{Tg2}T,$$
$$\mu_{Gj}(x,T) = \beta_{0gj} + \beta_{xgj}x + \beta_{Tgj}T (j>2)$$

Thus, in the first two age classes there is a second order effect of length, while in the older age classes this effect is not present. The variable $\mu_{Gj}(x,T)$ defines the mean of the growth distribution $g_j(y; x,T)$ from age class j to $j+1$. This is defined as a truncated normal distribution, truncated at x as individuals are not allowed (or expected) to shrink in length. The variance in growth σ^2_{Gj} is assumed to be constant within each age class.

A source of bias in the growth model is that the back-calculated length data represent a conditional sample of the underlying population, conditional on survival until capture (due to size-dependent gill netting most individuals avoid sampling until they are 3 years old). Because survival increases with size, this means that the growth model tends to overestimate size at age compared to the underlying population, as well as underestimate the variance in growth. Using the IPM (where survival is included) we can compare the mean size at age (at stable distribution) with the values from the growth model (where survival is not included), to get some idea of the magnitude of the bias (about 4 cm at the maximum bias in age 1). Here, the value is adjusted for this bias by reducing the growth rate at each age (more in the earlier ages) so that the mean size at age in the model matches the mean size at age in the data. Values reported in Table 2 are the adjusted values.

Figure 3(g) shows the mean length next year, $\mu_{ga}(x,T)$, as a function of current age class a and length x, for three different temperatures. In age 1 the largest individuals grow faster relative to their size than in older classes. This pattern can be explained by underlying heterogeneity among individuals in the growth rates (Vindenes and Langangen, 2015), which is not included here. Individuals who are relatively large when young tend to remain larger for a long time, but may also experience a slightly higher mortality (Vindenes and Langangen, 2015). Temperature has a positive effect on growth in all age classes, but the effect is strongest in ages 1 and 2. Figure 3(h) shows the resulting length at age from the unadjusted growth model, for three different temperatures.

In the growth model of Vindenes *et al.* (2014) that did not include age, there was an interaction with temperature and length so that temperature had a negative effect on growth for the largest individuals. This is not the case in the current

Table 2 Values of underlying parameters in the vital rate functions. The survival and fecundity models were calculated by Vindenes *et al.* (2014). Values given in parentheses are standard errors (where applicable)

Survival	
β_{s0}	−13.533 (3.570)
β_{sx}	0.510 (0.038)
β_{sx^2}	−0.004 (0.000)
β_{sT}	−193 (0.175)
β_{sxT}	−0.007 (0.003)
Offspring number (*fecundity, probability of maturity, offspring survival*)	
β_{m0}	−79.67 (395.439)
β_{mx}	5.659 (1.096)
β_{mT}	−15.064 (7.239)
β_{mxT}	0.247 (0.102)
β_{p0}	−16
β_{px}	0.4
β_{se0}	−8.3
β_{seT}	0.01
Somatic growth (*mean and variance of next year's length*)	
β_{G10}	2.051 (16.758)
β_{G20}	−4.802 (18.777
β_{G30}	13.878 (14.977)
β_{G40}	8.725 (16.331)
β_{G50}	3.426 (14.172)
β_{G60}	7.065 (9.099)
β_{G1x}	1.711 (0.164)
β_{G2x}	1.561 (0.055)
β_{G3x}	0.840 (0.005)
β_{G4x}	0.919 (0.007)
β_{G5x}	0.966 (0.008)
β_{G6x}	0.908 (0.005)
β_{G1T}	−0.559 (0.395)
β_{G2T}	0.975 (0.227)
β_{G3T}	0.363 (0.170)
β_{G4T}	0.294 (0.182)
β_{G5T}	0.384 (0.156)
β_{G6T}	0.358 (0.099)
β_{G1x^2}	−0.01929 (0.002)
β_{G2x^2}	−0.00873 (0.001)
β_{G1xT}	0.05243 (0.015)
σ^2_{G1}	3.32
σ^2_{G2}	3.28
σ^2_{G3}	2.70
σ^2_{G4}	2.55
σ^2_{G5}	2.07
σ^2_{G6}	1.55
Offspring length distribution	
β^2_{L10}	14.89 (6.43)
σ^2_{L1}	13.82

model, as temperature was found to have a positive effect on growth for all sizes and ages, although the effects are very small for older ages (**Figure 3**). The model is still largely in agreement with that of Vindenes *et al.* (2014), where the temperature effects were also very small for the largest individuals.

Projection Kernel and Population Dynamics

While the vital rate functions alone contain much information about the life history, understanding their combined effect on fitness and other life history variables requires a population model, like an IPM. A key element of an IPM is the projection kernel (analogue to projection matrix in matrix models), which is a function of all the vital rates. The projection kernel describes how the population density distribution (in this case, the age/size distribution) changes from one time step to the next.

Letting $n_j(x)$ represent the number (density) of individuals in age class j within a small length interval $[x, x+\mathrm{d}x]$, the size of age class j is given by $N_j = \int_0^\infty n_j(x)\mathrm{d}x$, and the total population size is given by $N = \sum_{i=1}^{6} \int_0^\infty n_j(x)\mathrm{d}x$ (Ellner and Rees, 2006). The population growth from year t to $t+1$ is described by the projection kernel $K_{ij}(x,y)$, giving the contribution from individuals in age j with length in the interval $[x, x+dx]$ to age class i and length interval $[y, y+dy]$ (unless $i=j+1$ this contribution is zero). Transitions between ages is a purely deterministic process, as individuals are always one year older the next ear. Growth transitions, on the other hand, are characterized by the variation around the mean growth (note that the model is still deterministic, this variation just describes the spread of individuals across length within each age class). For this model, the projection function is given by

$$\mathrm{K}_{ij,t}(x,\,y) = \begin{cases} s(x,T)g_j(y;x,T), & i=j+1 \text{ and } 1\le j\le 5, \text{ or } i=j=6 \\ b_j(x,T)f(y;T), & i=1, \\ 0, & \text{otherwise} \end{cases} \quad [1]$$

Figure 4 shows the projection kernel calculated at the mean temperature $T=10.5\,^{\circ}\mathrm{C}$. Notice that the projection kernel shows values for the growth and reproduction of individuals for the entire size range within each class (due to extrapolation of the vital rate models), however no individual will actually reach the larger sizes in the young classes.

Net Reproductive Rate and Generation Time

The net reproductive rate R_0 describes the generation-to-generation population growth rate, and if offspring are all born into the same state it also describes the expected lifetime reproduction of an individual (Caswell, 2001). In this model, offspring have different sizes, so the expected lifetime reproduction of an individual is conditional on its size at age 1. The generation-to-generation population growth is described by a matrix (kernel) $\mathbf{R}=\mathbf{B}(\mathbf{I}-\mathbf{S})^{-1}$ (Ellner and Rees, 2006). Additional requirements need to be fulfilled for the existence of a unique dominant eigenvalue R_0, the net reproductive rate for the population (Section 5.3.4 in Caswell, 2001).

Generation time is another important life history characteristic, intuitively defined as the time between subsequent generations. However, when the generations are overlapping different generation time measures exist. A commonly used measure is the time it takes for the population to increase by a factor R_0, $T_0 = \frac{\ln R_0}{\ln \lambda}$ (Caswell, 2001). Another measure is the cohort generation time T_c, that is the mean age of parents of offspring produced by a cohort over its lifetime (for a definition for IPMs, see Ellner and Rees, 2006). A third measure is the mean age of

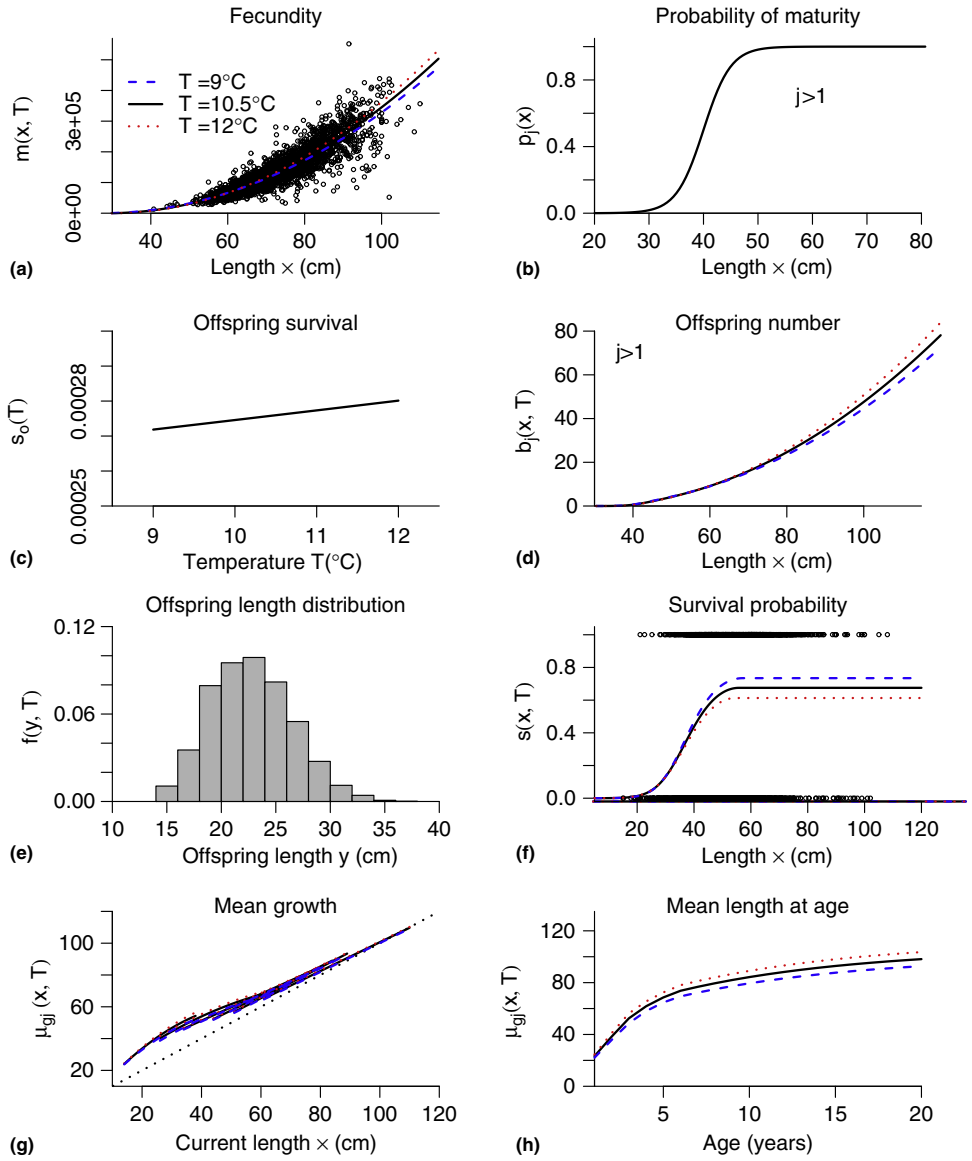

Figure 3 The vital rate functions defining the integral projection model (IPM). (a) Egg number $m(x,T)$ as a function of female length x, shown for three values of the temperature T. (b) Probability of maturity $p_j(x)$ as a function of female length x, for age $j>1$. (c) Offspring survival probability $s_O(T)$ as a function of temperature T. (d) Offspring number, $b_j(x,T)=0.5p_j(x)s_O(T)m(x,T)$ (number of 1-year-old female offspring produced) as a function of female length x, shown for three different temperatures. (e) Length distribution of offspring (at age 1), shown for three different temperatures. (f) Survival probability as a function of female length x, shown for three different temperatures. (g) Mean length next year $\mu_{gj}(x,T)$ as a function of current length x, shown for three different temperatures. The six clusters of lines correspond to the age classes, where the leftmost represents growth from age 1–2, the second leftmost from age 2–3, etc. (h) Mean length at age predicted from the growth model, shown for three different temperatures.

parents in a population at the stable age/size distribution, T_M (Caswell, 2001). Using a Markov chain definition of the population dynamics in a general stage structured population, Bienvenu and Legendre (2015) demonstrated that this value can be calculated as $T_M = \frac{\lambda}{\mathbf{vBu}}$ (adapting their formula to the scaling of the eigenvectors used here, $\mathbf{vu}=1$).

Discretization of the Model for Numerical Calculations

IPMs are typically discretized to a (large) matrix model before model calculations are done, so that the continuous projection kernel surface becomes a large projection matrix. The number of mesh points determines the accuracy of the model calculations, and should be chosen to obtain the desired level of accuracy. Many techniques are available for this numerical integration (e.g., using the midpoint rule; Easterling *et al.*, 2000; Ellner and Rees, 2006; Merow *et al.*, 2014; Rees *et al.*, 2014). Importantly, the discretization of an IPM is done after the vital rates are estimated, and only represents a method for numerical calculation of the model outputs (Ellner and Rees, 2006).

The discretized version of the projection kernel is the matrix $\mathbf{K}$, with 6 age classes and k size classes within each age class (each of width dx). Thus, the dimension of $\mathbf{K}$ is $6k \times 6k$.

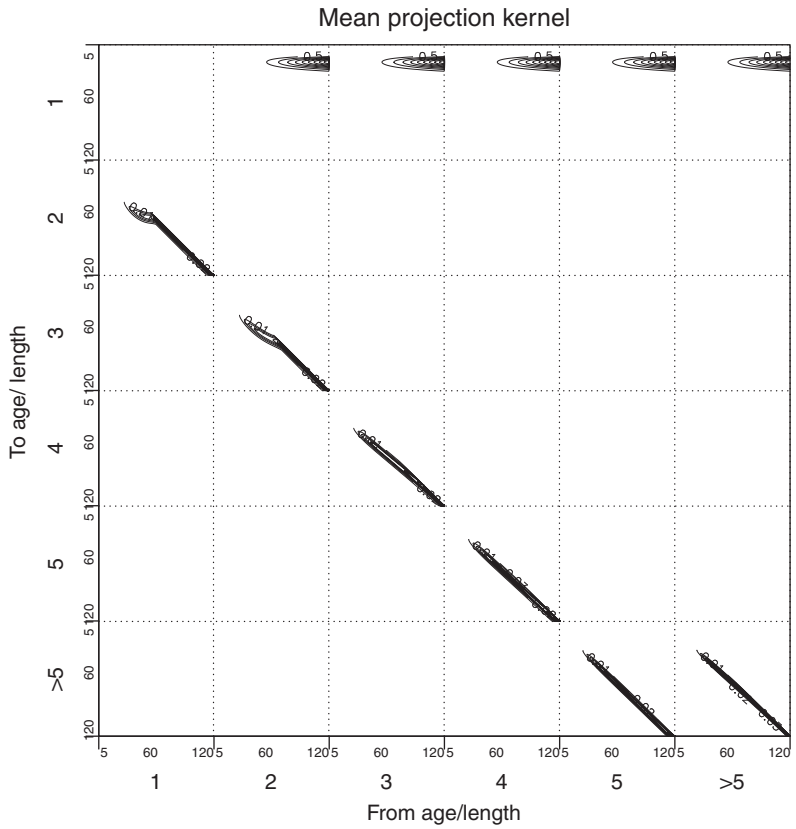

Figure 4 Mean projection kernel K for the temperature $T = 10.5\ ^{\circ}\mathrm{C}$ (note that the size values on the axes are approximate).

Results

Mean Growth Rate (fitness), Stable Structure, and Reproductive Value

An eigen analysis of the discretized matrix **K** yields three populaton parameters that describe the long-term asymptotic behavior of the population: the average population growth rate, the stable structure, and the reproductive value (Caswell, 2001). The long-term growth rate λ, which also measures the fitness of the life history in constant environments, is given by the dominant eigenvalue of **K**. The corresponding right and left eigenvectors provide the stable age/size structure $u_j(x)$ and the reproductive value function $v_j(x)$. Here, these are scaled so that

$$\sum_{j=1}^{6} \int_0^{\infty} u_j(x)\mathrm{d}x = 1$$

and

$$\sum_{j=1}^{6} \int_0^{\infty} u_j(x)v_j(x)\mathrm{d}x = 1$$

Figure 5(a) shows how λ changes with temperature in this model. As in the model of Vindenes *et al.* (2014) the effect is positive. Figure 6 shows the stable age and length distribution calculated for three different temperatures. This analysis suggests that the proportion of large and old individuals in the population will decline with warming (from age 5 and above), while the proportion of small and intermediate-sized individuals will increase, in particular for ages 2–4. The proportion of offspring (age 1) does not change much with temperature, but there are slightly fewer (and larger) individuals under warm conditions. Figure 7 shows the reproductive value as a function of age and length, calculated for three different temperatures. In all age classes and for all temperatures, the reproductive value increases with length, as is expected when neither survival nor offspring number declines with length.

Net Reproductive Rate and Generation Time

Figure 8 shows the kernel **R** for this model, calculated at the mean temperature $T = 10.5\ ^{\circ}\mathrm{C}$. It is equal to zero for all elements corresponding to sizes outside of the offspring size range, and for all elements corresponding to contributions to ages other than 1. Each value $R_{1j}(y, x)$ represents the expected lifetime number of size y offspring for an individual who has reached age j and size x.

Figure 5(a) shows how R_0 varies with temperature in this model. It is equal to λ when $\lambda = R_0 = 1$ (as it should be). As the temperature increases R_0 increases at a rate higher than λ. At warm temperatures individuals have reduced expected lifetimes, but leave a higher number of offspring on average. This is a good example that R_0 and λ are not equivalent and that R_0 is not, in general, a good measure of fitness (Caswell, 2001).

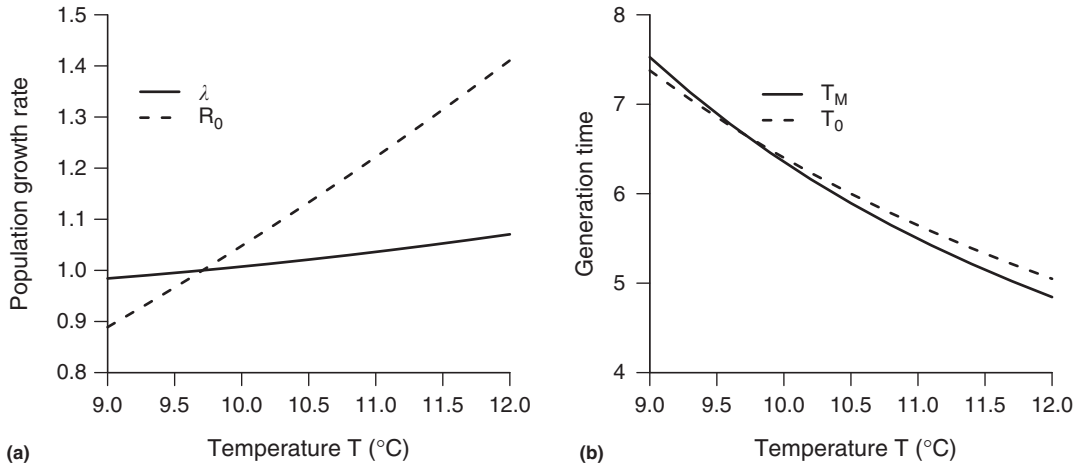

Figure 5 Some model outputs from analysis of the integral projection model (IPM) in different temperatures. (a) The expected population growth rate λ and the net reproductive rate R_0. (b) The two measures of generation time. T_m is the mean age of mothers in the stable population, whereas T_0 is the time for the population to grow by a factor R_0.

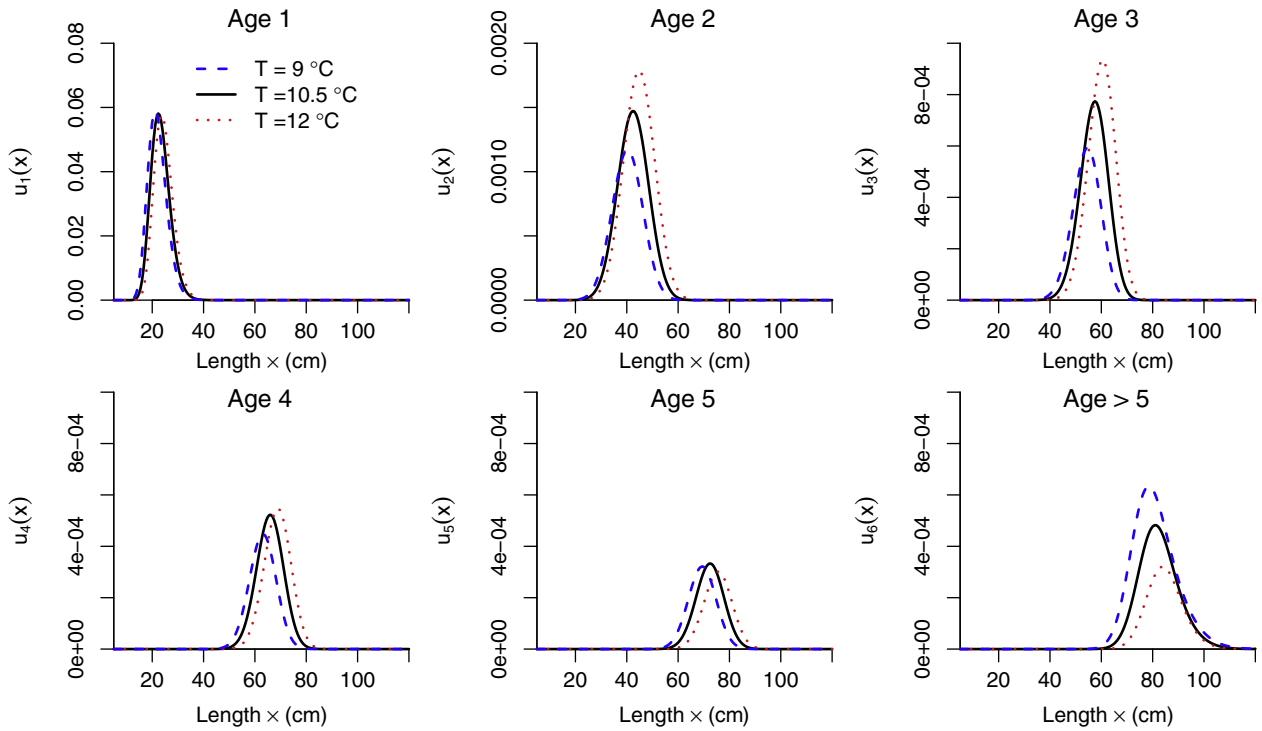

Figure 6 Stable age and size distribution calculated from the projection kernel, for three different temperatures.

The net reproductive rate R_0 is an important life history characteristic and a component to fitness, but it does not account for changes in generation time.

Figure 5(b) shows T_M and T_0 calculated for this model, as functions of temperature. The two measures are similar and both decline with temperature, but T_M declines a bit more rapidly. The figure also confirms that $T_M = T_0$ for $\lambda = R_0 = 1$, as expected.

Conclusions

This article has shown how the vital rates of female pike from Windermere depend on size, age, and temperature (Figure 3), and how these vital rates in turn affect population growth rate and other demographic outputs (net reproductive value and generation time; Figure 5). Temperature is a key environmental driver affecting many aspects of the life history of pike,

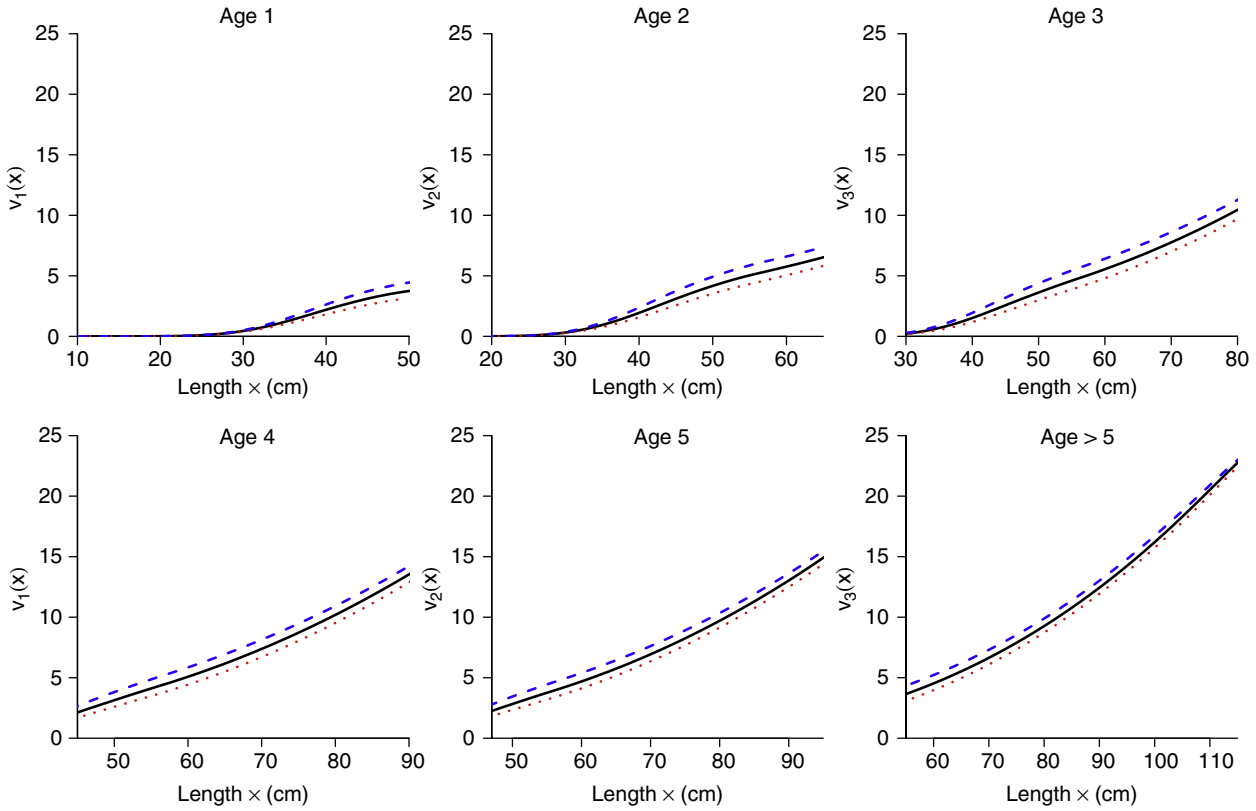

Figure 7 Reproductive value as a function of length x for each age class, calculated from the projection kernel at three different temperatures (see legend in **Figure 6**).

but is particularly important in the early life stages when individuals are small. Overall, these results indicate that warming may 'speed up' the life history of pike, as the net reproductive rate R_0 increases but the generation time decreases with temperature (**Figure 5**). Moreover, warming may shift the stable age/size distribution toward younger individuals, but larger individuals within each age class (**Figure 6**).

The underlying mechanisms of the thermal responses of vital rates are complex, and likely reflect both indirect and direct responses. Direct responses include physiological mechanisms (e.g., increasing metabolic rate) and behavioral mechanisms (e.g., increased aggression or feeding activity), while indirect mechanisms include responses mediated by effects on other species in the ecosystem (Winfield *et al.*, 2008). Some of the observed vital rate responses to temperature are also likely due to trade-offs in the life history. More research is needed to reveal the underlying mechanisms for these vital rate responses of Windermere pike.

Estimating vital rates for a demographic model can be challenging as it generally requires detailed individual-based data, preferably across the entire life cycle and over a long time period (especially for long-lived species), that captures a sufficiently large range of the environmental variability experienced by the population (Clutton-Brock and Sheldon, 2010). Unfortunately, such data are unavailable for most species. When some aspect of the life cycle is unobserved, such as the offspring survival probability in this model, available prior information from other studies can be used. Sometimes, as in this study, the missing parameter can be predicted or inferred.

Pike are relatively long-lived with a high fecundity, and the vital rates largely reflect this general life history. The high mortality of young and small pike leads to a strong selection pressure for rapid early growth. In a largely unpredictable environment where the average offspring survival is very low (only about 3 in 10000 pike survive the first year), having a long life with many reproductive events, and where many offspring (eggs) are produced in each event can be a good life history strategy (Roff, 1992). Having many reproductive years increases the probability that at least some will be favorable to the offspring, where potentially a much higher proportion survives than in the average year. This strategy is reflected in the high survival probability and fecundity of large pike.

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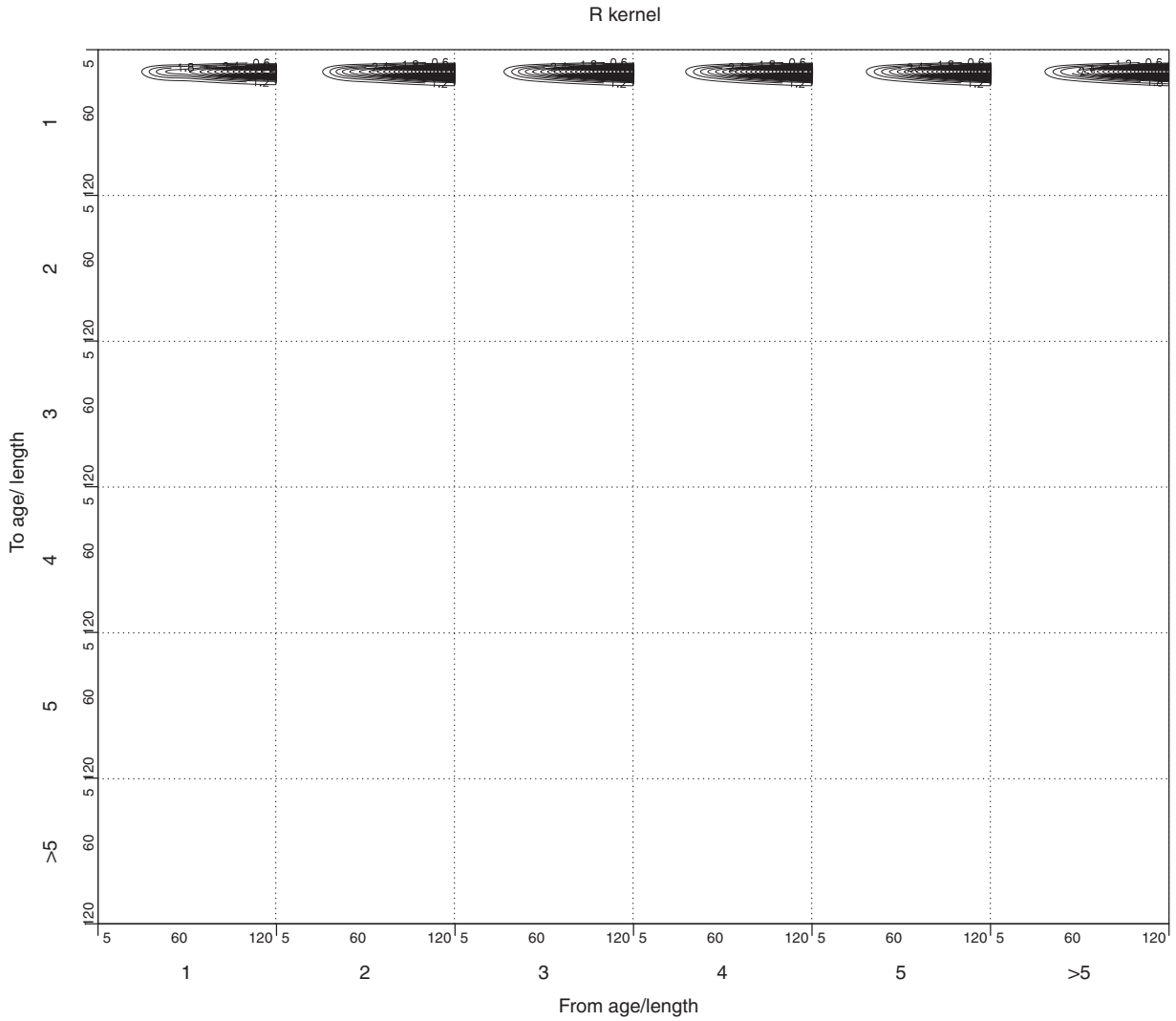

Figure 8 The generation-to-generation population projection kernel **R**, calculated at the mean temperature $T = 10.5$ °C. The dominant eigenvalue of **R** is the net reproductive rate ($R_0 = 1.107$ in this example). Note that the size values on the axes are approximate.

long-term data. This work was supported by the Research Council of Norway (grant number 224738).

See also: Life History: Age and Stage Structure

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Life History Theory: Basics

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Connection between Population Dynamics and Life History

There is a deep connection between population dynamics and life history, not surprisingly, since populations are comprised of individuals. "Population dynamics" refers to a time series of population size (and structure). At any one point in time, a population may be comprised of individuals of different ages or life stages. Individuals of different age or 'stage' may do different things; they may make different kinds of contributions to the size of future population and to the relative numbers of each age and stage in the next time period. Examples of 'stage' include: ontogenetic stage (immature vs. mature, larva vs. pupa, …), size (biomass, height, …), breeding status (nesting currently, prior mating experience, …), type of recruit (vegetative vs. sexual, …), or essentially any characteristic of an individual that can be measured at a point in time. Individuals can be represented in the future by themselves or their offspring, or both. Population dynamics thus depend upon the collective contributions of all the individuals of all ages and stages at each point in time. We model population dynamics by matrix projection methods and analyses (Caswell, 2001). The main variable of interest is the population size (and structure) at a given time and their dynamics. Time is measured from some initial point to some future point, not constrained by the lifetimes of individuals. The time line of the population intercepts individuals at different points of their lives and individuals come and go from the population.

'Life history,' in contrast, refers to the timing of 'key events' across the life cycle of individuals. Key events include death as well as milestones such as maturity, starting to reproduce, ceasing to reproduce, reproducing by different amounts, and may include changing form or location like entering and exiting dormant periods or migrating. Some events are of special interest for particular organisms. For trees in forests, for example, achieving canopy height (and thereby accessing constant high light) is a milestone. Folded into the idea of life history is that the occurrence of these key events is accompanied by changes in allocation of energy and time to different functions. Patterns of life history are diverse. Differences in timing of key activities is thought to be adaptive as it is thought to be related to expected differences in how environment varies over the life course of individuals. In this context variables of interest include the 'status' and 'activity' of the individual at a certain point in its life and the time (age) at which particular events occur. 'Status' refers to whether a particular 'event' has or has not yet occurred (or recurred) during the lifetime of the individual. Time for each individual is measured from birth. We model the process of individuals undergoing events and traversing life stages as a Markov chain process (Cochran and Ellner, 1992; Caswell, 2001). Each individual's life is a trajectory across time or a realization of the process, also called a sample path. Consideration of the trajectories of many individuals (or many sample paths) gives rise to the estimation of many different kinds of parameters.

Here how the same basic empirical data form the core quantitative basis for estimating both population dynamics and life history parameters is shown. Both realms of interest are grounded in having estimates for probabilities of 'status' change (e.g., from live to dead) and of 'stage' change (e.g., change in size) over a time step. The connection between the two realms can be established by clarifying the meaning of the time step and identifying a birth stage. To do this, matrix models have been used (both projection models and Markov chains) that classify individuals by age or stage and treat time as discrete, noting that discrete time formulations are relevant for a wide array of organisms. For organisms where birth and death occur at particular seasons, it is natural to model population and life history processes in discrete time. However, even when birth and death occur continuously over the year, census data are taken at discrete times; thus modeling population and life history processes from discrete time data are of interest for them as well.

Population Projection Structured by Discrete Age or Stage Classes

Populations are said to be structured when birth, death, and growth rates depend upon some trait(s) that characterize the individuals in a population. Commonly, populations are structured by age or stage which can be either continuous or discrete. Populations structured by discrete age classes are modeled by Leslie matrices (Leslie, 1945), whereas those structured only by discrete stage classes are modeled by Lefkovitch matrices (Lefkovitch, 1965). Some populations are structured by a combination of age and stage (Steiner *et al.*, 2014). This article focuses on populations structured by either discrete age categories or discrete stages. In either case, the matrix $\mathbf{A}$ has entries a_{ij} (j=column, i=row). Most entries provide the probability that individuals in the jth age (or stage) at time t survive and become individuals in ith age (or stage) at $t+1$. Some entries provide the number of individuals in the ith age (or stage) produced at time $t+1$ by individuals who were counted in the jth age (or stage) at time t. All matrix entries are average rates for individuals in a particular stage, they are expressed per capita per time step, i.e., survival rate, recruitment rate, etc. The matrix is used to project a population vector $\boldsymbol{n}(t)$ forward through time from any initial population vector,

$$\boldsymbol{n}(t+1) = \boldsymbol{A}\boldsymbol{n}(t) \qquad [1]$$

The vector has structure; it is the number of individuals in each age- (or stage-) class at a given time. The dimension of the matrix is the number of age- (or stage-) classes. Given certain characteristics of the matrix $\boldsymbol{A}$ (non-negative, irreducible,

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primitive), there will be convergence asymptotically to a constant per capita growth rate (per time step) λ with a stable stage distribution $\boldsymbol{u}^*$ and a vector of reproductive values $\boldsymbol{v}^*$, given, respectively, by the dominant eigenvalue of the matrix with its corresponding right and left eigenvectors (Cohen, 1979).

Both kinds of matrices can be decomposed into a sum of two matrices, one that includes only the single time-step fates of already existing individuals $\boldsymbol{Q}$ (with entries q_{ij}) and another that includes only the single time-step production of new individuals $\boldsymbol{F}$ (with entries f_{ij}),

$$\boldsymbol{A} = \boldsymbol{Q} + \boldsymbol{F} \qquad [2]$$

The column sums of $\boldsymbol{Q}$ provide the probability of survival over one time step for each age (or stage), constituting the entries p_j of the vector $\boldsymbol{p}$. The corresponding entries $m_j = 1 - p_j$ in the vector $\mathbf{m}$ provide the probability of death for each age (or stage). The matrix $\boldsymbol{T}$ (with entries $t_{ij} = q_{ij}/p_j$) provides the probabilities of transitioning from age (or stage) j to age (or stage) i during the time interval t to $t+1$, conditional on surviving the time interval. The column sums of $\boldsymbol{T}$ are all 1s for any ages (or stages) that have nonzero survival.

The difference between Leslie and Lefkovitch matrices is structural, i.e., the permissible locations for nonzero entries. In Leslie matrices the only nonzero entries are just below the diagonal in the first sub-diagonal (all survivors advance by one age class) and in the top row (newborns are all produced into the first age class) (**Figure 1(a)**). In Lefkovitch matrices, any entry can be nonzero (**Figure 1(b)**). Survivors can potentially be in any stage at the next time step (stay the same, advance or regress when they survive); newborns may occur as different sizes or types. Consequently, for a Leslie matrix $\boldsymbol{A}$, the corresponding $\boldsymbol{F}$ has zeros everywhere except the top row (**Figure 1(c)**), $\boldsymbol{Q}$ has zeros everywhere except the first sub-diagonal (**Figure 1(e)**), and $\boldsymbol{T}$ has zeros everywhere except the first sub-diagonal which are all 1s (**Figure 1(g)**). While for a Lefkovitch matrix **A**, there are no such structural restrictions for the corresponding $\boldsymbol{F}$, $\boldsymbol{Q}$, and $\boldsymbol{T}$ (**Figures 1(d), 1(f),1(h)**), some populations are structured by continuous state variables in which case an integral linear operator rather than a matrix $\boldsymbol{A}$ is used to project the population forward in time (Easterling *et al.*, 2000; Ellner and Rees, 2006). There are analagous quantities to λ_{dom}, $\boldsymbol{u}^*$, $\boldsymbol{v}^*$, $\boldsymbol{F}$, $\boldsymbol{Q}$, $\boldsymbol{T}$, $\boldsymbol{p}$, and $\boldsymbol{m}$ in that context.

Leslie matrix ***A***

Age class at time t+1	Age class at time t: 1	2	3	4
1	0	0	f3	f4
2	p1	0	0	0
3	0	p2	0	0
4	0	0	p3	0

(a)

Lefkovitch matrix ***A***

Stage at time t+1	Stage at time t: 1	2	3	4
1	a11	a12	f3	f4
2	a21	a22	a23	a24
3	a31	a32	a33	a34
4	a41	a42	a43	a44

(b)

Leslie ***F***

Age class at time t+1	Age class at time t: 1	2	3	4
1	0	0	f3	f4
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0

(c)

Lefkovitch ***F***

Stage at time t+1	Stage at time t: 1	2	3	4
1	0	0	f3	f4
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0

(d)

Leslie ***Q***

Age class at time t+1	Age class at time t: 1	2	3	4
1	0	0	0	0
2	p1	0	0	0
3	0	p2	0	0
4	0	0	p3	0
pj	p1	p2	p3	0

(e)

Lefkovitch ***Q***

Stage at time t+1	Stage at time t: 1	2	3	4
1	a11	a12	0	0
2	a21	a22	a23	a24
3	a31	a32	a33	a34
4	a41	a42	a43	a44
pj	p1	p2	p3	p4

(f)

Leslie ***T***

Age class at time t+1	Age class at time t: 1	2	3	4
1	0	0	0	0
2	1	0	0	0
3	0	1	0	0
4	0	0	1	0

(g)

Lefkovitch ***T***

Stage at time t+1	Stage at time t: 1	2	3	4
1	a11/p1	a12/p2	0	0
2	a21/p1	a22/p2	a23/p3	a24/p4
3	a31/p1	a32/p2	a33/p3	a34/p4
4	a41/p1	a42/p2	a43/p3	a44/p4

(h)

Figure 1 The general structure of the ***A***, ***F***, ***Q***, and ***T*** matrices for a Leslie matrix (in (a), (c), (e), and (g), respectively) and for a Lefkovitch matrix (in (b), (d), (f), and (h), respectively).

Markov Chains for Individuals Traversing the Life Cycle

The central idea: given the current state of an individual entity, there are probability rules that govern its state at the next point in time. These probability rules are contained in a matrix that governs/constitutes the Markovian process. When these rules are applied repeatedly over time to an individual entity, a sequence of states over time is experienced by each individual. The sequence of states for an individual is *a* 'sample path' or *a* 'realization of the process.' For some states, there is zero probability of exiting that state; these are called 'absorbing' states. The other states are called transient states. For example, in our application to demographic analysis, death is an absorbing state while particular ages or stages are not absorbing states; they are transient states. In the Markov chain literature (Iosfescu, 1980; Kemeny and Snell, 1976), the transition matrix is written so that the row state at t determines the column state at $t+1$, but here we follow Caswell (2001) and Keyfitz and Caswell (2005) in writing the transition from column state at t to row state at $t+1$, since here we are interested in drawing the connection to population projection matrices which are structured this way.

The Markov chain model for individual trajectories across the life span for the populations structured by either age or stage is

$$\boldsymbol{P} = \begin{bmatrix} \boldsymbol{Q} & 0 \\ \boldsymbol{m} & 1 \end{bmatrix} \quad [3]$$

where $\boldsymbol{Q}$ is the matrix of transient state transitions and $\boldsymbol{m}$ is the vector of transitions into death, the absorbing state. There is one absorbing state, death. The transient states are ages (or stages). This $\boldsymbol{Q}$ and $\boldsymbol{m}$ used in the Markov chain is the link from one realm of analysis to another since they are the ones described above, calculated from population projection matrices.

Age-Patterns of Survivorship and Fertility in Age-Structured Populations

Death – Consider the significant event of death. It happens to all living things and it happens once in a lifetime. Of interest for the individual at a given age is its 'status' (dead or alive). Considering a collection of individual life trajectories from a cohort point of view, where time starts at birth for each individual (age = 0), the collection of individual trajectories can be used to calculate the proportion still alive at age x ('survivorship' to age x, or l_x) which estimates the 'probability' that newborn individuals will survive to age x. Since life trajectories track individuals from one age to the next (= one time to the next), they also contain information on the probability that individuals of given age survive for one time step (p_x). Information about the 'probability of surviving' from one age to the next is also information about the 'probability of dying' ($m_x = 1 - p_x$) during the interval. Identified with each individual trajectory is the age of death. The mean or expected age of death for the collection of individual trajectories is also known as the 'life expectancy' from birth (e_0).

Reproduction – Consider the significant lifetime events of starting and stopping reproduction. Not all individuals reproduce during their lifetime. Some die before they reproduce. Of interest for the individual who is still alive at age x is not only its 'reproductive status' (reproducing or not), but also 'how many offspring' it will make during the age interval x to $x+1$ (the 'fertility at age x, or f_x). Each individual trajectory shows whether or not an individual reproduced before dying, as well as the ages of first reproduction and last reproduction for that individual. Considering a collection of individual life trajectories, the average contribution of each individual of the initial cohort to the production of offspring at that age is estimated by the probability that an individual is still alive weighted by the per capita fertility at that age, $l_x f_x$. Summing over all ages provides a measure of the expected number of offspring in the next generation for each individual of the initial cohort; it estimates 'population growth rate per generation' and it is known as the 'net reproductive rate,'

$$R_0 = \sum_x l_x f_x \quad [4]$$

'Generation' is a unit of time, but how long is a generation, how many time steps constitute a generation? Conceptually, the idea of generation time is the average separation in time between the birth of a mother and the birth of her children. Coale (1972) presented three distinct metrics, fully discussed by Keyfitz and Caswell (2005). The measure that focuses on what a cohort does over its lifetime, call it T_μ, weights the $l_x f_x$ by how many years have passed since the mother's birth (i.e., the mother's age), sums over all ages, and divides by R_0,

$$T_\mu = \frac{\sum_x l_x f_x}{\sum l_x f_x} \quad [5]$$

Later the focus will be on the one that relies on the relationship between per capita population growth rate per time step (λ) (obtained from the matrix analysis above) and per capita population growth rate per generation, $\lambda^T = R_0$. Then solve for T,

$$T = \frac{\log\ R_0}{\log\ \lambda} \quad [6]$$

Connecting life table parameters to Leslie matrix parameters – The life table schedule of age-specific survivorship l_x, the probability of surviving from birth to age x, for all ages after 0 ($l_0 = 1$ by definition), can be calculated as a product of annual survivals,

$$\begin{aligned} l_1 &= p_0 \\ l_2 &= p_1 p_2 \\ l_x &= p_{x-1} \ldots p_2 p_1 p_0 \end{aligned} \quad [7]$$

These annual survivals p_x (re-indexed to match matrix subscripts such that they start at p_1 rather than p_0) are found on the sub-diagonal matrix entries of the Leslie matrix **A** and its corresponding **Q**. The life table schedule of age-specific fertilities f_x (re-indexed to match matrix subscripts such that they start at f_1 rather than f_0) are essentially (we will assume they are) found in the top row of the Leslie matrix and its corresponding **F**. Depending upon the timing of reproduction with respect to the census interval, the actual matrix entries may be products of survivals of adults or juveniles with these

fertilities (see discussion of how birth pulse vs. birth flow, pre-breeding vs. post-breeding censuses affect the entries in the top row in Caswell (2001)).

Age-Patterns of Survivorship and Fertility in Stage-Structured Populations

Even when demographic rates are structured strictly by stage, individuals do have ages at particular times and cohorts have emergent age-patterns of survivorship and fertility. However, the probabilities of death and the probabilities and amounts of reproduction at each age are determined purely by the stage structure at that age.

Death – For purely stage-structured populations, the probability of death depends solely upon stage; for stage j this probability is $m_j = 1 - p_j$, where p_j is the annual probability of survival for a given stage given by the sum of the jth column of $\mathbf{Q}$. For a cohort then, the probability of survival at a given age p_x depends solely upon its stage structure at that age. The survival probability is essentially a weighted average of the p_js, where the weights are determined by what proportion of the cohort is in each stage. Imagine starting a cohort in a particular stage (or distribution of stages) at birth (age = 0), then use the $\mathbf{Q}$ matrix to project the cohort forward. The $\mathbf{Q}$ matrix acts on the stage structure of the cohort, transforming it at each age. As some individuals of each stage die, the cohort decreases in size at each age. Reshuffling of the survivors among stages is also governed by this matrix. Over the long term (at late ages), if there are any individuals remaining in the cohort, the stage distribution will reach a quasi-equilibrium, age-specific survival l_x will become stabilized (Steinsaltz and Evans, 2004) and instantaneous mortality rate ($\mu_x = \log(l_x) - \log(l_{x+1})$) will reach a plateau (Horvitz and Tuljapurkar, 2008) (**Figure 2**).

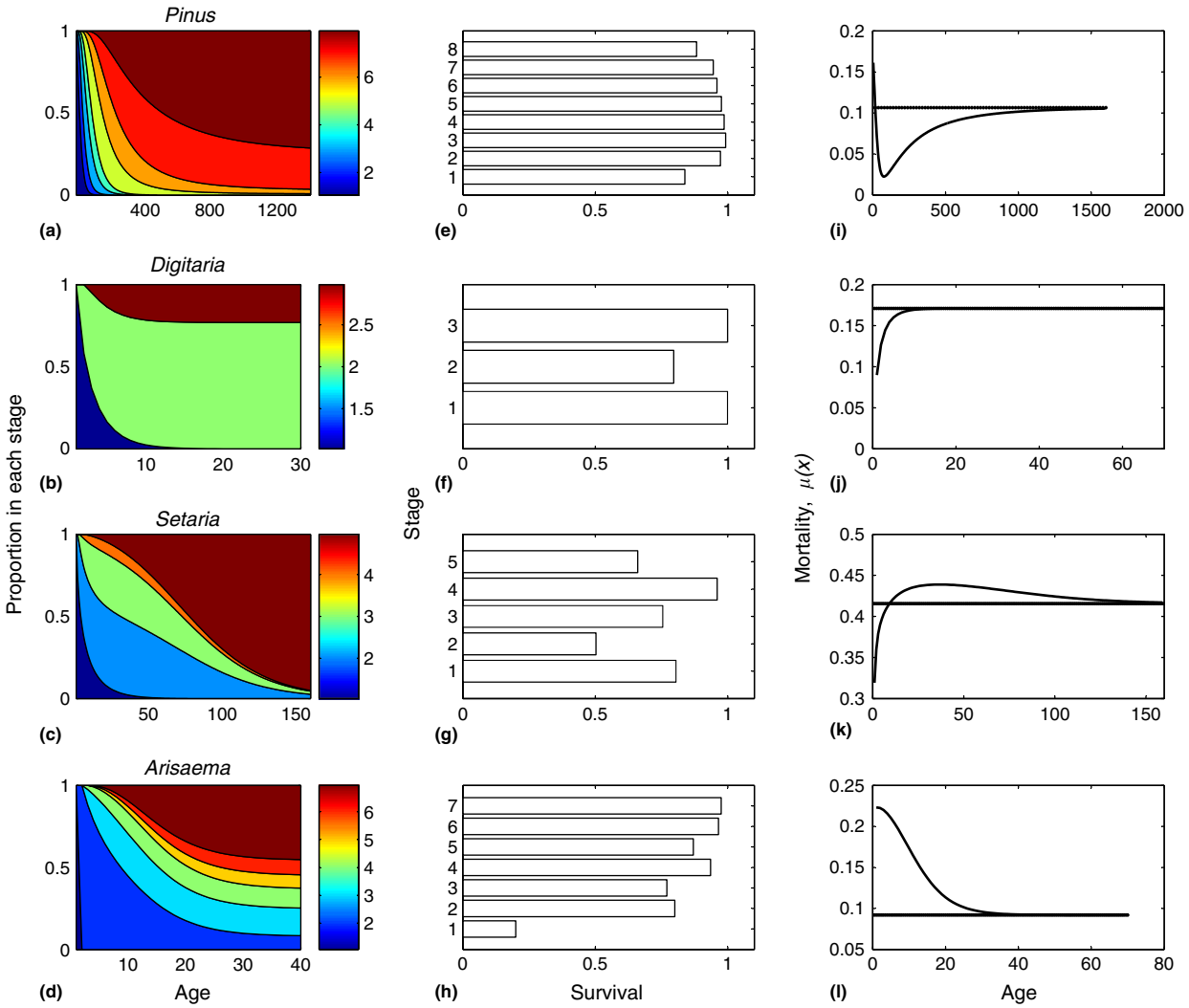

Figure 2 Stage distribution at each age for each of four plant species (*Pinus*, *Digitaria*, *Setaria*, and *Arisaema*) with stage-structured demographic rates (in (a)–(d)). One-period stage survival p_j for each species (in (e)–(h)). Age-specific instantaneous mortality μ_x (calculated from age-specific survivorship l_x as follows: $\mu_x = \log(l_x) - \log(l_{x+1})$) and the mortality plateau (dotted lines) for each species (in (i)–(l)). Reprinted from Horvitz, C. C., Tuljapurkar, S., 2008. Stage dynamics, period survival and mortality plateaus. American Naturalist 172, 203–215, with permission from ©2008 by C. C. Horvitz.

Survivorship to each age l_x, is the product of the prior age-specific survivals as in the age-structured case. Of course, underlying these cohort patterns are collections of individual trajectories. The Markov chain analysis below will provide further insights and a mechanism for calculating life expectancy, the average age at death.

Reproduction – For purely stage-structured populations, the probability of reproducing and the amount of reproduction depends solely upon stage; for stage j this is f_j the annual amount of offspring made by a given stage, it is an entry in **F**. For a cohort, the number of offspring expected to be produced at age x f_x depends solely upon its stage structure at that age and a kind of weighted average of the f_js across stages provides this metric. The Markov chain analysis below will provide further insights and a mechanism for calculating net reproductive rate and generation time.

Markov Chain Analysis for Stage-Structured Populations

Markov chain analysis is concerned in general with how long individual entities spend, on average, in different states before being absorbed and on first passage times to absorbing states. The examples presented here focus on death as the absorbing state, but other events of interest (like reaching a critical threshold size) can also be analyzed in the same framework. The framework is stochastic, so analytical methods provide not only means, but variances. Here the focus is on means only. More details and applications can be found in Caswell (2001) and Keyfitz and Caswell (2005). The general context is that the sequence of states experienced by individuals is governed by the state they start in and the process encapsulated in the matrix. Thus Markov chain analysis is ideal for providing insights on life history or anything related to timing. These include age-specific patterns of survivorship and fertility and life expectancy and similar life history parameters for stage-structured populations. One underlying feature of this analysis is that survival and transitions among stages at each time step are summarized in the $\boldsymbol{Q}$ matrix (the matrix of transient state transitions). Each year transitions are independent of previous years. The process is multiplicative over time.

Age-specific survivorship – Raising $\boldsymbol{Q}$ to the power x provides the probability that individuals from an initial cohort will be alive and be in a particular stage at age x, in other words, after x years. Therefore, to find out the total number still alive at age x, sum over all stages, meaning sum over the column. Considering each of the column sums of $\boldsymbol{Q}^x$, the sum of the first column provides the 'age-specific survivorship l_x' for individuals who were in stage 1 (that is to say, who were newborns) initially.

Life expectancy – The fundamental matrix in Markov chain analysis provides a measure of expected time in each state before being absorbed. It is defined as the summation of a series starting with the identity matrix and then having terms that are subsequently higher powers of $\boldsymbol{Q}$ and it is calculated as follows:

$$N = I + Q + Q^2 + Q^3 + \ldots$$
$$= (I - Q)^{-1} \qquad [8]$$

The entries in column 1 of this matrix are the number of years that an individual who was in stage 1 initially (a newborn) will spend in each stage before it dies. The summation over the whole column is the 'life expectancy of newborns,' e_0. The summation over each of the other columns is the 'remaining' life expectancy for individuals that start in each of the other stages.

Age-specific fertility – A matrix can be calculated from the **Q** and the $\boldsymbol{F}$ matrices that will provide the mean number of offspring of stage i produced at age x by an individual who began in stage j at age 0 (i.e., as a newborn). Start with $\boldsymbol{Q}^x$ and find all its column sums. Then normalize by dividing each matrix entry by its own corresponding column sum. So each resulting column is a measure of stage structure at age x for those who started in the stage of that column. Call that normalized matrix of stage structures $\mathbb{Q}(x)$ and multiply it on the left by the **F** matrix, essentially weighting fertilities by stage structure as follows,

$$\phi(x) = F\mathbb{Q}(x) \qquad [9]$$

The matrix entry $\phi(x)_{11}$ is the fertility for age x. Repeating this procedure for each age and harvesting the single entry of interest, obtain age-specific fertilities for the stage-based model (Caswell, 2001; Keyfitz and Caswell, 2005).

Net reproductive rate – A matrix $\boldsymbol{R}$ can be calculated from the fundamental matrix $\boldsymbol{N}$ and the $\boldsymbol{F}$ matrix. The entries r_{ij} provide the expected lifetime production of type i offspring by an individual who starts life in stage j,

$$R = FN \qquad [10]$$

$\boldsymbol{R}$ can incorporate reproduction by more than one means, for example seeds and vegetative offshoots. The dominant eigenvalue of $\boldsymbol{R}$ provides a measure of R_0, the net reproductive rate for the stage-structured population, since $\boldsymbol{R}$ projects the population from one generation to the next (Caswell, 2001; Keyfitz and Caswell, 2005).

Generation time – There are at least three measures of generation time for age-structured models. The one that most readily lends itself to stage-structured models is the one that relies on the relationship between per capita population growth rate per time step (λ) (obtained from the projection matrix analysis above) and per capita population growth rate per generation, $\lambda^T = R_0$, which was calculated in the previous paragraph. Then solve for T exactly as before in eqn [6],

$$T = \frac{\log R_0}{\log \lambda} \qquad [11]$$

Conclusion

Population projection matrices are clearly related to age-specific survivorship and fertility schedules of life tables, directly for age-structured populations and through Markov chain analysis for stage-structured populations. Connecting population projection matrices, life histories, life tables, and Markov chains for age- and stage-structured populations provides insight that populations are collections of individuals of different ages (or stages) at any given (externally defined) time, individuals each of whom has a particular life path. As key life

history events may occur at different ages for different individuals, this connection requires that we specify the relationship among age, stage, and (externally defined) time. Also revealed is the multiplicative nature across the lifetime of annual demographic rates for a cohort of newborns. Furthermore, elucidating these links in age-only or stage-only models (as done here) provides a context for further examination in greater depth of the joint effects of stage and age as well as for a definition of generation time that takes such joint effects into account (Steiner *et al.*, 2014).

See also: Age-Specific Survivorship and Fertility, Estimating. Life History: Age and Stage Structure. Life History: Pike

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Life History Trade-offs

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What Are Trade-offs and Why Do They Exist?

Trade-offs play a central role in life history theory and occur when a beneficial change in one trait comes at a (fitness) cost through a concurrent detrimental change in another trait. In life history terms, this entails that selection will act to simultaneously maximize the $l(x)$ and $m(x)$ schedules, which are, respectively, the probability of surviving from birth to the start of age class x and the expected number of offspring for a female in age class x (see 'What is a life history' in this article). However, selection is prevented from achieving this because of direct trade-offs between the $l(x)$ and $m(x)$ schedules, or because an indirect trade-off exists with a third trait. If there were no trade-offs, then selection would drive all traits correlated with fitness, and thereby the $l(x)$ and $m(x)$ schedules, to limits imposed by history and design (Stearns, 1989), resulting in so-called Darwinian demons that reproduce directly after being born, produce an infinite number of offspring and live forever. However, many, if not all, traits are well within these limits, which means that trade-offs must exist.

Trade-offs occur at both the phenotypic and genetic level, but can also be shaped by mechanisms that connect the latter two levels (e.g., physiological mechanisms that determine resource allocation to growth, survival, maintenance, and reproduction) (Stearns, 1989). Trade-offs can be divided into two groups: the first group comprises direct trade-offs between the $l(x)$ and $m(x)$ schedules, which arise through physiological constraints as energy is a limiting resource and allocation to one trait means deprivation of another. van Noordwijk and de Jong (1986) summarized this concept in their acquisition-allocation model (also called Y-model), which was first formulated by James (1974). Roff (2002) refers to this as the partitioning of resources model (after James (1974) and Riska (1986), who derived this model independently), as the variable-proportion model. The second group comprises indirect trade-offs, which occur when the trade-off between the $l(x)$ and $m(x)$ schedules is mediated by separate relationships that each schedule has with, for example, body size. For example, a positive correlation between offspring production and body size and a negative correlation between survival and body size will necessarily lead to a negative correlation between the $l(x)$ and $m(x)$ schedules.

Examples of Important Life History Trade-offs

The trade-off between 'current reproduction and survival' was the first trade-off to be analyzed (Williams, 1966). This trade-off is easy to visualize: reproductive individuals might be more vulnerable to predation as they are more visible, slower moving or less maneuverable. In addition, the energy diverted into reproduction might increase the risk of death, for example, through reduced immunocompetence. The empirical evidence for the existence of this trade-off, however, is mixed. In arthropods and nematodes, virgins and individuals that mate less live longer, but, in rats and mice, only one of four studies found that virgins live longer (Bell and Koufopanoum, 1986). Results from other taxonomic groups, including birds and insects, also give mixed results (reviewed in Stearns, 1989). Evidence for genetic trade-offs between survival and reproduction have also failed to reveal a general pattern. Results from breeding experiments also do not support the existence of this trade-off, while those from selection experiments do (Stearns, 1989).

Just as important a determinant of fitness as the effect of reproduction on survival is the direct effect of 'current reproduction on future reproduction.' Again, however, empirical studies investigating the existence of this trade-off show mixed results. In their overview of 11 laboratory studies (eight species) investigating the phenotypic correlation between current and future reproduction, Bell and Koufopanou (1986) listed only one study that found evidence for a negative correlation between the two traits. Field studies, mainly on birds, have been more successful in finding evidence for a negative relationship between current and future reproduction (Warner, 1984; Bell and Koufopanou, 1986). Gustafsson and Pärt (1990), for example, manipulated clutch sizes of one-year-old collared flycatchers (*Ficedula albicollis*) and monitored their reproductive output in the following 3 years. They found a negative correlation between current and future reproduction as bird pairs whose clutches were enlarged had smaller clutches over the next 3 years than control pairs. Breeding experiments have rarely found evidence of a negative correlation whereas selection experiments have been far more successful (Bell and Koufopanou, 1986).

The final example concerns the trade-off between 'offspring size and offspring number.' If we look across species, we find that some species produce thousands of small offspring (e.g., marine invertebrates such as bivalves and crustaceans), tens to hundreds of intermediate sizes offspring (e.g. most plants and insects), or only a few large offspring per reproductive event (e.g., most birds and mammals). The trade-off between offspring size and number plays an important role in explaining these differences in offspring size. This trade-off has been detected in many semelparous species (which reproduce only once in their lifetime) but has been detected less frequently in iteroparous species (e.g., many vertebrates and perennial plants that have multiple reproductive events) or in species with parental care (reviews in Roff, 1992; Fox and Czesak, 2000). Messina and Fox (2001) argue that one important reason for this discrepancy is that a negative relationship between offspring size and number is only expected to occur if the amount of resources available to invest into reproduction is fixed. However, in nature, this amount is often variable, for example, because individuals differ in their juvenile growth rate as a result of which they mature at different sizes, which in turn influences individual reproductive effort.

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In summary, there is no general pattern of negative genetic or phenotypic correlations for the major life history trade-offs described here. It is therefore imperative to investigate whether a trade-off exists and not simply assume that it does.

Measuring Trade-offs

Trade-offs have been measured through field observations (e.g., Clutton-Brock *et al.*, 1982, 1983), in experiments conducted in the laboratory (e.g., Partridge and Farquhar, 1981) and in the field (e.g., Askenmo, 1979), through phenotypic correlations in the laboratory (e.g., Bell, 1984a,b), and through genetic correlations (e.g., Rose and Charlesworth, 1981a,b). Reznick (1985) divides these different methods into four categories (Reznick, 1985):

1. Phenotypic correlations: A correlation is measured between two traits at the level of the phenotype but there is no manipulation of the organism or of its environment.
2. Experimental manipulations: A single factor is manipulated in an experiment and all else is kept constant (or randomly assigned).
3. Genetic correlations from sib analysis: The genetic correlation between two traits is estimated using covariation between individuals within or between families, or using covariation between clones or inbred lines.
4. Genetic correlations from selection experiments: The genetic correlation between two traits is estimated from correlated changes in one trait in response to selection on another.

These different methods of measuring trade-offs have received criticism (Tuomi *et al.*, 1983; Partridge, 1987) and controversy (Bell, 1984a,b; Reznick *et al.*, 1986). Reznick (1985) argues that categories 1 and 2 are flawed because causation is inferred from observed correlations and because there is no demonstration that the trade-offs are under genetic control. Stearns (1992), in turn, classifies categories 2 and 4 as the most reliable and informative methods for measuring trade-offs. Roff (2002) argues that both phenotypic and genetic correlations are important in shaping the evolution of two traits in a trade-off if each trait is subject to selection. His argument is that, if we take a phenotypic correlation to be a component of the set of trade-offs that together determine fitness, then there is no problem with either category, although it is preferable to investigate a possible trade-off by manipulating the traits in question. Roff (2002) warns, however, that problems can arise if potentially confounding third variables are ignored, as this could lead one to infer an incorrect causal mechanism determining the trade-off. Finally, it is important to realize that trade-offs are contingent on conditions. If no evidence is found for a trade-off between two traits in a particular environment and at a particular time, this does not necessarily mean that the trade-off does not exist in general. First, trade-offs are often the result of physiological or ecological factors; and second, trade-offs may manifest themselves differently in different organisms, or differently across different environments within the same organism. This means that a life history trade-off is likely only apparent in an organism under a particular set of circumstances, for example, under conditions of stress (Reznick, 1985).

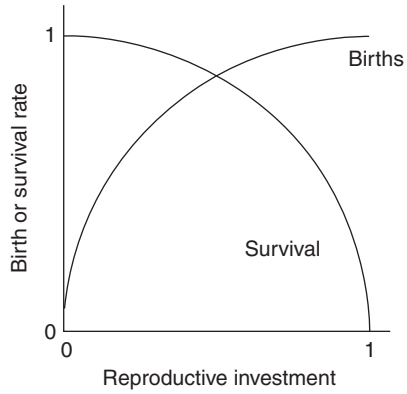

Figure 1 A schematic illustration of one of Schaffer's model of reproductive trade-offs. Reproductive investment varies from 0 to 1, at which point the individual produces the maximum possible number of offspring (birth rate equals 1) and the individual dies (survival rate equals 0). Other shapes are possible which affects reproductive investment. Modified from Schaffer, W.M., 1974. Selection for optimal life histories: The effects of age structure. Ecology 55, 291–303.

It is furthermore important to note that, when measuring trade-offs, one should not only focus on identifying the sign of the correlation between two life history traits – i.e., is there a negative correlation (in which case a trade-off is inferred), no correlation, or a positive one – but also on the shape of the correlation. The simplest shapes that a negative relationship between two traits can have include linear, concave, or convex (concave up) associations. Schaffer (1974) demonstrated theoretically that such differences in shape can critically influence predicted evolutionary responses. In his model of reproductive trade-offs, Schaffer (1974) plots reproductive investment at the current age against the probability of surviving to the next age (an example is shown in **Figure 1**). Which reproductive strategy is predicted to be optimal (e.g., semelparity or iteroparity) and what the optimal age for reproduction is, depends on the shape of the reproduction and survival curves. Werner and Anholt (1993) developed a model to investigate how the optimal foraging activity in the presence of predators depends on the shape of the trade-off between current and future reproduction. They found that, depending on whether the trade-off is of a concave or convex shape, the optimum allocation to current reproduction may stay the same, increase or decrease in response to the presence of predators. Hence any prediction on optimal life history strategies is possible but meaningless in explaining responses to perturbations unless the trade-off relationship can be specified.

Why Do We Observe a Positive Correlation Where We Expect a Negative One Between Traits?

Life history theory predicts negative correlations between traits such as survival and reproduction. However, observations often indicate that these traits are positively correlated, particularly when these are observations obtained from different

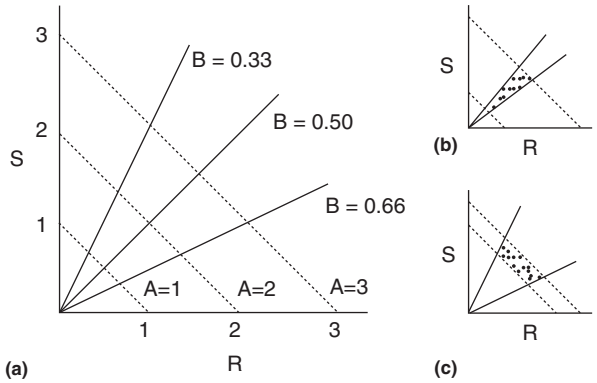

Figure 2 A schematic illustration of the partition of resources or acquisition-allocation model. The total amount of energy A that an organism can allocate to reproduction, R, and survival, S, is $A = R + S$. An individual i allocates a fraction B_i of energy A_i to reproduction: $R_i = B_i A_i$. Panel (a) shows different scenarios for different values of B (solid lines) and A (dashed lines). Panel (b) shows a scenario where variation in A is large between individuals and variation in B small; as a result there is a positive correlation between reproduction and survival. Panel (c) shows a scenario where variation in A is small between individuals and variation in B large, which results in a negative correlation between reproduction and survival. Modified from van Noordwijk, A.J., de Jong, G., 1986. Acquisition and allocation of resources: Their influence on variation in life history tactics. American Naturalist 128, 137–142 and Stearns, S. C., 1989. Trade-offs in life-history evolution. Functional Ecology 3, 259–268.

individuals within a population (Bell and Koufopanou, 1986). van Noordwijk and de Jong (1986) have shown how variation between individuals can lead to a positive, zero, or negative correlation between two traits. Their premise is based on their acquisition-allocation model (see above) in which each individual has a certain amount A of food available that can only be allocated to either reproduction, R, or survival, S (Levins, 1968; Sibly and Calow, 1986):

$$A = R + S$$

This model implies that all individuals allocate energy to R and S in the same manner. However, individuals typically differ in the amount of energy that they are able to acquire as well as in the fraction of the energy acquired that they allocate to reproduction, B, in which case we have:

$$R_i = B_i A_i, \text{ and}$$

$$S_i = (1 - B_i) A_i$$

where the subscript i indicates each individual. The sign of the correlation between the traits R and S depends on the relative degree to which each trait varies between individuals (van Noordwijk and de Jong, 1986) as is illustrated graphically in **Figure 2**. This illustration highlights the importance of asking critical questions when theory predicts a correlation between two traits to be negative and instead a positive correlation is observed. For example, how does variation in A and B change with changing environmental conditions in nature? Is between-individual variation in A higher than between-individual variation in B and vice versa? Answers to these questions are necessary to decide whether the trade-off in question plays an important role in the functioning of natural populations (Stearns, 1989).

Evolution of Life History Trade-offs

Although no one doubts the existence of trade-offs and the central role they play in the evolution of life history strategies, there is little understanding of how such trade-offs evolve (Chippendale *et al.*, 1996; Reznick *et al.*, 2000; Roff and DeRose, 2001; Roff, 2002). Roff and Fairbairn (2007) identified four issues that contribute to the lack of progress in understanding the evolution of trade-offs. The first issue is one of semantics: what exactly do evolutionary biologists mean by the term 'constraint'? Roff and Fairbairn (2007) suggest clearly distinguishing between using the term to denote a bias or to denote combinations of traits that are biologically not possible (e.g., unavailable to selection). The second issue concerns a misunderstanding of the central equation of the acquisition-allocation model (van Noordwijk and de Jong, 1986; **Figure 2**), which has led to incorrect predictions and invalid tests. That is, Roff and Fairbairn (2007) mathematically showed that the sign of the correlation between reproduction and survival (e.g., **Figures 2(b)** and **2(c)**) depends on more than only the relative variances in energy acquisition and allocation. The latter, however, is what authors commonly infer from the acquisition-allocation model (e.g., Brown, 2003; Ernande *et al.*, 2004). The third issue focuses on the difficulty of deriving accurate predictions on the evolution of trade-offs and the precise testing of these predictions, whereas the fourth issue deals with an essential ingredient for the evolution of trade-offs to occur: there has to be variation in a trade-off upon which selection can act. The latter raises the crucial question of what maintains variation in a trade-off. Roff and Fairbairn (2007) identify four candidate mechanisms for this (mutation-selection balance, antagonistic pleiotropy, correlated selection, and spatio-temporal heterogeneity in genetic variation), but, again there are few empirical studies to determine which mechanisms may be at play in nature. Addressing the four issues identified by Roff and Fairbairn (2007) is imperative to further progress in the study of life history trade-offs.

See also: Age-Specific Survivorship and Fertility, Estimating. Aging: Why Do We Age?. Life Histories, Axes of Variation in. Life History, What is?

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Life History, What is?

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Life History and Life History Traits

The Atlantic salmon (*Salmo salar*) inhabits rivers and streams that flow into the northern Atlantic Ocean in Europe and enter the sea on the east coast of North America. Salmon spend part of their life-cycle feeding in the ocean but return to the streams where they hatched to spawn. They can live up to 11 years, with longer living individuals spawning several times during their lifetimes. In contrast, the Pacific salmons (*Oncorhynchus* spp.) occur in streams and rivers in North America and Asia that drain into the Pacific Ocean. Like the Atlantic salmon, Pacific salmons migrate to the ocean to feed and grow, and return to the streams where they hatched when they are 5–7-years-old; however, they spawn just once and then die.

The flatfish (*Hippoglossoides platessoides*) in the Grand Banks of New Foundland, Canada, grow slowly and reach an adult length of 60 cm, mature at about 14 years of age, can live for 30 years and have low reproductive rates. The same species of fish inhabiting waters off the coast of Scotland grow faster and mature earlier (3 yrs), have smaller body sizes (30 cm), live for 6 years, and have much higher reproductive rates compared to the flatfish inhabiting the Grand Banks (Roff, 2002).

The Asian elephant (*Elaphus maximus*) female is reproductively mature at 14 years of age, produces a single calf that weighs nearly 100 kg every seven years, and can live for a century. Under favorable environmental conditions, some species of vole (*Microtus* spp.) can attain reproductive maturity soon after they are weaned (about 1 month of age) and can produce litters of up to 12 small, altricial pups.

Why don't elephants produce four calves at each breeding attempt with each weighing 25 kg? Why don't voles produce fewer, larger and better-developed pups that may have better prospects of surviving to adulthood? How could a seemingly counter-productive strategy of dying after a single reproductive event have evolved in the Pacific salmons? Why do flatfish in the Grand Banks live for five times as long than those off the coast of Scotland? These are just a few examples of questions biologists ask about the diversity of life history patterns. Each species seems to do its own thing, and sometimes even individuals of the same species do different things depending upon the environment in which they find themselves. Understanding the causes and consequences of the mind-boggling diversity of life history patterns has long been a major focus of ecology and evolutionary biology.

So, how to define life history? A life history is the schedule of key events in an organism's life cycle, and is usually defined in terms of life history traits calculated across individuals within a population. Life history traits (also called *fitness traits*) are phenotypic characters that affect fitness, and include size at birth, age or size at maturity, and the sex ratio of offspring, and age- or stage-specific rates of growth, reproduction, survival, and investment in offspring (Stearns, 1992, 2000; Roff, 2002; Braendle *et al.*, 2011). Life history theory is a collection of ideas proposed to explain both the diversity of life history patterns, and the mechanisms by which natural selection has shaped life history traits. It is generally accepted that life histories are shaped by complex interactions between extrinsic ecological factors like species interactions and intrinsic factors that include trade-offs among life history traits and design and phylogenetic constraints. Because the currency of natural selection is fitness, and the focus of life history theory is traits that are directly related to fitness, life history theory has grown to provide an explicit link between ecology, genetics, and natural selection. Examples of questions life history theorists attempt to answer include (Stearns, 1992, 2000): Why are organisms small or large? Why do they grow slowly or rapidly? Why do they mature early or late? Why do they reproduce only once or many times during their lifetimes? Why do they produce small or large offspring? Why do they produce few or many offspring per reproductive attempt? Why do they have short or long life spans? Why must organisms get old, and ultimately die? And what are the fitness consequences of variation in life history patterns within a population?

Life Tables and Euler–Lotka Equation

Consider a hypothetical age-structured population in which survival and reproduction is dependent upon age. The hypothetical organism attains reproductive maturity at some age α (age at first reproduction), and continues to reproduce until some age ω (age of last reproduction). They may reproduce at some interval Υ (inter-birth interval), and at each reproductive attempt, produce ψ offspring (litter or clutch size). The life history of such an organism can be compactly summarized in a life table, which provide tabular summaries of age-specific survivorship and reproduction (Caughley, 1977; Stearns, 1992; Roff, 2002).

In its basic form, a life table consists of three columns: age (x), age-specific survivorship (probability at birth or hatching of surviving to age x, l_x), and age-specific fecundity (the average number of daughters born to a female of age x, m_x). Most vertebrate life tables tend to focus on fecundity of the female segment of the population, primarily because male reproductive output is difficult to measure. In cases where reproductive output of males can be accurately quantified, there is no reason why life tables cannot be compiled for males.

How does one go about compiling a life table? One can use any reasonable data and statistical approach that permits unbiased age-specific estimates of survivorship and fecundity. Caughley (1977) provides a thorough discussion of methods that can be used to derive l_x. The best approach is to capture, individually mark and follow a cohort from birth until death. One can then record the number of individuals alive at each successive age (n_x). Then l_x is obtained by dividing the number of individuals alive at age x by the original size of the cohort,

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n_0 (i.e., $l_x = n_x/n_0$). Alternatively, but equivalently, one can record the number of individuals that die during each age interval (d_x). This information can then be used to calculate survivorship along with various other measures of age-specific survival (or its compliment, mortality) including age-specific survival (P_x) and mortality (q_x) rates. These quantities are related to each other as follows (Caughley, 1977):

$$l_x = \frac{n_x}{n_0} = \prod_{i=0}^{x} P_i$$

$$d_x = n_x - n_{x+1}$$

$$q_x = \frac{d_x}{n_x} = 1 - P_x$$

$$P_x = \frac{n_{x+1}}{n_x} = 1 - q_x.$$

Given any one of these variable, all others can be calculated as shown in **Table 1**.

Life tables compiled by following a cohort from birth until all members have died are called 'cohort life tables.' However, compiling cohort life tables can be very challenging, especially for long-lived organisms. Most ecological field studies last only a few years, yet many organisms live for tens or hundreds of years. Catching, marking and following members of a cohort can be logistically difficult and expensive. Because of these difficulties, most life tables for long-lived species are 'static life tables.' Static life tables are compiled from a snap-shot of the population, based on either the standing age distribution or age at death data. Static life tables assume a static (or stable) age structure of the population, and require that the population growth rate is known. The age-structure assumption is rarely met in free-living populations and the population growth rate can be challenging to measure (Caughley, 1977), and these constraints make static life tables of limited value for life history studies. For the remainder of this article we focus on cohort life tables.

The method of compiling cohort life tables discussed above assumes perfect detectability – that is, all individuals alive at a given point in time are detected and their identity accurately recorded. When the detectability is <1.0 (which is often the case in field studies), statistical estimation approaches that allow explicit consideration of imperfect detection, such as capture-mark-recapture (CMR) or live recapture – dead recovery methods should be employed to estimate survivorship (Lebreton *et al.*, 1992; Williams *et al.*, 2001). Life tables compiled using CMR-type approaches can be described as 'pseudo-cohort' life tables because they are often not compiled by following a real cohort, but are based upon individual-based data collected across multiple cohorts. For all practical purposes, pseudo-cohort life tables are just as good as cohort life tables, and do not require the stringent assumptions inherent in static life tables (Caughley, 1977).

Table 1 Calculation of survival (l_x and P_x) and mortality parameters (q_x and d_x) based on information collected by following a cohort of song sparrow chicks. See text for details

Age (x)	n_x	$l_x = n_x/n_0$	$d_x = n_x - n_{x+1}$	$q_x = d_x/n_x$
0	115	115/115 = 1	115 − 25 = 90	90/115 = 0.783
1	25	0.217	6	0.24
2	19	0.165	7	0.36
3	12	0.104	10	0.83
4	2	0.017	1	0.5
5	1	0.009	1	1
6	0	0	0	–

Source: Smith, J.N.M., 1988. Determinants of lifetime reproductive success in the song sparrow. In: Clutton-Brock, T.H. (Ed.), Reproductive Success. Chicago: University of Chicago Press, pp. 154–172.

Methods of estimating age-specific fecundity rates (m_x) also vary widely depending on the study species, methods of tracking or monitoring study organisms, and time and resources at one's disposal. For example, age-specific fecundity rates for black bears (*Ursus americanus*) can be estimated by radio-tracking a large number of females of known age, following them to their winter dens where they give birth, and counting the number of cubs (McLean and Pelton, 1994; Garrison *et al.*, 2007). Reproductive rates of yellow-bellied marmots (*Marmota flaviventris*) can be determined by following individually marked females as they emerge from natal burrow with pups (Schwartz *et al.*, 1998). In contrast, reproductive rates of Soay sheep (*Ovis aries*) can be estimated by recording reproductive output of individually marked females during the birth-pulse (Clutton-Brock and Pemberton, 2004). Fecundity for some birds has been estimated by recording reproductive output of individually marked birds in artificial nest boxes or natural nesting sites. More invasive methods (e.g., based on shot samples) can be used for some harvested species (e.g., Caughley, 1970).

Regardless of the field or estimation methods employed, what is really needed is an estimate of the average number of daughters produced by a female of age x, m_x. Once the m_x are estimated, this information is combined with the table of age-specific survivorship l_x to construct a life table.

Nothing in nature remains constant in time for very long, but for the sake of simplicity, let's assume that l_x and m_x are constant with time. This implies that the environment is constant and that population is sufficiently large such that demographic stochasticity has no discernable effects. Under this assumption, the population will ultimately converge to a stable age distribution (SAD) – that is, the proportion of organisms of different ages remains constant over time, and the number of individuals of each age class as well as the entire population grow (or decline) at a constant rate, r. Under these conditions, l_x and m_x are related to the per capita or instantaneous growth rate r via the Euler–Lotka equation:

$$1 = \int_{\alpha}^{\omega} l_x m_x e^{-rx} dx$$

In practice, the discrete time version of the equation is often used:

$$1 = \sum_{\alpha}^{\omega} l_x m_x e^{-rx}$$

The Euler–Lotka equation, also called the characteristic equation or the basic equation, derived in a population context by Lotka (1924, 1956), links r to its determinants (l_x and m_x), and forms the foundation of the stable age theory that underlies much of modern plant, animal and human demography.

Under the assumptions of large population size and constant l_x and m_x, the 'per capita' population growth rate r (also called the instantaneous population growth rate and the Malthusian parameter) can be calculated iteratively as the solution to the Euler–Lotka equation (i.e., the value of r that makes the equality true); $r<0$ implies a declining population, whereas $r>0$ implies an increasing population. Note that r is asymptotic population growth rate assuming the population is at equilibrium and that l_x and m_x are constants. The Euler–Lotka equation also permits calculation or approximation of several biologically important quantities including the net reproductive rate, generation time, the SAD, reproductive value and age-specific life expectancies (Stearns, 1992; Charlesworth, 1994; Caswell, 2001; **Box 1**).

The net reproductive rate (R_0) is the average number of daughters produced by a female over her lifetime and is given by:

$$R_0 = \sum l_x m_x$$

Thus, R_0 is the sum of age-specific fecundity 'weighted' by the probability at birth of surviving to that age. $R_0>1$ implies that, on average, each female more than replaces herself with female offspring, and $R_0<1$ implies she does not; these correspond respectively to increasing ($r>0$) or declining ($r<0$) population size. R_0 is consequently a measure of the 'per generation' population growth rate. This definition of R_0 implies that a 'generation' can be defined as the time required for a population to grow by a factor of R_0, and can be characterized with the relationship

$$R_0 = e^{rG}, \text{ or } G = \frac{\ln R_0}{r}$$

Another commonly used measure of generation time, referred to as the 'cohort generation time,' is defined as the mean age at reproduction of a cohort of females, and is calculated as (Charlesworth, 1994):

$$T_0 = \frac{\sum x l_x m_x}{\sum l_x m_x} = \frac{\sum x l_x m_x}{R_0}$$

Finally, the generation time is also defined as mean age of the mothers of a new-born cohort at SAD. This measure of generation time can be calculated as:

$$T = \sum x l_x m_x e^{-rx}$$

Charlesworth (1994) notes that T arises naturally in different contexts, and argues that it provides the most appropriate measure of generation time. T has also been used to quantify the relative 'tempo' of life history along a fast–slow continuum (e.g., Jones *et al.*, 2008). Species that have short generation lengths tend to live fast lives and die young, while those characterized by long generation lengths live slow lives and reach older ages.

Once at the SAD, the proportion of individuals in different age classes will not change over time. This distribution is given by:

$$c_x = \frac{e^{-rx} l_x}{\sum_x e^{-rx} l_x}$$

Box 1 Example calculation of *per capita* population growth rate (or Malthusian parameter) and related quantities using the life table method

Barkalow *et al.* (1970) compiled age-specific survivorship (l_x) and fecundity (m_x) for a Gray squirrel (*Sciurus carolinensis*) population (reproduced below). We use these data to illustrate calculation of various life history quantities.

Life table data for a gray squirrel population in North Carolina, USA

Age (x)	l_x	m_x	l_x*m_x	l_x*m_x*x
0.0	1.000	0.000	0.000	0.000
1.0	0.253	1.280	0.324	0.324
2.0	0.116	2.280	0.264	0.529
3.0	0.089	2.280	0.203	0.609
4.0	0.058	2.280	0.132	0.529
5.0	0.039	2.280	0.089	0.445
6.0	0.025	2.280	0.057	0.342
7.0	0.022	2.280	0.050	0.351
Sum			1.120	3.128

As described in the text, the net reproductive rate is calculated as $R_0 = \sum l_x m_x$; we need only to multiply l_x and m_x columns, and add them up! For this squirrel population, $R_0=1.12$. So, on average, each female produces 1.12 daughters during her lifetime, implying that the population is increasing at the rate of 12% per generation. But how long is a generation? We find answer to this question by calculating the generation time for this population. There are several measures of generation time (see text); for this example, we will use a simple measure that is easy to calculate: $T_0 = \frac{\sum x l_x m_x}{R_0}$. All we have to do is to multiply x, l_x, and m_x columns together, and divide the sum by R_0 which we have calculated already. From the table above, $T_0=3.128/1.120=2.79$ years. So, we know that the population is increasing approximately 12% every generation (=2.79 years), which allows us to calculate the approximate value of *per capita* population growth rate: $r \approx \ln(R_0)/G = \ln(1.12)/2.79 = 0.040$.

Calculation of other quantities discussed in the text (e.g., stable age distribution, reproductive values) requires the exact value of r, which in turn can be found by solving the Euler–Lotka equation: $1 = \sum_\alpha^\omega l_x m_x e^{-rx}$ This can be easily accomplished by using software packages such as R or MATLAB; one should obtain identical results regardless of the software used. Using the example R code, we find the exact value of r to be 0.041 – the approximate value was very close indeed! The following R script can be used to solve the Euler–Lotka to find the exact value of r.

```
#Define the Euler-Lotka equation:
lotka=function(lx, mx, x, r) {y=(1 - sum
(lx*mx*exp(-r*x))) return(y)}
#Call R function "uniroot" to find the
root; r_approx is the approximate #value of r
as calculated #above
interval=c(r_approx - 0.5, r_approx+0.5)
r_exact=uniroot(lotka, interval=interval,
lx=lx, mx=mx, x=x)$root
```

Age-specific reproductive value v_x quantifies the relative contribution of females of different ages to population growth. It can be calculated as:

$$v_x = \frac{e^{r(x-1)}}{l_x} \sum_{y=x}^{\infty} e^{-ry} l_y m_y$$

In many applications, the –1 is omitted from the first exponent; this causes reproductive value for each age class to be off by a factor of e^r (Roff, 2002). This omission makes no difference because v_x is often expressed relative to the reproductive value of youngest age class such that $v_0 = 1$. Reproductive value can also be written as the sum of current reproduction and expected future (or residual) reproductive value (Williams, 1966; Stearns 1992). This decomposition can be useful in the context of costs of reproduction, especially those pertaining to the trade-off between current reproduction and future reproductive potential.

Finally, given l_x, one can calculate the age-specific life expectancy, E_x, which is an estimate of how much longer an individual of age x is expected to live given that it has already survived to age x (Charlesworth, 1994):

$$E_x = \frac{\sum_{y=x}^{\infty} l_y}{l_x}$$

A slightly different measure of life expectancy considers survivorship to the mid-point between age x and $x+1$ in the numerator (as opposed to survivorship to age x):

$$E'_x = \frac{\sum_{y=x}^{\infty} L_x}{l_x}$$

where

$$L_x = (l_x + l_{x+1})/2$$

Much demographic theory has been developed to study dynamics and persistence of populations of specific species. This means all of the quantities that can be calculated based on the stable age theory have a natural population ecological interpretation. For example, r quantifies instantaneous asymptotic growth of the whole population, while c_x gives the proportion of organisms at age x at the SAD, and R_0 measures per generation finite population growth. Much of the same theory applies to life history studies as well as to population growth studies; however, the quantities can have different interpretations. When applied in the life history context, they no longer refer to populations of a species, but instead are often applied to specific genotypes and strategies (Stearns, 1992). For an allele to spread through a population, individuals with genotypes that includes that allele must survive and reproduce more than competitor genotypes without the allele. Similarly, the fittest strategy will have highest value of r. The same theory can be applied in both population dynamic and life history contexts, but the results must be interpreted with respect to the group of organisms under focus.

Second, the stable age theory based on the life table approach is, in the strict sense, only appropriate for age-structured populations where survival and fecundity rates depend upon age. However, in many biological populations survival and reproductive rates are affected more strongly by something other than chronological age (Caswell, 2001). For example, some species are naturally structured by discrete life history stages (e.g., eggs, larvae, pupae, and adults in insects). Some plants and invertebrates have complex life histories, produce multiple types of offspring and the progression of life does not proceed deterministically as is observed in age-structured populations of birds and mammals. In other species, survival and reproductive rates may be strongly influenced not only by chronological age but also by some quantitative character like body mass, body size or stem diameter. For populations structured by discrete stages, or those exhibiting complex life histories, matrix population models provide a flexible and powerful framework for population modeling and estimating r (or its discrete time analogue, $\lambda = e^r$) and R_0 (Caswell, 2001). For populations in which rates are influenced by quantitative characters (e.g., body mass, body length, diameter), integral projection models (IPMs) offer similar tools (Easterling *et al.*, 2000; Ellner and Rees, 2006; Coulson, 2012). In later chapters we cover both classes of model, and provide examples of their application.

Third, much of the stable age theory – whether based on life tables, matrix models or IPMs – is applicable to populations inhabiting constant, unlimited environments. All quantities calculated from these analyses are 'asymptotic' measures and assume that (e.g., Roff, 2007, 2010): (1) survival and reproductive rates (or more generally, vital demographic rates) are constant over time, such that the population ultimately converges to a stable age, stage, or mass distribution. Both the entire population, and the size of each age or stage class, will grow at the same rate r (or it discrete time equivalent $\lambda = e^r$); (2) there are no stochastic, density-dependent or frequency-dependent effects on vital rates; and (3) the population is sufficiently large so that demographic stochasticity can be ignored. When these assumptions are violated, demographic or evolutionary quantities calculated based on stable age theory (e.g., r, R_0, E_x) should be interpreted with caution.

Many plants and animals occur in small numbers, so 'demographic stochasticity' may not always be ignored. Most natural populations are subject to the vagaries of nature, with components of the environment varying unpredictably with time, space or both ('environmental stochasticity'). Most living organisms inhabit finite environments and their growth will ultimately cease as the resource needs of the population exceed what the environment can provide; populations must cease to grow when that happens ('density-dependence'). Finally, the contribution of a character (genotype or phenotype) to the next generation often depends upon the frequency of that character in the population ('frequency-dependence') (Ayala and Campbell, 1974). Despite these restrictive assumptions, analyses based on stable age (stage, or mass) theory have provided the foundation of much of life history theory. Nonetheless, theoretical advances have been made that permit comparable analyses in the presence of demographic and environmental stochasticity, and density- and frequency-dependence (Tuljapurkar, 1990; Caswell, 2001; Rees and Ellner, 2009; Coulson *et al.*, 2010; Roff, 2010; Coulson, 2012). However, the complexity of models increases rapidly as new processes are included, and results obtained from them can be more challenging to interpret when compared to

those obtained from stable age (stage, or mass) theory-based analyses.

The Fitness Concept

Much of life history theory is concerned with identifying the fittest life history for a particular situation. For example, under what circumstance will a slow life history strategy outcompete a fast one? The answer to this question depends upon the relative fitnesses of the competing strategies. The fittest life history will always win. Unfortunately, there is no single measure of fitness that will always identify the fittest life history. Under the assumptions of stable age theory, and the absence of density- or frequency dependence, the Malthusian parameter r (or $\lambda = e^r$) is the appropriate measure of fitness. However, quantifying fitness in the presence of environmental stochasticitcty, and density- or frequency dependence is rather challenging. Fitness measures appropriate under such circumstances vary widely, and have been thoroughly discussed in the literature (e.g., Jong, 1994; Brommer, 2000; Roff, 2002, 2007, 2010; Metcalf and Pavard, 2006).

Trade-Offs and Constraints

Individuals are not always able to simultaneously maximize all components of fitness. If they could, we would see Darwinian demons – species with infinite lifespans producing infinite numbers of offspring starting immediately after birth (Law, 1979). However, such evolutionarily 'ideal' organisms do not exist because individuals are unable to simultaneously maximise all components of fitness. Individuals consequently trade-off different elements of their life history, within developmental or genetic constraints underpinning life history traits. The fittest life history is the point along this trade-off where fitness is maximized (Partridge and Harvey, 1988; Stearns, 1989, 1992).

Smallegange, later in this volume, provides an in-depth treatment of trade-offs.

Analytical Framework

Classical life history theory treats evolution of life history traits as an optimization problem (Stearns 1992, 2000; Roff, 2002). It assumes that life history patterns are determined by an interaction between extrinsic environmental (ecological) factors and intrinsic trade-offs and constraints including design and lineage-specific constraints. Extrinsic environmental factors are thought to shape life histories through their effects on age- or stage-specific survival, reproduction, and growth rates, subject to trade-offs and constraints. The 'Evolutionarily Stable Life History Strategy' (ESS) is the strategy that maximizes fitness. This approach to modeling life history evolution typically necessitates (1) an unambiguous definition of fitness; (2) a model that connects life history traits to fitness; (3) functional (or otherwise explicit) relationship among life history traits that represent trade-offs; (4) boundary conditions, representing developmental, design or lineage-specific constraints; and (5) optionally, relationships between intrinsic and/or extrinsic factors and life history traits. Each of these aspects is covered in chapters later within this section.

See also: Life History Evolution in Guppies, Experimental Studies of. Life History Trade-offs. Life-History Evolution in Island Populations of Birds

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Linkage Disequilibrium: Population Genetics of Multiple Loci

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Glossary

Disease association A genetic locus shows an association with a disease when there is a difference in allele or genotype frequencies between an ethnically matched set of patient and control samples.

Hitchhiking The 'hitchhiking effect' refers to genetic changes at 'neutral' loci which are not directly subject to selection, but can exhibit changes in allele frequencies and linkage disequilibrium due to their close physical presence to a selected locus. As an approximate generality, significant effects at a neutral locus are seen if the recombination fraction between the neutral and selected loci is smaller than the order of magnitude of the selected differences at the selected locus (Thomson, 1977).

Permutation test A permutation test is a type of statistical randomization test where the distribution of the test statistic under the null hypothesis is obtained by calculating all possible values of the test statistic (or a random subset thereof) based on shuffling the labels of the observed data. For LD studies, this is accomplished by randomly permuting the allele names at one locus, thus imposing the null hypothesis of no association, and recalculating the measure of LD for each rearrangement.

Recombination fraction Recombination refers to the shuffling of genetic material between pairs of homologous paternal and maternal chromosomes. The recombination fraction θ is calculated as the proportion of combinations of alleles in the offspring not seen in the parents. For loci which are very closely physically linked θ is very close to zero, while the maximum value of θ is 0.5 (50%).

Stratified or conditional analyses When two or more loci in a genetic region show an association with a disease, conditional analyses may indicate the primary effect. If the two loci are not 100% correlated, it is possible to stratify analyses on variation at one locus (performing a separate analysis for each subset at the conditioned upon locus) in order to help distinguish which locus is more likely to be directly involved in disease risk.

Causes of Linkage Disequilibriuim

Linkage disequilibrium (LD), also known as gametic disequilibrium, is the nonrandom association of alleles at two or more loci (see e.g., Slatkin, 2008). Patterns of LD are important to consider in evolutionary biology as well as medical genetic studies. LD can be created by various evolutionary factors: selection (including disease) either directly on one or both loci, or indirectly via a hitchhiking event, migration and admixture, inbreeding, or genetic drift. The most likely cause though is historical – when a new mutation arises there is a nonrandom association created with respect to variation at other polymorphic loci. This association is broken down over time by recombination, at a rate per generation of $(1-\theta)$ for neutral loci, where θ is the recombination fraction. The relationship between LD and the recombination fraction has been used to develop methods for dating mutations based on allele frequencies (Slatkin and Rannala, 2000). LD can remain for a very long time between loci which are physically very close (closely linked loci). This fact is used for mapping mutations related to disease associations and was part of the impetus for the International HapMap Project. This project revealed the block-like structure of LD across the genome. Note, however, that although rare, unlinked markers may show significant LD; also very closely linked loci may be in linkage equilibrium.

The original models of population genetics dealt chiefly with single loci, so that the genome was regarded as a collection of individual independent loci each undergoing its separate evolution. Theoretical and simulation studies of selection and of two- and three-locus systems demonstrated that LD of alleles could be maintained in a stable polymorphism (see Feldman *et al.*, 1974 for references). Simulations were made of multi-locus systems involving more loci and of simple selection schemes (Lewontin, 1964; Franklin and Lewontin, 1970) and it was thought that LD might be quite ubiquitous across the genome, but this turns out not to be the case.

The hitchhiking effects produced on the allele frequencies and LD of linked neutral loci as a selected locus evolves toward its equilibrium value were also studied (Smith and Haigh, 1974; Thomson, 1977; Stephan *et al.*, 2006). As an approximate generality, significant effects on the neutral locus were seen if the recombination fraction between the neutral and selected loci is smaller than the order of magnitude of the selected differences at the selected locus. Further, significant LD could be generated between neutral loci that initially showed no LD (Thomson, 1977).

Migration and admixture of populations with different genetic profiles can create transient LD. Again, this will be maintained for a longer time the more closely linked the loci are. The human leukocyte antigen (HLA) system in the human immune system is known for its strong and extensive LD. Distinguishing among demographic and selective explanations for patterns of variation observed with HLA genes is a challenge (Meyer and Thomson, 2001; Meyer *et al.*, 2006; Single *et al.*, 2007a). However, many features of HLA LD patterns and allelic and amino acid variability implicate selection acting on the classical HLA class I and II loci (summarized in Meyer and Thomson, 2001).

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Measures of Linkage Disequilibrium Strength

The definition of the LD parameter D_{ij} (also written as $D_{A_iB_j}$) of nonrandom association between a pair of alleles A_i and B_j at two loci (A and B) is the difference between the observed (or estimated) haplotype frequency ($f_{ij} = f(A_iB_j)$) and that expected under random association of the two alleles (linkage equilibrium). If the allele frequencies are given by p_{A_i} and p_{B_j}, then $D_{ij} = f_{ij} - p_{A_i}p_{B_j}$ (see **Table 1**, line 1). While this is the base of all measures of LD, defining the 'strength' of any observed nonrandom association is complicated by the fact that the maximum value D_{ij} can take is a function of the observed allele frequencies. In the bi-allelic case, the constraints based on allele and haplotype frequencies (see **Table 1**, footnote b) make it possible to characterize LD with a single disequilibrium parameter D, having an arbitrary sign depending on which are the focal alleles, since $D_{11} = -D_{12} = -D_{21} = D_{22}$.

There are several normalized measures of the strength of LD for 'bi-' and 'multi-allelic data' (Hedrick, 1987; Lewontin, 1988). Each of these statistics represents a single summary of multidimensional data, capturing different aspects of the underlying LD, and has different strengths and weaknesses for addressing specific research questions. The two most common measures of the strength of LD are: (1) the normalized measure of the individual LD values (Lewontin, 1964), $D_{ij}' = D_{ij}/D_{\max}$ (**Table 1**, line 2 for details); and (2) the correlation coefficient r for 'bi-allelic' data, often reported as $r^2 = D_{ij^2}/(p_{A_1}p_{A_2}p_{B_1}p_{B_2})$.

For bi-allelic data, $D' = 1$ whenever one or more of the four possible haplotypes are 'not' observed, irrespective of the expected frequencies. In contrast, r directly measures the correlation coefficient of the bi-allelic variation at two loci. Specifically, $r = 1$ only when the allelic variations at the two loci show 100% correlation, i.e., when both loci have equal allele frequencies and only two complementary haplotypes are observed. This correlation property is of interest to many research questions. For example, if two loci show associations with a disease but r is close or equal to one, then there is little or no variation between them that can distinguish which is more likely to be directly associated with disease risk. In other situations stratification or conditional analyses may allow testing for risk heterogeneity between two potentially disease predisposing genetic variants. For illustration, consider an example with $f_{11} = 0.2$, $f_{12} = 0.3$, $f_{21} = 0$, $f_{22} = 0.5$ for the A_1B_1, A_1B_2, A_2B_1, A_2B_2 haplotypes, respectively. $D' = 1$, since not all haplotypes are observed. However, the value of $r = 0.5$ reflects the variation at the B locus on haplotypes carrying A_1, and variation at the A locus on haplotypes carrying B_2.

Hedrick (1987) extended the D' measure for multi-allelic data as a weighted average over all alleles at each locus of the individual normalized LD values (see **Table 1**, line 4). The multi-allelic extension of the r^2 measure is denoted W_n^2 (see **Table 1**, line 5). In the statistics literature it is known as Cramer's V statistic (Cramer, 1946), defined on the contingency table relating two categorical variables and is a reexpression of the Chi-square statistic, normalized to be between zero and one (Hill, 1975; Hedrick, 1987; Single *et al.*, 2007b).

When there are 'different numbers of alleles' at the two loci, the direct correlation property mentioned above for the r measure is not retained by its multi-allelic extension W_n. The complementary pair of conditional 'asymmetric' LD (ALD) measures, $W_{A/B}$ and $W_{B/A}$ (see **Table 1**, line 6) was developed to extend the W_n measure, and is especially useful when there are 'different' numbers of alleles at the two loci (Thomson and Single, 2014). This leads to cases where W_n may equal or be close to one while one of the two ALD measures is substantially less than one. For example, consider a scenario with two and three alleles at the first and second loci, with $f_{11} = 0.3$, $f_{22} = 0.5$, $f_{23} = 0.2$ for A_1B_1, A_2B_2, and A_2B_3 haplotypes. $W_n = 1$, however there is variation at the B locus on haplotypes containing the A_2 allele. The correlation cannot be 100% when the number of alleles at the two loci differs. In this example, the two ALD measures reflect that while there is no variation of A locus alleles on any of the haplotypes conditioned on the B locus alleles ($W_{A/B} = 1$), there is variation at the B locus on haplotypes carrying A_2 ($W_{B/A} = 0.73$). The ALD measures show that with appropriate sample size, stratification analyses could

Table 1 Linkage disequilibrium and genetic diversity measures

Description	*Definition of measures*[a]
1. Pairwise multi-allelic LD[b]	$D_{ij} = f_{ij} - p_{A_i}p_{B_j}$
2. Individual normalized LD[c]	$D_{ij}' = D_{ij}/D_{max}$
3. r^2 (bi-allelic)	$r^2 = D_{ij}^2/(p_{A1}p_{A2}p_{B1}p_{B2})$
4. Multi-allelic D' extension	$D' = \sum_i\sum_j p_{A_i}p_{B_j}\lvert D_{ij}'\rvert$
5. Multi-allelic r^2 extension[d] (overall LD squared)	$W_n^2 = \left[\sum_i\sum_j D_{ij}^2/\left(p_{A_i}p_{B_j}\right)\right]/\min(k_A-1,\ k_B-1) = [X_{LD}^2/2N]/\min(k_A-1, k_B-1)$
6. Multi-allelic squared ALD[e] (overall asymmetric LD squared)	$W_{A/B}^2 = \left[\sum_i\sum_j\left(D_{ij}^2/p_{B_j}\right)\right]/(1-F_A)$
	$W_{B/A}^2 = \left[\sum_i\sum_j\left(D_{ij}^2/p_{A_i}\right)\right]/(1-F_B)$

[a] Summation is over all i=1, 2, …, k_A and j=1, 2, …, k_B, where k_A and k_B are the number of alleles respectively at the A and B loci. The observed sample size is denoted by N individuals ($2N$ alleles/haplotypes).

[b] $\sum_j D_{ij} = 0,\ \sum_i D_{ij} = 0,\ \sum_j f_{ij} = p_{Ai},\ \sum_i\sum_j f_{ij} = 1$

[c] $D_{\max} = \min\left[p_{Ai}(1-p_{Bj}), (1-p_{Ai})p_{Bj}\right]$ if $D_{ij} > 0$

$D_{\max} = \min\left[p_{Ai}p_{Bj}(1-p_{Ai})(1-p_{Bj})\right]$ if $D_{ij} < 0$. The range of D_{ij}' is (–1, 1).

[d] The range of W_n^2 is (0, 1).

[e] F_A and F_B are expected homozygosity values under Hardy–Weinberg proportions: $F_A = \sum_i p_{A_i}^2$ $F_B = \sum_j p_{B_j}^2$.

be carried out for specific comparisons. An appealing property of the ALD measures is that $W_{A/B} = W_{B/A} = W_n$ when there is symmetry in the observed data and thus for bi-allelic single nucleotide polymorphisms (SNPs).

Several conditional LD measures have been proposed for mapping disease predisposing genes via the association of marker genes near the putative disease predisposing locus (Nei and Li, 1980; Chakravarti *et al.*, 1984; Hudson, 1985; Guo, 1997). The difference in proportions statistic, $d_* = (f_{A_2B_1}/p_{A_2} - f_{A_1B_1}/p_{A_1})$, of Nei and Li (1980) measures the association between alleles at a marker locus (B) and a disease locus (A). It is applicable to study designs of rare diseases where individuals are not randomly sampled from a single population, but sampling intensity varies within disease categories (Kaplan and Weir, 1992; Maiste and Weir, 1992). Other conditional measures have been applied related to the presence and strength of disease risk in a genetic region (Levin and Bertell, 1978; Bengtsson and Thomson, 1981). Devlin and Risch (1995) summarize these and other measures used in LD mapping.

Multi-Locus Linkage Disequilibrium Measures

When a large number of loci are involved, it is common to use graphical techniques to explore patterns of LD based on all pairwise combinations among loci (e.g., Abecasis and Cookson, 2000; Barrett *et al.*, 2005). The measures described below can be used in the two-locus case and easily extend to more than two loci. Nothnagel *et al.* (2002) proposed a multi-locus LD measure based on the degree of structure in a set of haplotypes as measured by entropy. Entropy measures the disorder or lack of predictability in a system and is thus maximal when all haplotypes are equally likely and zero when there is only one haplotype (i.e., complete predictability). For a set of observed haplotype frequencies, f_i, the entropy, S_{obs}, is defined as $-\Sigma_i\, f_i\, \log(f_i)$. The normalized entropy difference is defined as $(S_{eq} - S_{obs})/S_{eq}$, where S_{eq} is the entropy based on haplotype frequencies expected under linkage equilibrium. Sabatti and Risch (2002) describe the use of haplotype homozygosity, $\Sigma_i \Sigma_j f_{ij}^2$, as a measure of LD. This measure works equally well with bi-allelic and highly polymorphic data as its definition does not depend on the number of alleles at either locus. Another measure of overall multi-locus LD is based on the observed likelihood-ratio statistic, S_{obs}, for testing LD significance. The standardized statistic is defined as $\xi = \left(\sqrt{2df}/N\right)([S_{obs} - \mu_S]/\sigma_S)$, where μ_s and σ_s are the mean and standard deviation of the permutation distribution for S (Zhao *et al.*, 1999). Details of the permutation distribution are provided in the next section on significance testing.

Linkage Disequilibrium Significance

The statistical significance of individual LD coefficients, D_{ij}, can be tested using $X_{ij}^2 = (2N)D_{ij}^2/p_i(1-p_i)q_j(1-q_j)$ (Slatkin and Excoffier, 1996; Weir, 1996), which is the number of haplotypes times the r^2 measure in the SNP setting with two alleles at each locus. For multi-allelic loci, the X_{LD}^2 statistic, listed in the alternate definition for W_n, in Table 1 (line 5) can be used to test the significance of overall LD. However, for highly polymorphic data a permutation test is recommended (Slatkin and Excoffier, 1996).

Likelihood-ratio tests relate the likelihood of the observed data with no constraints (L_1), to the likelihood of the data under the null hypothesis of linkage equilibrium (L_0), where haplotype frequencies are computed as the product of allele frequencies. The likelihood-ratio statistic, $S = 2\log(L_1/L_0)$, has an asymptotic Chi-square distribution with $(k_A - 1) \times (k_B - 1)$ *df*. The Chi-square approximation for S and X_{LD}^2 can be poor for highly polymorphic loci. A better approximation for the distribution of S under the null hypothesis of no LD can be generated by first permuting phenotypes at each locus between individuals, thus imposing equilibrium. In the second step, the likelihood of the permuted data, L_1, and a corresponding new value of S is computed for the permuted sample. These two steps are repeated a large number of times to give the permutation distribution of S, which can be used to compute a p-value for the test of no LD.

Linkage Disequilibrium and Natural Selection

The homozygosity F statistic ($F = \Sigma_i f_i^2$) can be used to detect selection at a single locus (Ewens, 1972; Watterson, 1978; Slatkin, 1994, 1996). The Ewens–Watterson test compares the observed sample homozygosity to the distribution of values expected under a null hypothesis of no selection. Haplotype homozygosity was described above as one measure of multi-locus LD. Sabeti *et al.* (2002) defined the extended haplotype homozygosity (EHH) in order to examine signatures of recent positive selection using a collection of SNPs. A stepwise approach was used to study the breakdown of LD over increasing distances from a 'core' region by including an increasing number of SNPs at each step. The haplotype homozygosity is computed for each of the core SNP-defined haplotypes. At each step of the EHH computation, additional SNPs are considered with the effect of smoothing the results compared with considering the markers individually. While this is a good approach for neighboring SNPs, it is not practical for more broadly spaced polymorphic markers such as microsatellites or HLA. The conditional ALD measures described above were originally derived as a weighted average of haplotype specific homozygosity values and are informative for detecting selection acting independently on loci in high LD (Thomson and Single, 2014).

The 'disequilibrium pattern analysis' (DPA) (Klitz and Thomson, 1987; Thomson and Klitz, 1987) and 'constrained disequilibrium values' (CDV) (Robinson *et al.*, 1991a) methods are two complementary approaches that have been used to detect selection acting on sets of HLA loci. These methods also apply to other closely linked loci; the DPA method requires more than bi-allelic variation. Results from the two methods have identified specific HLA haplotypes that show signs of past selection in specific populations.

The DPA method identifies patterns of pairwise LD that are consistent with present or recently past selective events in the genetic region. Suppose that selection was acting directly on the A_1B_1 haplotype. Related haplotypes which share an allele with a selected haplotype (i.e., A_1B_j $j \neq 1$ and A_iB_1 $i \neq 1$) then have an expected value of pairwise LD that is proportional to

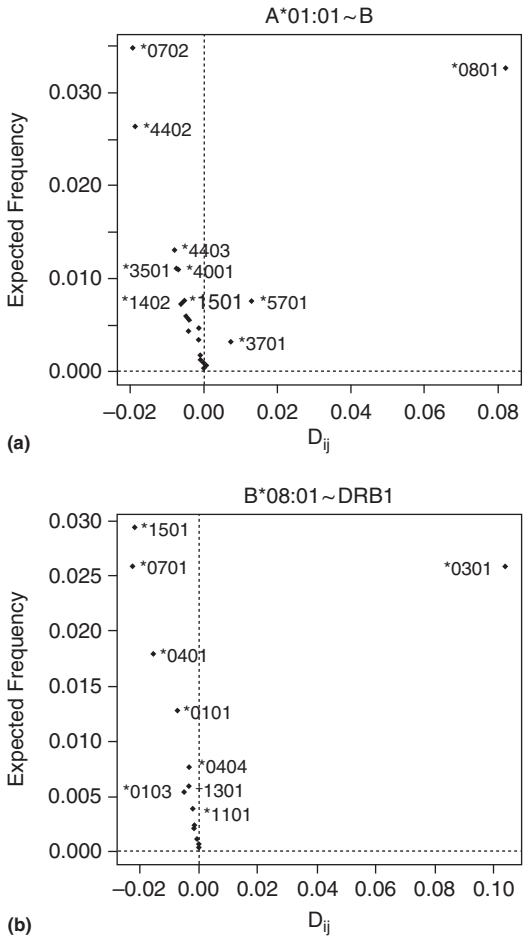

Figure 1 DPA analysis for pairwise combinations of the class I HLA-A and B loci, and the class II DRB1 locus with the alleles A*01:01, B*08:01, and DRB1*03:01 constituting the putative selected haplotype. The allele in the figure title is the one that is conditioned on (e.g., A*01:01 in (a)) with frequencies and LD values plotted for each allele at the second locus listed in the figure title (e.g., B alleles found with A*01:01 in (a)). In each case, a single allele pair in the upper right quadrant (A*01:01 ~ B*08:01 in (a)) showing high positive LD and frequency along with the linear pattern for the negative LD values is indicative of selection. This linear pattern is not expected if selection is not acting, in which case a random scatter of values is typical. Adapted with permission from Williams, F., Meenagh, A., Single, R., *et al.*, 2004. High resolution HLA-DRB1 identification of a Caucasian population. Human Immunology 65, 66–77.

the frequency of the unshared allele. This is illustrated in Figure 1 for HLA data, adapted from Williams *et al.* (2004), for HLA class I data in a Caucasian population.

Three-locus systems impose additional constraints on the range of possible pairwise LD values. In the simplest case of three bi-allelic loci (*A*, *B*, and *C*) there are eight haplotypes which can be completely specified by seven parameters: three allele frequencies p_{A_1}, p_{B_1}, and p_{C_1} (with $p_{X_2} = 1 - p_{X_1}$), three pairwise LD parameters denoted D_{AB}, D_{AC}, and D_{BC} (with $D_{XY} = D_{X_1Y_1} = -D_{X_1Y_2} = -D_{X_2Y_1} = D_{X_2Y_2}$) and one three-locus LD parameter denoted D_{ABC} (with $D_{ABC} = D_{A_1B_1C_1} = -D_{A_1B_1C_2} = -D_{A_1B_2C_1} = D_{A_2B_1C_1} = -D_{A_2B_1C_1} = D_{A_2B_1C_2} = D_{A_2B_2C_1} = -D_{A_2B_2C_2}$). The general formulation (Geiringer, 1944; Bennett, 1954; Feldman *et al.*, 1974) for three-locus haplotypes is:

$$f_{ijk} = f(A_iB_jC_k) = p_{A_i}p_{B_j}p_{C_k} + p_{A_i}D_{B_jC_k} + p_{B_j}D_{A_iC_k} + p_{C_k}D_{A_iB_j} + D_{A_iB_jC_k}$$

The CDV method has been used to detect selection events in the HLA region (Robinson *et al.*, 1991b; Grote *et al.*, 1998) by examining the pattern of pairwise LD values imposed by a three-locus system (D'') compared to those in the respective two-locus system (D'). The difference between the two measures, $\Delta = |D'' - D'|$, has a distribution which can be indicative of selection (e.g., if one of the three Δ values is positive and the other two are zero or negative – see Robinson *et al.*, 1991a; Grote *et al.*, 1998 for complete details). The DPA and CDV methods do not capture all signals of selection, but often show agreement in these conditional analyses.

See also: Inbreeding and Nonrandom Mating. Population Structure and Gene Flow

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Lophotrochozoa, Diversification of

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Introduction

Lophotrochozoa is a monophyletic group of animals that includes annelids, molluscs, bryozoans, brachiopods, platyhelminthes, and other animals that descended from the common ancestor of these organisms. Lophotrochozoa is one of the three major clades that comprise bilateral animals, or Bilateria. Another superphylum Ecdysozoa, comprising nematodes, arthropods, and their relatives and which are defined by molting, are the sister taxon to Lophotrochozoa. The third major bilateral group is Deuterostomia, which includes hemichordates, echinoderms, and chordates (vertebrate organisms belong to this group). Of the three major bilaterian groups, Lophotrochozoa possesses the greatest morphological disparity as judged by the greatest diversity of distinct body plans.

The lophotrochozoan hypothesis was first posited based on 18S nuclear ribosomal subunit gene DNA data by Halanych *et al.* (1995). Prior to the 18S results, lophophorate phyla, that is brachiopods, phoronids, and bryozoans (**Figure 1**) were generally considered to be more closely allied to deuterostome animals than annelids (**Figure 2**) and molluscs (**Figure 3**). The lophophorate taxa are characterized by the presence of a ciliated tentacular apparatus that surrounds the mouth but not the anus and is invaded by mesoderm. Ribosomal gene data showed that these three taxa were actually nested well with protostome taxa challenging concepts about evolutionary importance and conserved nature of developmental processes such as spiral cleavage, coelom formation, and blastopore fate. The name 'Lophotrochozoa' was chosen to refer to the fact that taxa with a lophophorate feeding structure were allied to those with a trochophore larva (a larva most typically characterized by annelids and molluscs that possess specific sets of ciliated feeding bands).

In 1997, Ecdysozoan paper was published by Aguinaldo *et al.* which established that nematodes, arthropods, and their allies formed a clade, but it also affirmed support for the Lophotrochozoa hypothesis (Aguinaldo *et al.*, 1997). This paper was also based on 18S ribosomal gene data. Importantly, Aguinaldo and colleagues placed platyhelminth flatworms within Lophotrochozoa and put forth an expanded definition of the group. In the 1990s, molecular phylogenetics was still a relatively new field and these results met considerable skepticism. In particular, the Articulata concept, which posited that segmented annelids and arthropods are closely related, was firmly entrenched as a popular hypothesis prior to the molecular revolution. Although several papers also using 18S ribosomal data consistently supported the lophotrochozoan and ecdysozoan hypotheses over others, skepticism persisted until de Rosa and colleagues showed that the Hox genes, transcription factors used in early development, supported placement of brachiopods within protostome animals (de Rosa *et al.*, 1999). Within the Hox complex there are ortholog groups (e.g., Post 1, Post 2) that show fidelity to either Lophotrochozoa, Ecdysozoa, or Deuterostomia. Shortly thereafter, topologies based on other sources of molecular data (e.g., 28S nuclear ribosomal gene, myosin II heavy chain – Mallatt and Winchell, 2002; Ruiz-Trillo *et al.*, 2002, respectively) also confirmed the 'New Animal Phylogeny' (Halanych, 2004). As this consensus was emerging, multigene studies based on expressed sequence tag (EST) data were becoming feasible. Some of the early genome scale studies (e.g., Philippe *et al.*, 2005; Dunn *et al.*, 2008) confirmed that the existence and membership of these three animal super groups: Deuterostomia, Ecdysozoa, and Lophotrochozoa.

Unlike ecdysozoans, where the shared derived trait of molting has been hypothesized to define the group, there is not a single well-defined feature that can be used to circumscribe lophotrochozoans. Given that organisms were placed in separate phyla due to the lack of similarities, this lack of a common character is indicative of the great diversity of animal forms. The clade Lophotrochozoa has incorrectly been equated to 'Spiralia' (e.g., Giribet, 2008). The term 'Spiralia' is problematic, due to its long and variable use, the fact that numerous lophotrochozoan taxa do not have spiral cleavage, and that early development is more variable than traditionally realized.

Members and Relationships

Lophotrochozoa is perhaps the least understood 'superphylum' in terms of evolutionary relationships within the group. As such our understanding of the group has been changing and will continue to do so as more data is brought to bear on issues of memberships and evolutionary relationships. Here, a general consensus at the time of writing this article is presented, and **Figure 4** serves as a guide to our current understanding of lophotrochozoan evolution. Lophotrochozoa includes two major groups of phyla, Trochozoa and Platyzoa. The names and even monophyly of these groups varies among different studies. Trochozoa includes molluscs, annelids, and nemerteans (aka ribbon worms). Additionally, brachiopods and phoronids, which are sister taxa, are also clearly associated with these taxa, but were not typically considered trochozoans. Exact relationships among these groups are less certain. Dunn *et al.* (2008) placed annelids sister to a brachiopod, phonorid, nemertean clade with molluscs just outside this group. Hejnol *et al.* (2009) recovered molluscs as sister to a brachiopod and nemertean clade with annelids sister to these taxa. Kocot *et al.* (2011) on the other hand found annelids and molluscs to form a clade with brachiopods as the sister taxon and nemerteans outside of this group. Thus, relationships of the Trochozoa group are, at present, still unclear. Sipunculids (peanut worms), echiurid (tongue worms), and myzostomids (parasites of echinoderms) have been shown to be within the annelid radiation (Struck *et al.*, 2007; Weigert *et al.*, 2014) and thus are not considered individually here.

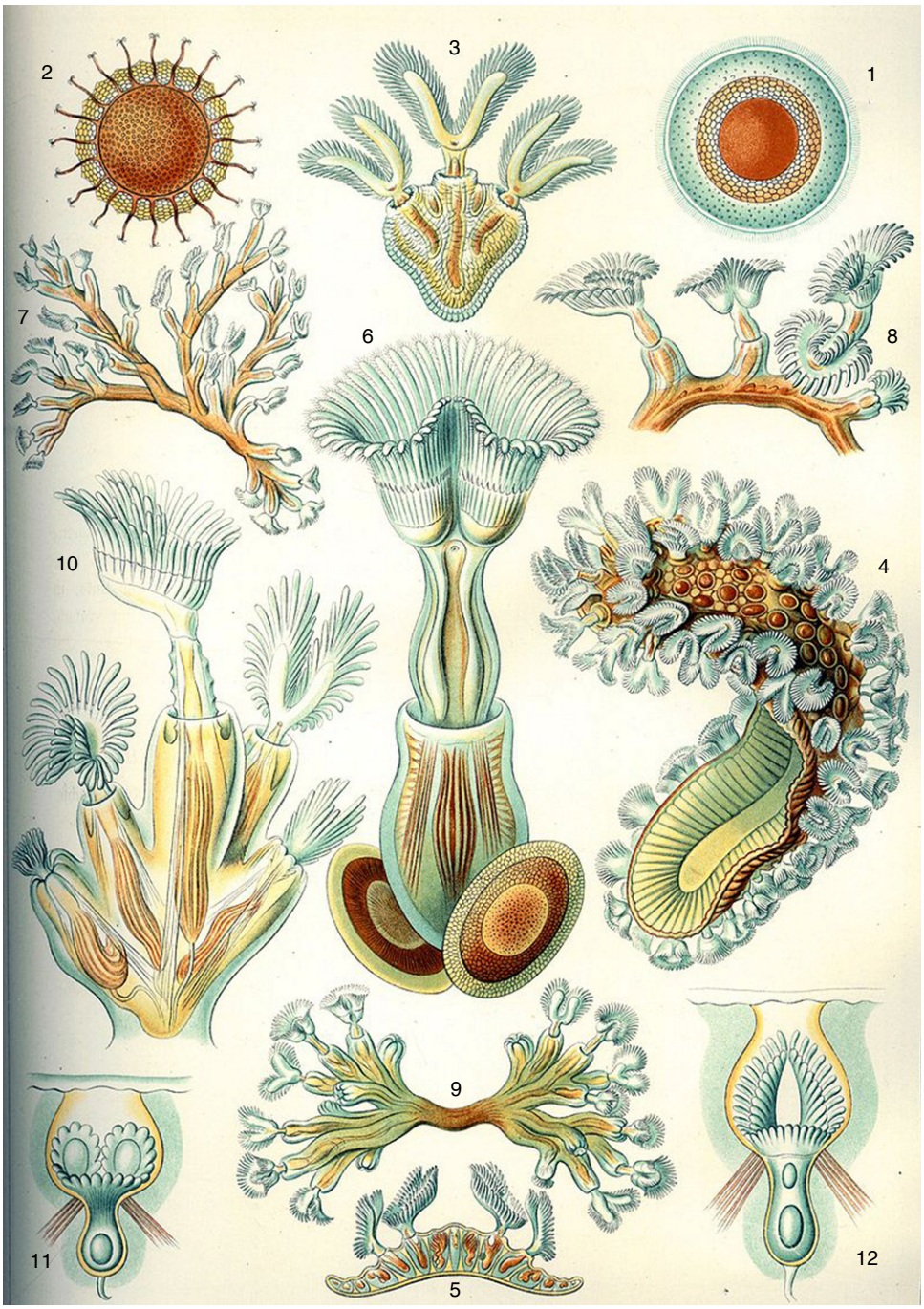

Figure 1 Haeckel's plate for Bryozoans, also known as moss animals. Reproduced from Haeckel, E., Bibliographisches Institut Leipzig, 1904. Kunstformen Der Natur. Leipzig und Wien: Verlag des bibliographischen Instituts. Available at:https://en.wikipedia.org/wiki/Bryozoa#/media/File:Haeckel_Bryozoa.jpg (accessed 21.12.15).

Both reviews and primary data papers (Halanych, 2004; Giribet, 2008; Hejnol *et al.*, 2009; Kocot *et al.*, 2010) report a Platyzoan clade that consists of at least Platyhelminthes (exclusive of Acoelomorpha), Gastrotricha, Gnathostomulida, Micrognathozoa, and Syndermata (which includes Rotifera and Acanthocephala). However recent phylogenomic work (e.g., Struck *et al.*, 2014) argues that Platyzoa is not a monophyletic group and that a platyhelminth plus gastrotrich clade is more closely related to trochozoans than syndermatans. Unfortunately, this region of the animal tree is not well understood for several reasons: (1) many platyzoans have reduced morphology and modified lifecycles due to a parasitic or meiofaunal lifestyle, (2) taxa in this region of the tree have been poorly sampled for molecular work, and (3) many taxa have elevated rates of nucleotide substitution relative to other metazoans. In particular, the issue of elevated substitution

rates can lead to artifacts in phylogeny reconstruction (e.g., long-branch attraction) that can be very hard to correct.

Other taxa that are clearly within Lophotrochozoa are bryozoans, entoprocts (or kamptozoans), and cycliophorans. There is considerable evidence that cycliophoran (symbionts that live on lobsters lips) are closely allied if not within entoprocts (Hejnol *et al.*, 2009; Kocot *et al.*, 2011; Struck *et al.*, 2014). However, this group and bryozoans are typically very unstable in phylogenetic analyses. Their exact position varies within the topology based on taxon and gene sampling and the choice of phylogenetic reconstruction method. For example, some analyses place bryozoans next to platyzoans

Figure 2 Annelids are segmented representatives of lophotrochozoans that include this earthworm, *Eisenia fetida*. https://en.wikipedia.org/wiki/Eisenia_(annelid)#/media/File:Redwiggler1.jpg

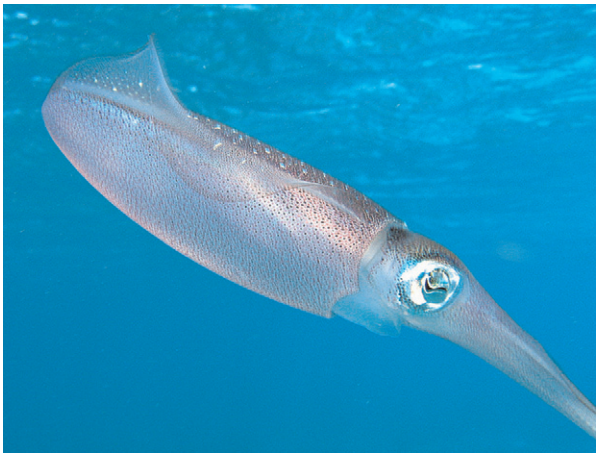

Figure 3 Molluscs, including this squid, have a long fossil history dating back to the Cambrian. https://en.wikipedia.org/wiki/Lophotrochozoa#/media/File:Caribbean_reef_squid.jpg

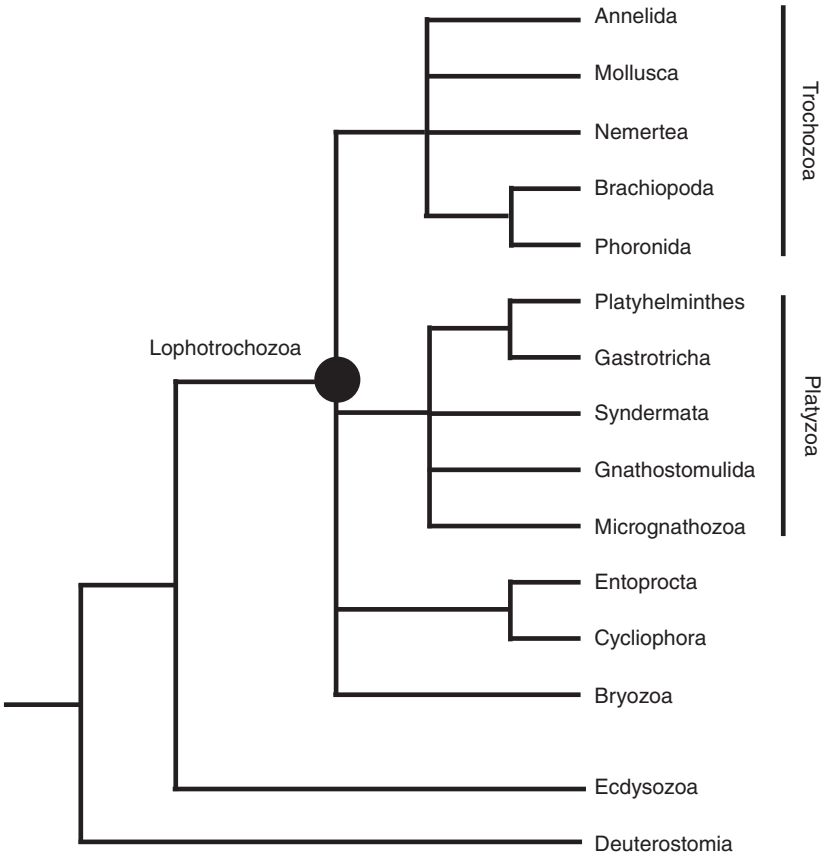

Figure 4 Summary of the present understanding of Lophotrochozoan relationships. Some regions of the tree are shown as unresolved as placement of some groups is currently under debate.

whereas other studies place them next to trochozoans. At present, the position of these taxa in the tree is not certain.

Origins

All three major bilaterian clades arose prior to the Cambrian period, which marks the beginning of the Paleozoic Era at 542 million years ago. Fossils from this period show a marked increase in the number of multicellular organisms. Moreover, the Cambrian explosion near the middle of the period is renowned for the diversity and complexity of animal fossils with many phyla and taxa belonging to modern-day crown groups. Organisms that belong to molluscs, brachiopods, and annelids are reported from the Cambrian implying the origin of the group is older. Although lophotrochozoan clearly existed before the Cambrian, whether Ediacaran fossils can be assigned to extant lophotrochozoan groups is less certain. For example, much debate has surrounded *Kimberella*, a sluglike form first found in the Edicara Hills of Australia that has been debated to be mollusc. Similarly, *Wiwaxia* is concerned within the lophotrochozan radiation but placement in a crown group has been debated.

See also: Adaptive Radiations: Insights From Evo-Devo. Bacterial Species Concepts. Hybrid Speciation. Reinforcement

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Glossary

Adaptive peak A mathematical model for evolution toward an adaptive optimum symbolized by a hill peak, the top of which represents the point of maximum fitness.

Brownian motion A pattern of evolution in which a trait changes randomly from one generation to the next, thus serving as a null hypothesis for the outcomes expected from random evolutionary processes. Brownian motion produces evolutionary outcomes that have similar statistical properties as diffusion processes in physics and chemistry.

Drift Random evolutionary change with a Brownian motion pattern. Neutral drift occurs because of chance sampling in the survival of an offspring generation, with a rate that is determined by the reproductive population size. Selective drift occurs by natural selection in which the direction and magnitude of selection change randomly over time.

Evolutionary trajectory The evolutionary path of a quantitative trait through a morphospace.

Macroevolution Evolution over longtime scales. It can refer to the long-term history of a population lineage through time (anagenesis), to divergence between populations after reproductive isolation, or to processes other than the microevolutionary processes associated with drift, selection, and reproductive isolation.

Morphospace A mathematical space, usually multidimensional, with axes that represent the range of possible values of phenotypic traits. One of the simplest morphospaces is a bivariate plot of simple measurements such as the length and width of a structure. Morphospaces are frequently used to show the relative similarity of individual organisms, populations, or species.

Neo-Lamarckian A school of evolutionary thought that was dominant in the late nineteenth and early twentieth centuries that placed importance on the now-discarded theory of inheritance of acquired characters. Neo-Lamarckism was abandoned in light of discoveries about Mendelian inheritance and the development of population genetic theory leading to the Modern Evolutionary Synthesis.

Ornstein–Uhlenbeck process A process that produces a pattern of evolution in which the phenotypes evolve around an optimum and are constrained from diverging too far from it. Evolution on an adaptive peak is an example of an Ornstein–Uhlenbeck process.

Orthogenetic An evolutionary process, now largely disproven, in which the phenotype evolves in a continuous direction because of factors internal to the organism, eventually driving the species to extinction as the phenotype becomes less and less fit. Orthogenesis arose from early twentieth century discoveries about genetic inheritance, especially homeotic mutations, and largely replaced Neo-Lamarckism until orthogenesis itself was replaced by the Modern Synthesis evolutionary theory in which selection and drift act on mutations within a population structure.

Parsimony An algorithm for reconstructing phylogenies from discrete (noncontinuous) characters in which the number of character changes is minimized.

Phylogeography Phylogenetic divergence among geographically separated populations that occurs within species and between closely related species.

Saltationist An evolutionary process in which phenotypic changes occur in large bursts without continuous intermediates between ancestor and descendant. Like orthogenesis, saltationism arose from twentieth century work on homeotic mutations and was replaced by Modern Synthesis population-based theory.

UPGMA An early algorithm for constructing trees from continuous phenotypic traits, now superseded by Neighbor-joining and maximum-likelihood algorithms.

Introduction

Whether quantitative genetic processes can explain macro-evolutionary processes has been an important question in evolutionary biology since paleontologist George G. Simpson applied the concept of an adaptive landscape to explain the origin of grazing horses by natural selection and environment-driven changes in fitness (Simpson, 1944). While Simpson did not literally apply quantitative genetic models to his fossil data, he did adopt quantitative genetic concepts like adaptive peaks

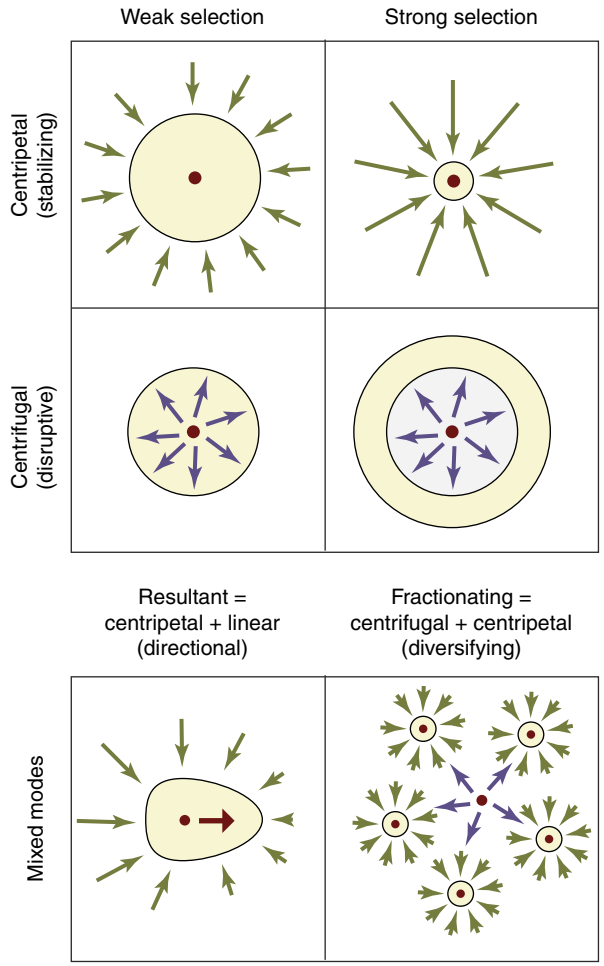

Figure 1 Modes of selection used by Simpson (1944) to explain macroevolutionary patterns, with today's equivalent terms. The top four examples result in no net change in the group (red dot shows the group mode), but affect the pattern of within-group variation (cream colored area). Centripetal selection, which is the same as stabilizing selection, pushes variation toward the mode; centrifugal selection, which is the same as disruptive selection, pushes variation away from the mode equally in all directions. Simpson envisioned many combinations of selective patterns, two of which are shown in the bottom panels. Centrifugal selection that is unequal results in directional change in the group mode. A combination of centrifugal selection on individual groups but centripetal selection between groups, perhaps as a result of intergroup competition, results in fractionating selection (diversifying selection in current terminology).

from Sewall Wright's work on mutation, allele frequencies, selection, and population divergence (Wright, 1932). Simpson argued that the long-term evolution documented by the fossil record – macroevolution, including not only the origin and evolution of species, but also their phylogenetic and phenotypic diversification – could be explained by the same microevolutionary population processes that are studied by quantitative geneticists (Figure 1). Simpson's goal was to demonstrate that the varied rates of evolution in phenotypic traits measured from sequences of fossils could be explained by varied regimes of natural selection. His integration of genetics, paleontology, and macroevolution made him one of the architects of the Modern Synthesis theory of evolution, which in the 1930s and 1940s replaced the orthogenetic, saltationist, and neo-Lamarckian theories that were current at the beginning of the twentieth century (Bowler, 1992).

Controversies

The Modern Synthesis view that macroevolution is the result of population-level microevolutionary processes quickly dominated the field but not without controversy. Simply put, the Synthesis view is that evolution is a population-level process, that phenotypic variation in populations arises from mutation and recombination, that population means change from one generation to the next by natural selection that differentially preserves those phenotypes that increase individual fitness or by the chance sampling process of drift. Coupled with factors that isolate one population from another, these processes are arguably sufficient to account for macroevolutionary patterns observed at a geological time scale such as the origin and diversification of major clades, changes in taxonomic diversity through time, or phenotypic changes within a long-lived species through time.

Challenges to this Synthesis view have come primarily from scientists who have argued that additional, non-population processes are required to explain some macroevolutionary patterns. Examples that are relevant to this discussion are punctuated equilibrium, the hypothesis that most species do not change much during their history except for rapid bursts of change at speciation (Eldredge and Gould, 1972); developmental constraints, the hypothesis that processes of organismal development channel and constraint variation and thus strongly influence macroevolutionary outcomes (Alberch, 1982); neutral evolution, the hypothesis that most evolutionary change occurs by random fixation of mutations or variants rather than by selection based on improved fitness (Kimura, 1983); and species or clade selection, the hypothesis that selective processes not only at the level of individual fitness but also on emergent traits of species and clades (Jablonski, 2008). Scientific debate about the impact of these three processes on macroevolution has encouraged the development of fully quantitative models for phenotypic evolution and their application to comparative and paleontological data to test whether quantitative genetic patterns observed at the population level are commensurate with long-term macroevolutionary patterns observed in comparative phylogenetic and paleontological data.

Nonmetric Traits

Early applications of quantitative genetics to macroevolution fell into two categories: nonmetric trait analysis, which was modeled on the analysis of allele frequencies, and continuous trait analysis, the theory for which was slower to develop. Nonmetric trait analysis focused on the frequencies of minor variations of the phenotype, such as numbers of foramina (small openings in bones for vessels and nerves), numbers of cusps on teeth, numbers of bristles on fruit flies, and numbers of spots on moth wings. The frequencies of

these traits, which were either known or assumed to be heritable, were compared within and between populations and species to determine the amount of divergence and whether drift alone, including founder effects, could explain the divergence or whether selection needed to be invoked. The nonmetric trait approach was grounded in the early genetic work of Sewell Wright (e.g., Wright, 1934) and extensively elaborated by Ford (1945), whose seminal evolutionary work on industrial melanism in moths used it; Berry (1978), who used it to study founder effect, selection, and phylogeography in mainland and island populations; and Sjøvold (1977), who enlarged the statistical toolkit for analyzing nonmetric trait divergence at macroevolutionary scales. Theory and process of the evolution nonmetric phenotypic traits were developed at length in the book *Phenetics* by Yablokov (1986), a student of the prominent geneticist Timofeev-Ressovsky. Despite the potential of nonmetric traits for studying the relationship of quantitative genetic processes and macroevolution, this approach has received little attention since the 1980s.

Continuous Quantitative Phenotypic Traits

Selection Regimes and Rates of Evolution

While nonmetric traits were important in early research on evolution of the phenotype, most studies of genetic processes and macroevolution have been based on quantitative traits. Advances in multivariate morphometrics and phylogenetic theory of quantitative traits have allowed complex new questions to be asked, as described in more detail below. Simpson's early work, and those who followed his lead, involved relatively simple phenotypes, usually sizes of teeth and bones that could be measured in both living and fossil animals, and focused on questions that could be answered by comparing rates of evolution, direction of selection, or intensity of selection.

Simpson, Haldane, and Kurten made early contributions to our understanding of how selection changes as environments change by measuring rates of evolution in living taxa from different environmental regimes and measuring sequences of fossils through major environmental transitions (Simpson, 1944; Haldane, 1949; Kurtén, 1959). Simpson (1944) classified evolutionary rates into three classes based on the distribution of their magnitudes within a taxonomic group or clade: horotelic distributions are the norm, with many bursts of rapid evolution interspersed with lower rates; bradytelic distributions are characterized by slow rates with most near zero, arising in situations where evolution is constrained by stabilizing selection, diminished genetic variation, or other factors; tachytelic distributions are unusually rapid and include exceptionally fast bursts of 'quantum evolution' as lineages cross from one adaptive zone to another. Simpson explained all three of these modes of evolution – stabilizing, random, and directional modes of selection are their parallels – in terms of population genetic processes, especially variation in the environmental and evolutionary context controlling the intensity of selection (**Figure 1**). Wright's (1932) shifting balance explanation for how a species could shift from one adaptive peak to another was an important model for Simpson's explanation for the origin of higher taxa, or adaptive radiations, by colonization of new adaptive zones, an idea that has been tested by comparing rates of evolution (Kurtén, 1959), comparing modes of selection in a quantitative phylogenetic framework (Schluter, 2000), and by measuring phylogenetic evolution of multivariate phenotypic traits in morphospaces (Polly, 2008a). The study of rates of evolution was formally placed in a quantitative genetic framework by Gingerich, who proposed that rates be measured in units of haldanes, which is the average change in standard deviations per generation, that they be scaled to take into account the probability of evolutionary reversals that occur over longtime periods, and that measurements of macroevolutionary divergence be scaled in a comparable way (Gingerich, 1993, 2001). This work made it possible to convert phenotypic rates of evolution measured over thousands or millions of generations to quantitative genetic parameters such as heritability and selection intensity. Selection has also been measured directly from sequences of well preserved fossils from which demographic age structures and, hence, individual survivorship can be estimated (Van Valen, 1965; Bell, 1988).

Adaptive Peaks

Although the adaptive landscape was used as a metaphor for the processes governing variation in rates of phenotypic evolution and the origin of taxa, it was not placed in a fully quantitative framework until Lande's work in the late 1970s. Lande presented equations that describe the intensity and direction of selection on the population mean of a quantitative phenotypic trait at any point on an adaptive peak (**Figure 2**), derived estimates of the

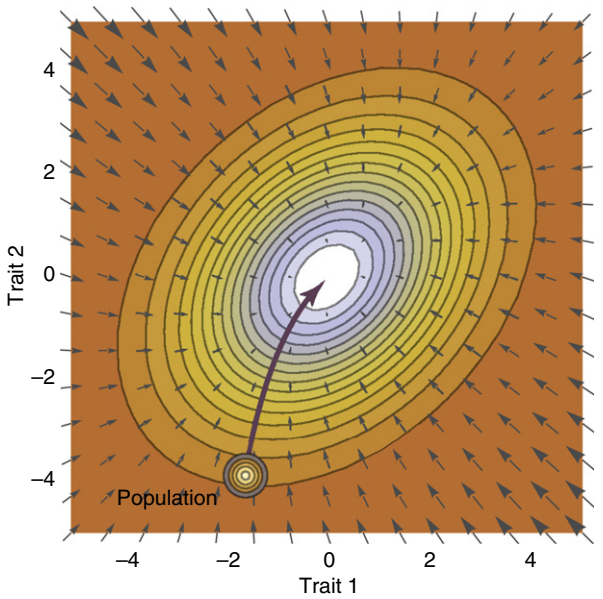

Figure 2 Graphic depiction of evolution on an adaptive peak. Joint fitness of two phenotypic traits defines the contoured peak (lighter colors indicate higher fitness). Selection vectors (small arrows) show the direction and intensity of selection at different points on the peak. A population is shown as a smaller set of contours that show the mean and variance of phenotype. Over time, selection will push the population in a predictable path up the peak (heavy arrow).

rate and direction of evolutionary response to that selection, and described the trade-offs between drift and selection on the evolution of the phenotype (Lande, 1976). Lande's equations were based firmly in population quantitative genetic principles of population variance, heritability, fitness, and multilocus genetic underpinnings of phenotypic traits. One of the goals of the quantitative framework was to determine whether challenges to the Modern Synthesis, especially the hypotheses that most evolution was neutral or random, that developmental and other constraints channel the direction of long-term evolution, and that most phenotypic change occurs at speciation, were consistent with quantitative genetic principles (Charlesworth *et al.*, 1982), so it was elaborated to include adaptive peak shifts as model for long-term changes in the environmental context that defines fitness (Lande, 1986) and for correlated phenotypic traits (Lande and Arnold, 1983). Lande's own conclusions were that drift, selection, and a phenotypic version of Wright's shifting balance theory were adequate to explain, perhaps even to predict, patterns of punctuated equilibrium and that developmental and other constraints on evolution were consistent with quantitative genetic models for multivariate phenotypic traits (Lande, 1985, 1986). Importantly, expansions of Lande's model now form the basis for most of the quantitative analysis of phenotypic evolution (Arnold *et al.*, 2001; Gingerich, 2009; Hansen, 2013; Doebli, 2013; Bell, 2013).

Phylogenetic Comparative Methods

In parallel to adaptive peak models, methods for analyzing traits in a phylogenetic context were also developed. Felsenstein (1973) proposed a maximum-likelihood algorithm for reconstructing phylogeny from continuous phenotypic traits when they have evolved by a Brownian motion process such as drift or randomly changing selection. Unlike previously existing morphometric tree-building algorithms such as the unweighted pair-group averaging (UPGMA) method, or then-emerging discrete-trait algorithms such as parsimony, Felsenstein's method was explicitly grounded in probabilistic evolutionary quantitative genetic theory and balanced the probability of character reversals with character change using the statistical properties of Brownian motion. In the simplest situations, the phenotypic divergence between taxa evolving by Brownian motion is a function of their rate of evolution and shared phylogenetic history. A single phenotypic trait evolving in an ancestor-descendant lineage by Brownian motion will have a possible distribution of descendant phenotypes whose mean is equal to the ancestor's phenotype and whose variance is equal to the squared rate of change per generation times the number of generations elapsed (Felsenstein, 1988). Because the statistics of Brownian motion are nondirectional, the same distribution describes the range of possible ancestor phenotypes given an observed descendant phenotype. The likelihood of phenotypes of the ancestor of two observed descendants is described by the joint probabilities of the descendants. By taking into account the ancestral likelihood distributions of many taxa, the overall likelihoods of competing phylogenetic hypotheses can be estimated and the most likely one identified. Using the same logic, the likelihood that traits evolved by Brownian motion can be evaluated if the phylogenetic tree is already known, their rate of evolution can be estimated, the likely ancestral phenotypes can be estimated, and the covariances that arise from shared common ancestry can be removed in order to study non-phylogenetic factors that cause trait covariance, such as genetic correlations or developmental constraints (Felsenstein, 1988; Harvey and Pagel, 1991). Furthermore, the statistical framework can be parameterized so that non-Brownian motion modes of evolution can also be evaluated (Martins and Hansen, 1997; O'Meara *et al.*, 2006; Revell and Collar, 2009; Hunt and Rabosky, 2014). These other modes include Ornstein–Uhlenbeck processes that arise from centrifugal (stabilizing) selection associated with an adaptive landscape with a broad peak, and diversifying (directional) selection. Collectively, these tools for analyzing phenotypic traits among taxa whose phylogeny is known are called phylogenetic comparative methods.

Hypothesis Testing

Because they are underpinned by a common quantitative genetic framework, the tools derived from Lande's quantitative theory for the evolution of phylogenetic traits and Felsenstein's phylogenetic methods can be combined to test quantitative genetic hypotheses about macroevolution, or to generate hypotheses about population-level genetic processes from comparative phenotypic data. Population-level parameters such as phenotypic variance, heritability, population size, and selection intensity can be extrapolated over hundreds, thousands, or millions of generations using any one of the several existing models of evolutionary pattern (Brownian motion, stasis or stabilizing selection, directional or diversifying selection, shifting adaptive peak, etc.) and the resulting predictions can be compared to data collected from species with comparable phylogenetic divergence times. The expected rate of phylogenetic divergence in the phenotype will be a function of population-level phenotypic variance, population size, and heritability (Lande, 1976). If these parameters are known for all the species being compared, then the average selection intensity and the long-term pattern of selection can be inferred. Conversely, the population parameters can be inferred if the long-term patterns are known from paleontological or phylogenetic data and tested at the population level.

An example of macroevolutionary hypothesis testing based on quantitative genetic predictions is Gingerich's (2001) study rates of evolution in lineages of fossil species tracked through millions of years. He found that average per-generation selection intensity was about 0.2 phenotypic standard deviations per generation, similar to the rapid rates of change measured in lab selection experiments for large and small body size. Interestingly, long-term diversification (maximum range of phenotypes in a clade) was less than expected if evolution continued directionally at this rate over millions of generations, suggesting that, while evolution is not constrained over short timescales, there are large-scale constraints on phenotypic disparity, a finding that has been replicated in other macroevolutionary studies covering many taxonomic groups at a several phylogenetic timescales (e.g., Hunt, 2007; Evans *et al.*, 2012).

Units of Analysis

An important requirement for comparing data drawn from different evolutionary scales is that the units of analysis must be comparable (Gingerich, 2001; Houle *et al.*, 2011). Evolutionary divergence times, for example, can be measured in units of millions of years (meganna), in molecular sequence differences, or in phenotypic character counts, but the time scale of the evolutionary process is the generation. Similarly, phenotypes can be measured in millimeters, kilograms, or Procrustes units, but it is the amount of change proportional to the genetic and phenotypic variance that is relevant to processes such as selection and drift. In order to compare population-level and macroevolutionary processes, the units of analysis must be standardized across the scales on which data are observed. Rates of phenotypic evolution are most easily compared across scales when they are measured as standard deviations per generation, a unit known as the 'haldane' (Gingerich, 1993), which is the same unit as quantitative genetic parameters such as selection intensity.

An example of how scaling matters is determining whether an observed Brownian motion pattern of species divergence arises from neutral genetic drift or selective drift. Neutral genetic drift is a process due to chance sampling from parent to offspring generation and is dependent on population size. Divergence between species due to drift is therefore a function of the average population size over time, the number of generations since common ancestry, and the genetic variance in the trait and will have a Brownian motion pattern (Lande, 1976). Selective drift is a process in which a trait is evolving by directional natural selection, but the direction and magnitude of selection are changed randomly over time (Kimura, 1954). Polly (2004) used this scaling relationship to show that divergence in tooth morphology in a species flock of shrews whose last common ancestor lived about 40 million years ago had diverged randomly (Brownian motion) but at a rate much faster than expected from neutral genetic drift, even if heritability in tooth structure was exceptionally high and population sizes were consistently small. Only selection could explain the observed divergences, yet there was no evidence that selection followed a consistent, directional trend, despite the clear functional role that teeth have.

Morphometrics and Multivariate Phenotypes

This comparative framework for hypothesis testing is especially powerful when it can be applied to complex traits that are commonly preserved in the fossil record. Quantitative genetic studies have traditionally focused on traits that are easy to measure in lab or field settings, especially univariate traits such as body size, color, bristle number, and fecundity, but these traits are rarely preserved in fossils or in the museum research collections that are used for large-scale comparative phenotypic studies. Instead, complex structures such as teeth, bones, shells, and leaves are commonly studied in macroevolution, even though we know comparatively little about their developmental and genetic underpinnings. Developments in geometric morphometrics now allow extraordinarily complicated structures to be measured efficiently, allowing multivariate structures such as these to be studied quantitatively in large numbers (Bookstein, 1991; Dryden and Mardia, 1998). Studies of the genetics, development, geography, phylogeny, and phenomics of complex traits have abounded in the last decade and the opportunity for studying phenotypic evolution across these scales has never been greater.

Complex traits present new challenges, both in terms of analytical methods and evolutionary theory. Morphometric analysis of the shapes of two-dimensional structures, even extraordinarily complex ones, has become trivially easy, merely requiring a source of digital photos and software for capturing Cartesian coordinates that represent the structure of interest (Bookstein, 1991). The mathematical dimensionality of the resulting morphometric traits is often very large, but nevertheless easily tractable in mathematical terms. The analysis of three-dimensional shape, which includes most real biological structures, is far more challenging. Capturing three-dimensional data requires specialized equipment such as CT-scanning or laser scanning, and the methods available for representing the full three-dimensional structure of a trait as Cartesian coordinates, whose orientation on the object must be homologous, are difficult to apply to varied structures and fraught with concerns about biological homology, algorithmic optimization, and unintentional weighting of one part of a structure over another (Gunz *et al.*, 2005; Polly, 2008a; Shen *et al.*, 2009).

The high dimensionality of morphometric traits itself is an issue, because the number of mathematical variables represented by the landmark coordinates used to represent the structure may be much greater than the trait's biological dimensionality. Three-dimensional morphometric representations of mammal teeth, for example, can easily have several hundred mathematical dimensions, each of which can be thought of as a potentially independent sub-trait, but quantitative trait locus (QTL) analysis and molecular developmental studies suggest that a much smaller number of genes may be involved in variation in tooth phenotypes (Workman *et al.*, 2002; Harjunmaa *et al.*, 2014). Because rates of evolution, standard deviation units, trait covariances, and other fundamental parameters are themselves dimensional, a new consensus about the concept of trait dimensionality is urgently needed. Multivariate traits might be thought of as coherent individual phenotypes with no sub-traits (Adams, 2014), they might be thought of in terms of the number of different genes underpinning them (Workman *et al.*, 2002), they might be thought of in terms of the number of developmental factors that control them (Salazar-Ciudad and Jernvall, 2010), or they might be thought of in terms of their number of functional units (Stinchcombe *et al.*, 2012).

An important challenge for quantitative phenotypic evolution is the study of evolutionary novelties – the gain and loss of limbs and digits, the multiplication of body segments, and the origin of eyes. The origin of novel structures is one of the most interesting aspects of macroevolution, and a better understanding of which is the primary goal of evolutionary developmental biology (Raff, 1996). However, the origin of new structures is almost impossible to quantify in morphometrics because each homologous point on the structure must be present in all structures in the analysis. Some morphometric approaches, such as outlines and surfaces, are 'homology free' in that they can accommodate the gain and loss of features on

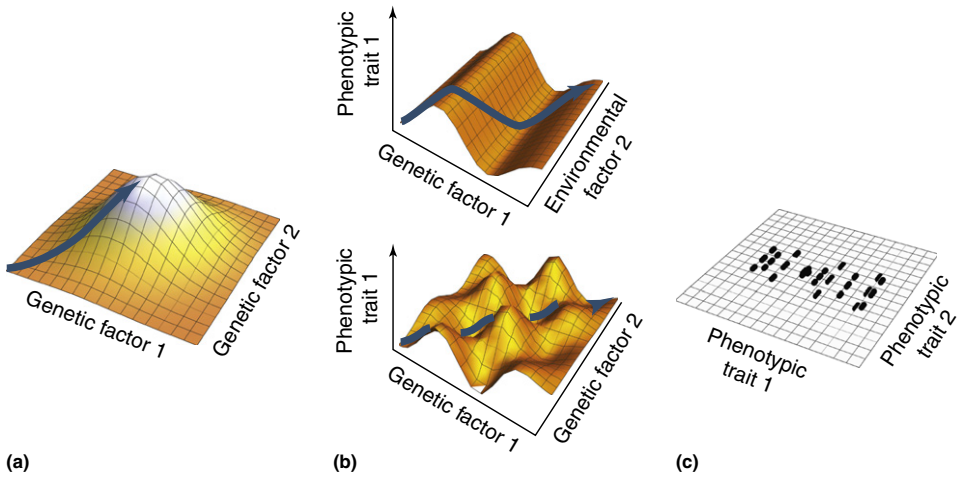

Figure 3 Diagram showing how genotype–phenotype maps might be combined with geometric morphometrics to model evolutionary trajectories in which developmental interactions result in discontinuous phenotypic change. (a) Continuous, linear evolution toward an adaptive peak in genetic space. (b) Two genotype–phenotype maps that describe the relationship of phenotypic traits to genetic and environmental factors. The same evolutionary trajectory is shown crossing both surfaces. (c) The genotype–phenotype maps result in a nonlinear, discontinuous distribution of phenotypes in the morphospace shown in this panel. This figure is based on ideas presented in Polly, 2008b.

a larger homologous structure, but even these cannot be used to represent an evolutionary transformation such as the evolution of limbs (Polly, 2008b). The most promising approach for studying evolution that involves the origin and loss of traits is the nonmetric approach described above, but no new developments have been made in this area.

An even more important challenge is that the evolutionary transformations that are reconstructed as shortest paths through a multivariate mathematical morphospace may not be the shortest biological paths from one phenotype to another (Polly, 2008b). The quantitative genetic and morphometric frameworks that were introduced above are based on trajectories through mathematical spaces, but as evolutionary distances increase to the macroevolutionary scale, the correspondence between mathematical and biological transformations becomes less certain. Dynamic relationships between tissues and gene signaling during development are known to produce nonlinear transformation in phenotypes that jump across gaps in morphometric spaces (Salazar-Ciudad and Jernvall, 2010). Thus, a continuous linear change in the level of expression of a developmental signaling gene may result in a discontinuous jump in the phenotype, one that may involve the origin of novel features. This phenomenon has important implications for quantitative theory for phenotypic evolution and is the object of ongoing study (Wolf *et al.*, 2001; Hansen, 2008; Rice, 2008). Morphometric algorithms may require elaboration to provide a nonlinear mapping between developmental genetic spaces and morphospaces (**Figure 3**).

Conclusion

The challenges raised about the ability of the Modern Synthesis to reconcile microevolutionary processes with macroevolutionary patterns have as yet only been partially answered, in large part for want of data and analytical methods. The rapid advances in both now offer opportunities for comprehensively addressing these questions. For instance, the preponderance of data suggest that phenotypic evolution is usually more rapid than expected by truly neutral processes like drift over short timescales, but over broad timescales there appear to be constraints operating that prevent lineages from becoming as disparate as they might, given their per-generation rates of evolution (Gingerich, 2001; Hunt, 2007; Evans *et al.*, 2012). However, despite the huge variety of data that underpins that observation, most of the traits that have been analyzed are essentially proxies for body size. Body size has fairly obvious constraints imposed on the small end by the requirements of cellular function and on the large end by the force of gravity (McNeill Alexander, 1998). Data for other kinds of traits are incomplete, but those that are available suggest that the pattern is different.

Rates of evolution in complex traits, such as the form of teeth and bones, for example, is still usually faster than expected from drift and long-term divergence often appears to be constrained; however, the constraints appear to change to allow lineages to move in new directions that their ancestors and relatives could not. The lifting of constraints is associated with large, seemingly correlated changes in form and function. For example, ankle structure in mammalian carnivores has evolved for about 50 million years constrained to a relatively small area of morphospace within which lineages have evolved and revolved similar structures; only the pinniped lineage (seals and sea lions) have broken out of those constraints, and out of the terrestrial locomotor system of their relatives (Polly, 2008a), consistent with Simpson's concept of adaptive zones (Simpson, 1944; **Figure 4**). The evolutionary dynamics of developmental systems appear to generate similar patterns in which phenotypes evolve within constrained areas of morphospace then jump to new areas at points where the system passes a threshold (Salazar-Ciudad and Jernvall, 2010). Added to the complexity of these processes is the complexity of selection surfaces and adaptive landscapes for

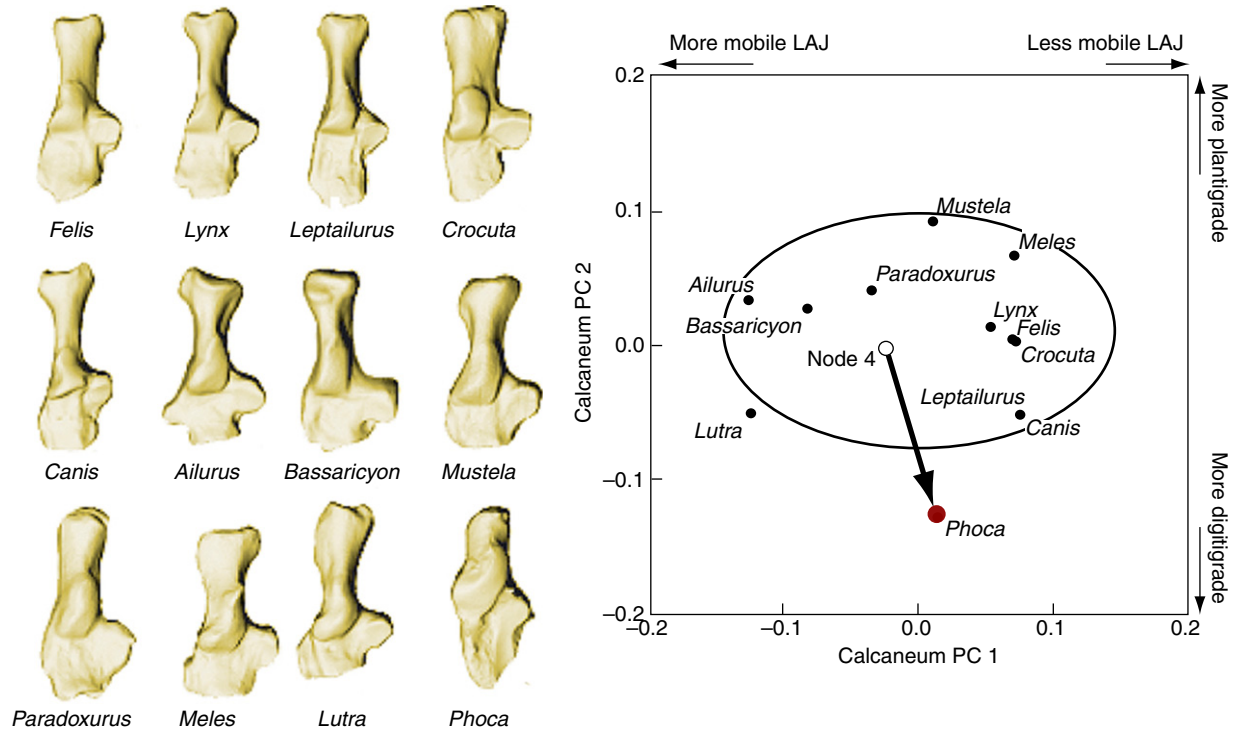

Figure 4 Complex patterns of evolution in multivariate phenotypes illustrated with the evolution of the ankle in mammalian carnivores. The calcaneum, the main supporting bone in the mammalian hind foot, of 12 taxa are shown at left, including cats (*Felis*), dogs (*Canis*), otters (*Lutra*), weasels (*Mustela*), and seals (*Phoca*). The morphospace at the right shows the distribution of their shape. Most of these species have evolved in a limited area of the morphospace defined by the functional constraints of supporting their weight and moving in their terrestrial environment (ellipse). The dark arrow shows an evolutionary trajectory estimated with maximum-likelihood for the seal lineage from its last common ancestor with terrestrial carnivores (Node 4). The rate of evolution of ankle structure in the seal lineage is similar to the rates in terrestrial species, but the seal's ankle is able to become more different by passing out of the constraints of terrestrial locomotion. This figure is based on research presented in Polly (2008a).

highly multivariate traits, which may have 'holes' that cannot be crossed, isometric lines of equal fitness in which phenotypes can wander randomly, and complex peaks and valleys that defy the simple metaphor of a landscape (Gavrilets, 1999). Some processes, such as species selection, are only starting to be formulated in a quantitative framework that will allow their effects to be compared in the same equations. Notably, Simpson (2011) has used a modified version of Price's (1972) evolutionary equation to partition phenotypic change in clades into change arising from evolution within lineages and change that arises from turnover in traits due to differential survival of lineages.

Opportunities exist not only for empirical research on how evolution works, but on methodological developments to deal with the evolution of complex traits. In addition to the morphometric challenges raised above, new ways of conceiving evolutionary modes are needed for multivariate traits. For univariate traits, the only alternatives to Brownian motion are directional evolution and stasis. Multivariate traits evolving on multivariate selection surfaces behave in ways that hardly fit the latter definitions. The models used to estimate evolutionary modes are therefore difficult to apply to multivariate evolution in a meaningful way, even though considerable progress has been made to develop multivariate equations (Arnold *et al.*, 2001; Gavrilets, 1999). Furthermore, most of the quantitative apparatus that is available for estimating rates and modes of phenotypic evolution are algorithms that find a single optimal parameter. The complex patterns and processes of multivariate evolution require methods that allow the relative support for competing hypotheses to be compared, ones that use a maximum-likelihood or Bayesian statistical framework. Some such methods are already available (Schluter *et al.*, 1997; O'Meara *et al.*, 2006; Goldberg and Igić, 2008; Slater *et al.*, 2012), but more are needed, as are development of new models for the evolution of nonmetric traits and, ideally, synthesis with continuous multivariate traits.

Supplementary Material

[Note: This multimedia item is essentially an animated version of **Figure 4**.] Multimedia Animation 1 related to this article can be found online at doi:10.1016/B978-0-12-800049-6.000555-X.

See also: Adaptive Landscapes. Divergence and Diversification, Quantitative Genetics of. Systems in Evolutionary Systems Biology

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Mammalian Diversification

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Introduction

The spectacular morphological and ecological diversity of present-day mammals has long fascinated both scientists and the general public. Such diversity is even greater, and more fascinating, when extinct mammalian lineages are also considered. Living and extinct mammals include many small, generalist species along with lineages that have adapted to a variety of specialized life histories, including large-bodied terrestrial herbivores, aquatic and terrestrial predators, fossorial (i.e., underground-adapted) species, ant-eating specialists, as well as tree-dwelling, gliding, and even flying forms. Many of the specialized forms evolved multiple times in different mammalian lineages, providing some of the most remarkable and best-documented examples of evolutionary convergence across all animals.

Mammalian diversification has been an ongoing process for the last 200 million years, but some periods during this time have been particularly relevant. Many different mammalian lineages have arisen and subsequently diversified, while others seem to have maintained a much lower diversity for a long time. Even lineages that diversified substantially in some cases have gone extinct, being replaced by other diversifying lineages. There is therefore a very rich history of mammalian adaptive radiations, which have been extensively analyzed on the basis of their fossil record, as well as with the use of molecular approaches. This article presents an overview of the present understanding of mammalian diversification, attempting to summarize the main results from many recent studies. There will be a brief treatment of the early phases of mammalian diversification, pointing to relevant primary sources, and then a more extended description of the diversification of living (extant) mammalian groups. The final section addresses the evolution of morpho–ecological diversity in mammals, including some discussion on the occurrence of convergent morphological features in different lineages. The goals of the article are to provide a concise summary of the main conclusions emerging from recent studies in this field, and to point the reader to examples in the primary literature (as well as recent reviews) that provide more detailed information on particular topics.

Early Phases of Mammalian Diversification

Mammals arose in the Upper Triassic period (Mesozoic Era), *ca.* 220 million years ago (MYA), as a subgroup of the synapsid amniotes. A common perception in the earlier literature is that Mesozoic mammals were all very small and not particularly diverse, and that their burst of diversification occurred when dinosaurs went extinct at the end of the Cretaceous period (a time known as the Cretaceous–Paleogene (KPg) boundary), *ca.* 66 MYA. This view has been shown to be inaccurate by many recent studies. There is now abundant evidence that Mesozoic mammals were quite diverse, including small, tree-dwelling insectivores and rodent-like forms, along with burrowing and swimming forms, and even medium-sized predators (e.g., Hu *et al.*, 2005; Luo, 2007; Bi *et al.*, 2014; Close *et al.*, 2015). There were clearly many different lineages, occupying a variety of habitats throughout the planet. Although most of them remained quite small (around the size of a shrew or a rat), it is now known that some of them could achieve a much larger size, including a >4 kg carnivorous form that was even found to have preyed on small dinosaurs (Hu *et al.*, 2005). It is therefore correct to say that mammals already comprised a successful and quite diverse radiation of terrestrial vertebrates during the Jurassic and Cretaceous periods in the Mesozoic, when they coexisted with dinosaurs in many different ecological settings. At the same time, it is still accurate to say that their morphological disparity (implying ecological diversity) was still modest compared to the levels that would eventually be achieved in the Cenozoic, and that we still see today. For example, there were no flying mammals, nor highly specialized marine forms such as cetaceans or sirenians. Moreover, the vast majority of known Mesozoic mammals were indeed quite small, with the largest known forms estimated to have attained a mass of $\sim$14 kg. In contrast, many mammalian lineages that are known from the Cenozoic fossil record and from present-day faunas achieved much larger body sizes, surpassing 100 kg or even weighing several tons (e.g., the largest mammal, the blue whale, can weigh up to $\sim$130 t). The history of mammalian diversification thus includes both a turnover of many distinct phylogenetic lineages that often replaced each other over the last $\sim$200 million years, and a trend for increase in body size and morphological disparity that mostly occurred in the Cenozoic.

Origin and Diversification of Living Mammals

From the early phases of mammalian diversification in the Mesozoic, three main lineages have survived up to the present (**Figure 1**): Monotremata (the egg-laying platypus and echidnas), Marsupialia (marsupials such as kangaroos and opossums), and Placentalia (placental mammals). Recent molecular studies (e.g., Meredith *et al.*, 2011; Dos Reis *et al.*, 2012) have strongly supported the conclusion that Monotremata represents the earliest divergence of the three groups, having split from the other two clades *ca.* 180–220 MYA. Marsupialia (representing the broader clade Metatheria) and Placentalia (representing the broader clade Eutheria) are sister-groups, having diverged from each other *ca.* 170–190 MYA. The monophyletic group encompassing Eutheria and Metatheria is known as Theria. Fossil evidence demonstrates that each of the three extant lineages underwent considerable diversification during the Jurassic and the Cretaceous periods (see **Figure 1**), when they also coexisted with additional mammalian groups that did not leave any living

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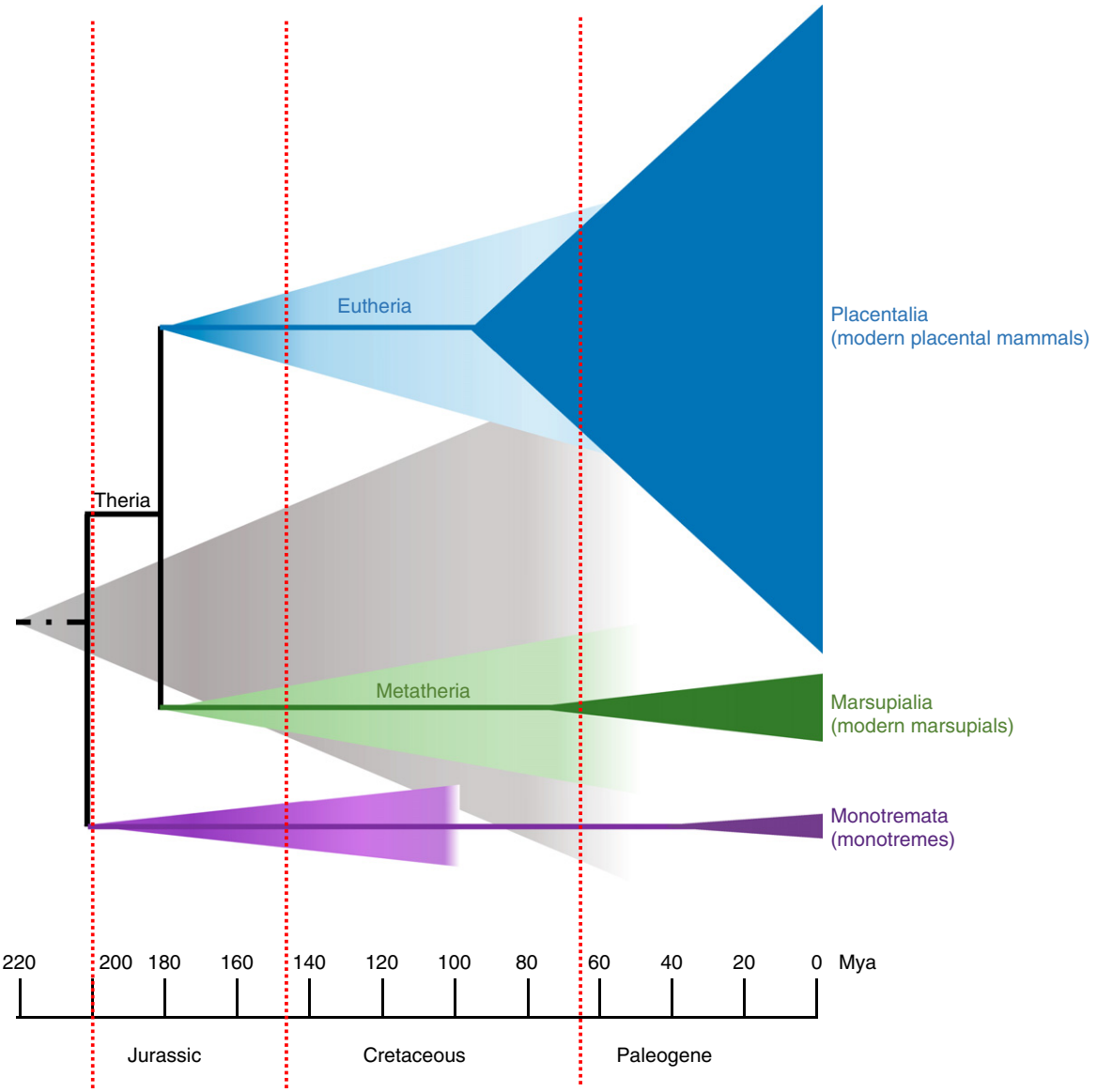

Figure 1 Schematic representation of the diversification of mammals. The timing of divergence (in million years ago (Mya)) among the three extant mammalian lineages (Placentalia, Marsupialia, Monotremata) is based on the mean between the values reported in two recent and comprehensive molecular analyses (Meredith *et al.*, 2011; Dos Reis *et al.*, 2012). The chronological range of stem taxa in each group is drawn from Luo (2007) and Bi *et al.* (2014). Within each of the three main lineages, the more recent triangle (shown in stronger color) represents the timing and intensity of diversification of the crown group (i.e., the most recent common ancestor of all living species and all the descendants from this ancestor). Also within each lineage, the older triangle (depicted in lighter color) represents the diversification of stem groups (i.e., having diverged prior to the origin of the crown group). The light gray triangle represents the diversification of mammalian stem groups that are not contained in any of the three extant lineages.

representative. Much of this early diversity encompasses what is known as 'stem taxa' within each of the three lineages, i.e., species that are less closely related to the living forms than the latter are to each other. The crown-group within each lineage (defined by the common ancestor of all presently living species and all its descendants) arose later, *ca.* 90–100 MYA in the case of Placentalia, 65–87 MYA in the case of Marsupialia, and only *ca.* 40 MYA in the case of Monotremata (Meredith *et al.*, 2011; Dos Reis *et al.*, 2012; Mitchell *et al.*, 2014).

Each of the three main lineages has achieved a different level of present day diversity. Extant monotremes comprise only two families and five species (MacDonald, 2001), and their known fossil record does not indicate that the crown-group has exhibited high levels of diversity in the past (Springer and Krajewski, 2009). Marsupials display a different pattern, with considerable present-day diversity (>300 species belonging to seven orders) and morphological disparity, including hopping, climbing, gliding, burrowing, predatory, and semiaquatic forms. Extinct marsupial lineages further extend this diversity, with various large forms that even included lion-like and saber-toothed predators (Savage and Long, 1983). Marsupial diversification mostly took place in the southern

continents, especially South America and Australasia. The most ancient among the extant lineages are all South American, and they are paraphyletic with respect to the Australasian radiation (Meredith *et al.*, 2011; Mitchell *et al.*, 2014). This phylogenetic pattern has been robustly supported, and implies a biogeographic reconstruction that postulates an origin of crown marsupials in South America, followed by colonization of Antarctica and Australia while these landmasses were still connected in the Late Cretaceous. The early divergences in South America have been dated at 69–87 MYA, while the diversification of the Australasian lineages was rapid, and clustered around the KPg boundary, 60–67 MYA (Springer *et al.*, 2009; Mitchell *et al.*, 2014). Such a pattern is suggestive of a rapid radiation following the colonization of new landmasses, but may also have been influenced by new ecological opportunities that arose in the aftermath of the KPg mass extinction.

The third main group of living mammals, the placentals, is the most speciose, comprising 19 orders, >100 families, and >5000 species (MacDonald, 2001; Wilson and Reeder, 2005). They have undergone a remarkable diversification since their origin in the Mesozoic (see **Figure 1**), giving rise to fantastically disparate forms that include whales, bats, tigers, moles, humans, anteaters, and elephants. How did they diversify? When did they diversify? And where did it happen? These questions have attracted the attention of numerous scientists, and the attempts to address them using a variety of approaches have led to some of the liveliest debates in evolutionary biology in the second half of the twentieth century. A key discussion revolved around the required resolution of the phylogenetic relationships among living groups of placental mammals, without which it would not be possible to test alternative hypotheses regarding the timing and biogeography of their diversification, as well as the sequence of morpho–ecological changes that accompanied the divergence of evolutionary lineages. Due to the rapid radiation of different placental lineages, as well as the major (often extreme) morphological specializations of extant groups, piecing together their relationships and reconstructing their history using traditional approaches (e.g., comparisons of their bones and teeth) proved to be remarkably challenging (e.g., Eisenberg, 1981; Novacek, 1992; Murphy and Eizirik, 2009). Different studies yielded different phylogenies, leading to conflicting hypotheses regarding the history of placental mammal diversification. Many controversies persisted up to the end of the twentieth century, including highly publicized conflicts between phylogenetic reconstructions derived from early molecular data sets and groups retrieved with morphological characters (e.g., D'Erchia *et al.*, 1996). As larger molecular data sets began to be assembled, improved resolution could be obtained for various sections of the mammalian tree, in some cases revealing phylogenetic groups that had not been anticipated based on morphology (e.g., Springer *et al.*, 1997; Stanhope *et al.*, 1998).

Diversification of Placental Mammals

In the early 2000s, analyses based on large molecular data sets finally allowed the resolution of most relationships among placental mammal orders (Eizirik *et al.*, 2001; Madsen *et al.*, 2001; Murphy *et al.*, 2001a,b). These studies corroborated some classical groups strongly supported by morphology (e.g., Glires, comprising Rodentia and Lagomorpha), as well as others revealed by previous molecular studies (e.g., Afrotheria). Many subsequent studies consolidated the resolution of the placental mammal tree, and progressively refined the estimation of divergence dates among its contained lineages (e.g., Springer *et al.*, 2003; Murphy and Eizirik, 2009). The following description of the timing and biogeography of placental mammal diversification is based on a compilation of sources that used large molecular data sets to directly estimate phylogenetic relationships and divergence times among extant lineages, and especially on two recent articles that included extensive sampling of taxa and genomic regions (Meredith *et al.*, 2011; Dos Reis *et al.*, 2012).

Extant lineages of placental mammals began to diversify in the late Cretaceous, 90–100 MYA (**Figure 2**). There are three well-supported lineages that emerged at this time: Afrotheria, Xenarthra, and Boreoeutheria (which in turn comprises two large and well-supported sub-lineages: Laurasiatheria and Euarchontoglires). The identification of these major groups and the timing of their divergence supported the view that early placental mammal diversification was strongly influenced by biogeographic processes, likely related to the breakup of supercontinents in the late Cretaceous (Eizirik *et al.*, 2001, Madsen *et al.*, 2001; Murphy *et al.*, 2001a,b). The geographic ranges of living and fossil representatives of these clades suggest that Afrotheria originated in Africa, Xenarthra in South America, and Boreoeutheria in the northern continents (Eurasia or North America). The timing of their divergence coincides with the period of final separation between South America and Africa (both formerly part of the supercontinent of Gondwana), and also with the likely intermittent connection between South and North America via the proto-Antilles corridor (Murphy *et al.*, 2001b).

The exact sequence of biogeographic events leading to the separation of these groups depends on the resolution of the phylogenetic root of living placental lineages (i.e., the relationships among Afrotheria, Xenarthra, and Boreoeutheria). Unfortunately, this has proven remarkably difficult to resolve. All three possible resolutions (Afrotheria diverging first, Xenarthra diverging first, or a sister-group relationship between these two groups) have been retrieved by various molecular studies, and in general, the alternative topologies could not be significantly rejected. An early view of placental evolution suggested that Xenarthra was the most ancient extant group, with the remaining lineages comprising a clade called Epitheria (McKenna and Bell, 1997). This topology is not retrieved very frequently as the best solution, but remains a viable alternative. Several studies have retrieved a sister-group relationship between Xenarthra and Boreoeutheria (e.g., Murphy *et al.*, 2001a), forming a clade named Notolegia (Springer *et al.*, 2007) or Exafroplacentalia (Waddell *et al.*, 2001). This resolution, with Afrotheria as the most divergent clade, suggests that the breakup between South America and Africa, *ca.* 100 MYA, would have caused the initial split in crown placental mammals. Afrotherian progenitors would have remained in Africa, while those of all other living placentals would be in South America, whence they would have colonized the northern continents via the proto-Antilles

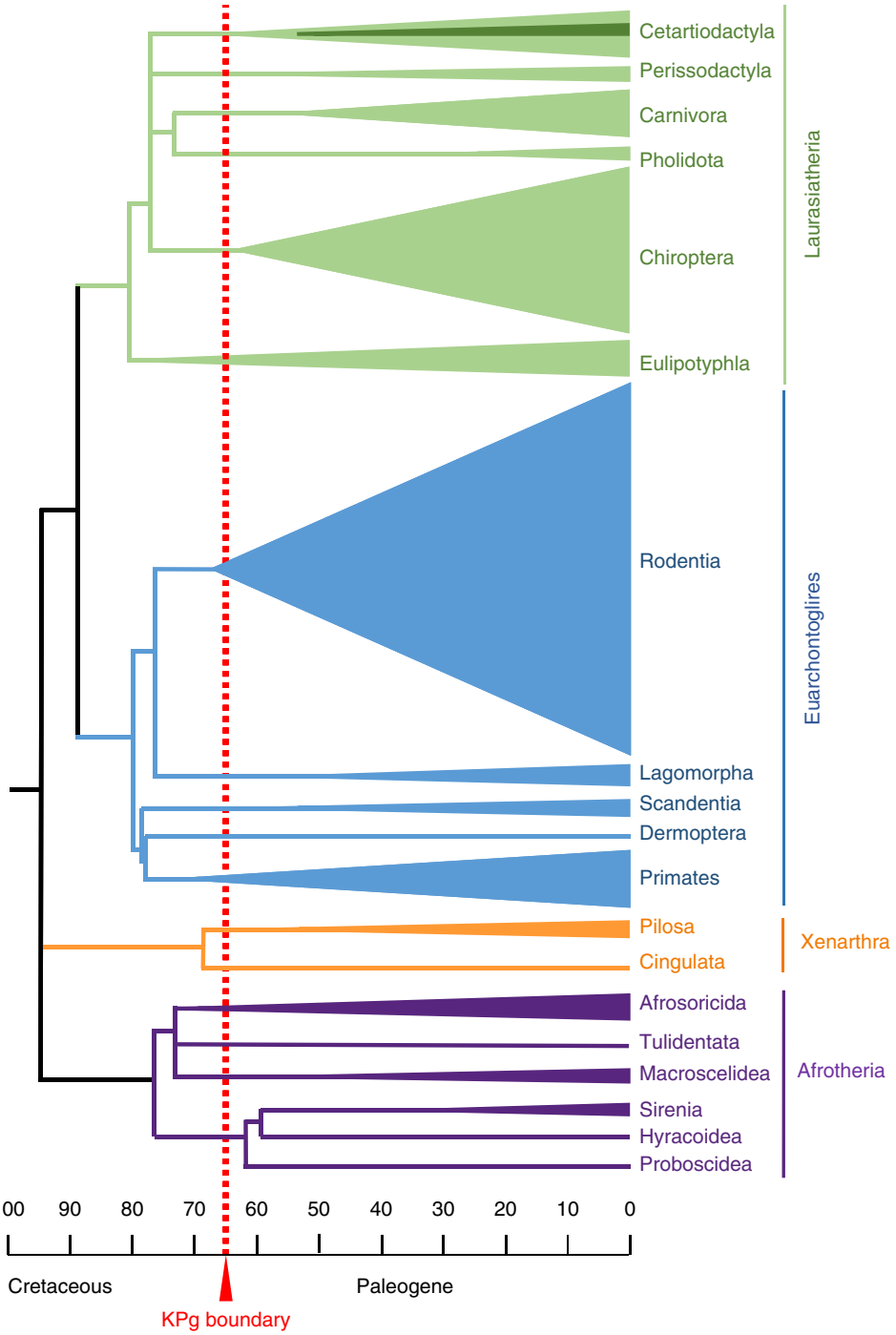

Figure 2 Schematic representation of the diversification of placental mammals. The relationships among extant placental orders are based on the tree reported by Meredith *et al.* (2011). The current diversity of each order is represented (approximately) by the width of its respective line or triangle. The timing of divergence among orders, as well as the timing of diversification within each order, is based on the node ages reported by Meredith *et al.* (2011) and Dos Reis *et al.* (2012). For nodes in which both of these studies reported dates, the average between their two values is depicted here. Each of the four main lineages of placental mammals (Laurasiatheria, Euarchontoglires, Xenarthra, and Afrotheria) is shown in a different color. Within the order Cetartiodactyla, the nested line in darker green represents Cetacea (whales, dolphins, and kin), indicating its origin and diversification from within the order that contains even-toed ungulates (cows, deer, hippos, camels, and kin).

corridor to North America and further to Eurasia (Murphy *et al.*, 2001b, Springer *et al.*, 2007). An alternative, non-exclusive scenario is that Notolegia was more widespread in Gondwana, and at least some of its members were present in India before it was separated and drifted northward; when India collided with Asia *ca.* 55–65 MYA, these lineages would then have colonized the northern continents *via* Eurasia (see Murphy *et al.*, 2001b – online supplemental item 15). The

third possible scenario has been retrieved most often in recent studies (e.g., Meredith *et al.*, 2011), and supports a sister-group relationship between Afrotheria and Xenarthra (which jointly form a clade known as Atlantogenata). Under this phylogenetic reconstruction, the initial split in Placentalia is between southern and northern groups, with the final breakup between South America and Africa likely underlying the separation between the two southern lineages. Given these intriguing biogeographic implications, the effort to resolve the position of the placental mammal root will likely remain a topic of active research in the coming years.

Morpho–Ecological Diversification and Convergent Evolution

A key aspect in the evolutionary analysis of mammalian diversification is to understand the history of the remarkable morpho–ecological adaptations that are a hallmark of many extant lineages. Once the main sections of the phylogeny of living mammals were resolved, new insights could be gleaned onto this topic. There were clearly periods of rapid phylogenetic diversification in the history of both placentals and marsupials (Meredith *et al.*, 2011; Mitchell *et al.*, 2014). In the case of crown placentals, recent analyses indicate that the main bursts of diversification occurred prior to the KPg boundary, and may have been associated with continental breakup but also to novel ecological opportunities associated with the Cretaceous Terrestrial Revolution (Meredith *et al.*, 2011). The main lineages of placental mammals were already established prior to the KPg boundary, and each of them underwent a rapid diversification *ca.* 80 MYA. An assessment of morphological diversity (both extant and fossil) within these lineages indicates that each of them likely still comprised primitive forms (i.e., small and insectivorous) at the time of this diversification (Eizirik *et al.*, 2001), and subsequently underwent a separate adaptive radiation giving rise to specialized forms (Madsen *et al.*, 2001). The timing of this morpho–ecological diversification (i.e., originating elephants and manatees in Afrotheria, or bats, whales, and moles in Laurasiatheria, etc.) is difficult to assess based on molecular data alone, but fossil evidence strongly suggests that it postdates the KPg boundary. It therefore remains a viable hypothesis to postulate that larger, specialized forms of modern mammals arose after the KPg mass extinction, driven by rapid adaptation to novel ecological opportunities that emerged after the extinction of nonavian dinosaurs. However, such processes would have occurred within lineages that were themselves a product of previous diversification processes.

It has been known for a long time that convergent evolution could be observed between lineages belonging to different mammalian groups. This was especially the case when one compared marsupial and placental morpho–ecological 'equivalents,' such as mole-like forms or gliding forms. These comparisons can also include monotremes, as in the case of forms specialized on feeding on social insects: echidnas clearly show morphological convergence with respect to placental and marsupial lineages that eat ants or termites. In addition to these classic cases, the resolution of the placental mammal phylogeny in the last 15 years has revealed many other remarkable case of morpho–ecological convergence (Madsen *et al.*, 2001; Springer *et al.*, 2004). For example, specialized fossorial (mole-like) and marine forms evolved independently in Afrotheria and Laurasiatheria. Specialized ant-eating forms evolved independently in Xenarthra, Afrotheria, and Laurasiatheria, and display remarkable convergence in their morphology (e.g., comparing South American anteaters, Old World pangolins, and African aardwarks). Such cases of extreme adaptations to very diverse life histories, repeated in separate lineages in the context of rapid radiations, contribute to fostering continuous fascination for mammals as a highly attractive subject to in-depth evolutionary analyses.

See also: Mammals Everywhere. Mammals, Origin of

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Mammals Everywhere

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Introduction

Mammals represent the smallest class of terrestrial vertebrates, comprising approximately 6000 known species. Despite their modest number, mammals are found on all continents, in all oceans, and in all biomes. They range from the abyssal depths to the tops of mountains, living in water, underground, on the surface, in trees, and some can even fly (Macdonald, 2009). Few in number, mammals exert far-reaching effects on the ecosystems in which they live and may serve as landscape architects (Wright *et al.*, 2002; Sukumar, 2003) or regulate trophic cascades (Estes *et al.*, 1998; Terborgh, 1988).

Hallmarks

The earliest mammals relied on hearing and smell to guide their largely nocturnal activities (Ji *et al.*, 2009), leading to the prominence of these sensory modalities in most modern mammals. Uniquely, mammals possess three middle-ear ossicles, which transmit sound waves striking the eardrum to the inner ear (**Figure 1**). Olfactory receptors located in the nasal cavities are well developed in most mammals (Buck, 2004) and are used in a variety of contexts: to find food, detect predators, and, in concert with a host of glandular secretions that are variously produced and expressed, in intraspecific communication and reproduction (Eisenberg and Kleiman, 1972; Doty, 1986). Vision is also well developed in some groups of mammals, especially among diurnal species and in Primates, the group that includes humans.

Hair, another mammalian hallmark, provides insulation, camouflage, a means of communication, and is a sensitive mediator of touch, particularly through sensory vibrissae on the face and extremities (Noback, 1951). All mammals nourish their young via mammary glands (Oftedal, 2002), which give the group its name. One lineage (monotremes) lays and incubates eggs while another (marsupials) gives birth to early-term embryos that complete their development attached to a teat. A specialized complex of fetal and maternal tissues – the placenta – nourishes embryos in the uterus (Wildman *et al.*, 2006). In different groups, this organ involves various degrees of intimacy between fetal and maternal bloodstreams.

Unlike most other vertebrates, mammalian teeth are structurally and functionally diversified (**Figure 2**): from front-to-back, nipping incisors, stabbing and tearing canines, and grinding and shearing premolars and molars (Stock *et al.*, 1997). This variety of functions has permitted mammals to efficiently utilize a wide range of diets. Mastication was particularly important in the exploitation of plants as food by homeotherms, because it quickly exposed ingested cellulose to

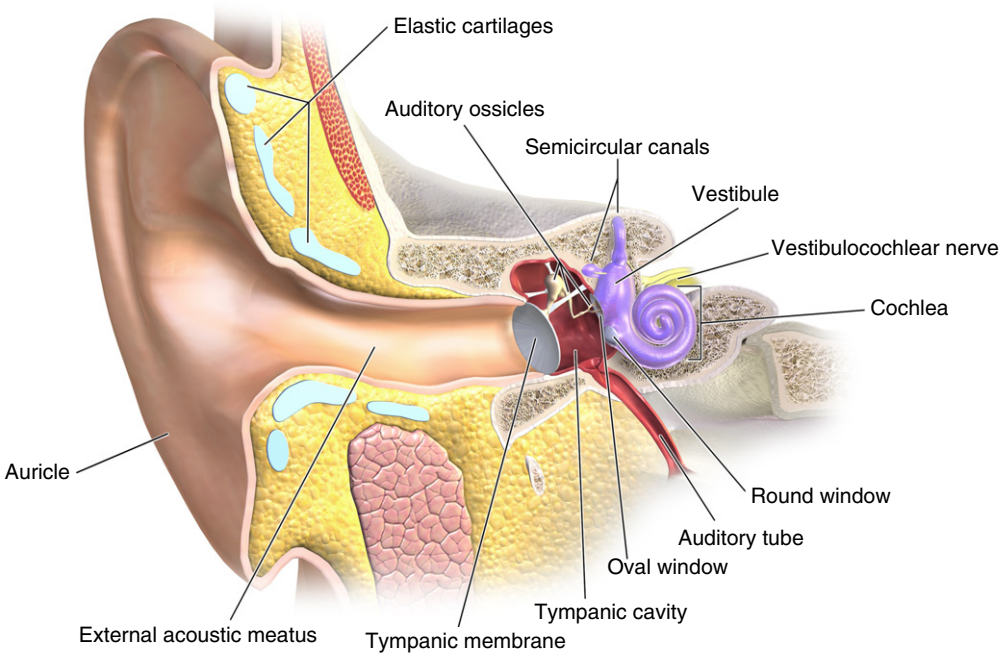

Figure 1 Structure of the mammalian ear, showing the position of the auditory ossicles (erstwhile jaw elements) between the external ear ('auricle' or ear pinna) and the inner ear ('cochlea'). Blausen.com staff. 'Blausen gallery 2014.' *Wikiversity Journal of Medicine*. doi:10.15347/wjm/2014.010. ISSN 20018762.

Figure 2 Skull of the Colombian weasel (*Mustela felipei*), in the family Mustelidae (Carnivora), showing functional differentiation in the dentition. The dental formula (numbers of maxillary/mandibular incisors, canines, premolars and molars, respectively) is 3/3, 1/2, 3/3, 1/2. Photograph of the holotype specimen, FMNH 70999 (Field Museum of Natural History) by B. D. Patterson.

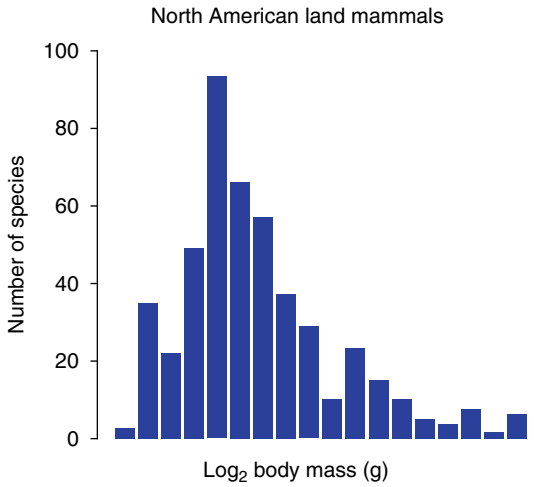

Figure 3 A frequency distribution of body sizes for North American land mammals (465 species). As is the case globally, most mammal species weigh between 50–100 g. The North American fauna shown here encompasses species ranging from 3 g to 393 kg in size (Public domain).

microbial action in the gut (Ley *et al.*, 2008). The gastrointestinal tract of plant-feeding mammals is also compartmentalized to aid cellulose digestion by microorganisms. Ruminants, sloths, kangaroos, and colobus monkeys use foregut fermentation in a compartmentalized stomach, whereas possums, elephants, horses, and many rodents rely on hindgut fermentation (Feldhamer *et al.*, 2007). Smaller species (e.g., rabbits, pikas, and rodents) with shorter digestive tracts and gut passage times often rely on coprophagy (ingestion of select fecal pellets) to extract more nutrients from their diet (Hirakawa, 2001).

Body size

Body size is correlated with so many features of organisms – from their metabolic rate and generation time to space use and diet – that body size has been called the single most fundamental organismal trait (Lomolino *et al.*, 2010). Mammals range over eight orders of magnitude in body size, from the 2 g pygmy shrew to the 180–200 t blue whale (Smith and Lyons, 2011). In this respect, they exceed all other animal groups. Even insects, which outnumber all others combined, only range over three orders of magnitude (Chown and Gaston, 2010).

Surely, this amazing variation in body size both underlies the ecological diversity of mammals and has helped give rise to it (**Figure 3**). It helps mammals exploit a range of ecological opportunities that are beyond the reach of less variable organisms. For example, elephants can detect infrasound waves as low as 10 Hz, whereas some insectivorous bats echolocate at over 200 kHz. Mammal diets are highly varied and include virtually all items eaten by other tetrapod groups and some that only mammals can exploit. The smallest mammals tend to exploit energy-rich resource patches needed to maintain their exorbitant metabolic rates, whereas larger mammals are more wide-ranging, have slower metabolisms and longer gut retention times, and can exploit lower-quality foods (McNab 2007; Brown and Maurer, 1989). Mammals seem to have an optimal body mass of about 1 kg, which may explain the 'Island Rule' in which small mammals evolve to become larger and larger ones are dwarfed (Damuth, 1993; Brown *et al.*, 1993). Nevertheless, various lineages of Cenozoic mammals, especially larger ones, show evidence of progressive increases in body size consistent with Cope's Rule (Alroy, 1998).

Mammals vary geographically in ways that help them exploit different habitats and ultimately foster their diversification. Some of the most conspicuous 'ecogeographic rules' – general patterns of geographic variation in response to ecology – are embodied by mammals, although their interpretations are often complex (Lomolino *et al.*, 2010). Bergmann's Rule posits that races of warm-blooded animals from colder climates tend to be larger than those from warmer regions (Mayr, 1956; Meiri and Dayan, 2003), because the volume of thermogenic tissue increases as the cube of body size, whereas heat loss scales with surface area (square of size). The wide-ranging puma illustrates this pattern, with smaller tropical forms flanked by large subspecies in both the Northern Rockies and the Southern Andes (Gay and Best, 1996). Allen's Rule was coined to describe the progressive shortening of appendages (both limbs and extremities) in progressively colder climates. It has been used to explain the faces and ears of North American foxes and hares (but not rabbits; Stevenson, 1986) and even humans (Hurd and Van Anders, 2007). Gloger's Rule, hypothesizing that animals from humid environments

are darker than their relatives from drier ones, also seems applicable to mammals (Kamilar and Bradley, 2011). These rules may even be used to forecast animal responses to global warming (Millien *et al.*, 2006).

Socially, mammals range from solitary and promiscuous to eusocial forms. Groupings offer different mammals protection from abiotic factors or from predators, help them find and harvest food, and even rear young via cooperative breeding (Silk, 2007). Although many species are solitary and even territorial except during the breeding species, others form intra-group alliances and coalitions. Kin and group selection are implicated in the more dramatic examples of mammalian sociality (Clutton-Brock, 2002). Maternity colonies of the Mexican free-tailed bat may number as many as 20 million individuals (McCracken, 1986).

Life Modes

The locomotory diversity of mammals is exceptional in itself and has also served to foster their diverse trophic, physiological, ecological, and social adaptations. Surface dwelling and quadrupedal, plantigrade (flat-footed) locomotion is primitive for synapsids (Luo *et al.*, 2011), but mammals quickly adopted various other forms (Ji *et al.*, 2006; Luo and Wible, 2005). Their innovations involved pronounced modifications of the limbs, transforming a largely common *bauplan* into radically different products, such as wings, paws, hoofs, and flippers (Cooper and Tabin, 2009). Most mammals are terrestrial and either walk or run. Functionally, locomotion by quadrupeds must provide propulsion, maneuverability, endurance, and support and stability while the feet make intermittent contact with the substrate (Hildebrand, 1985). Different gaits are used to move at progressively faster speeds: walking, pacing, trotting, and finally galloping and bounding, in which all four feet may become airborne. Mechanical constraints on the ribcage and diaphragm link breathing frequency to the different footfalls in these gaits, strictly coupling respiration and locomotion to maintain aerobic capacity (Bramble and Carrier, 1983; Bramble and Jenkins, 1993).

Most ungulates, some carnivores, and even some rodents have become truly cursorial, in which limb movements are restricted to a single plane, parallel to the body. Cursorial mammals have elongated limbs, often with bone reductions and fusions in the lower leg so that only the tips of the digits (in the Equidae, a single digit) contacts the ground (**Figure 4**). A proximal shift of appendicular musculature reduces weight and increases running speed (Hildebrand and Goslow, 2001). Fast, efficient movements give them large home ranges and the ability to pursue prey or escape predators. Ricocheting is a specialized form of locomotion typically employed by smaller, open-country species in which the hind limbs provide propulsion and the forelimbs are shorter and used in feeding and slower, more precise maneuvers (**Figure 5**). Lengthening of one or more hind limb segments provides mechanical advantage, and long, elastic tendons that transcend the knee and ankle store energy from one jump that can be used in the next one (Berman, 1985).

Relatively modest transformations carried mammals from substrates into the tree tops, which are home to many marsupial groups, tree sloths and anteaters, some pangolins, most primates, tree shrews, and many groups of carnivores and rodents. Typically, recurved claws or grasping hands and feet provide secure purchase far aboveground (**Figure 6**). Prehensile tails have evolved independently in both American and Australian marsupials, anteaters, pangolins, platyrrhine monkeys, porcupines, and binturongs. Arboreal mammals typically possess forward-directed eyes for good depth perception and many can rotating their ankles for secure descents. Most scramble over branches, but some (sifakas and tarsiers) are powerful leapers. Lesser apes and some ateline monkeys can travel rapidly by brachiation, or arm swinging, while other arboreal mammals relying on suspension (sloths) are notoriously slow. Various arboreal groups, including some marsupials, squirrels and anomalurid rodents, and colugos, have developed extensive patagial membranes for gliding between trees.

Many terrestrial groups have also undertaken evolutionary transitions to subterranean habits, often adopting similar convergent adaptations. Diggers typically have cylindrical

Figure 4 A roan antelope (*Hippotragus equinus*), in the family Bovidae (Cetartiodactyla), at full gallop. Cursorial locomotion offers many mammals rapid escape from predators and efficient long-distance movements that are involved in foraging and even migration. Photographed by B.D. Patterson in the Moremi Game Reserve, Botswana (© 2005).

Figure 5 A springhaas (*Pedetes capensis*), in the family Pedetidae (Rodentia). This ricochetal hopper uses energy stored from jumps in tendons for rapid, evasive movements in open biomes. Photographed by B.D. Patterson in the Moremi Game Reserve, Botswana (© 2005).

Figure 6 A sifaka (*Propithecus verreauxi*), in the family Indriidae (Primates), showing the prehensile hands and feet possessed by many arboreal mammals. In addition, sifakas have long legs and a tail for leaping between trees and across the ground. Photographed by B.D. Patterson in the Berenty Reserve, Madagascar (© 2005).

bodies and short limbs so as to fit compactly within their burrows, and tolerance for the hypoxia and hypercapnia that often develop there (Begall *et al.*, 2007). Loose skin helps them to turn within a confined space. Many burrow dwellers move backwards nearly as adeptly as they do forwards, aided by stiff hairs on the hind feet and tail that may help move dirt and transmit information through touch. In lineages long committed to underground life, such as golden moles (Chrysochloridae) and African mole-rats (Bathyergidae), the eyes become nonfunctional and the ear pinnae are lost or reduced in size. Excavations may be accomplished with either the pectoral girdle or with the teeth, and the soil is moved with either fore or hind limbs. Forms that use the teeth for digging (e.g., pocket gophers and mole-rats) have well-haired lip folds behind the incisors that prevent dirt from entering the oral cavity (Diaz *et al.*, 2000).

The bats are one of three vertebrate groups to evolve powered flight (Kunz and Fenton, 2003). Their wings consist of elongated arm, hand, and finger bones that support elastic skin membranes extending between them and the body, and variably to the legs and tail (**Figure 7**). Relative to birds, most bats fly slowly but are highly maneuverable, and most bat families are insectivorous and feed while flying. Bats emit high-frequency sound pulses through the mouth and/or nose to locate prey items, often via an ornate nose leaf to focus sound. Different wavelengths are sensitive to objects of different sizes, so that by exploiting different band-widths, species effectively partition their insect resources. Even the earliest known bats could both fly and echolocate, making it controversial to gauge which innovation came first (Gunnell and Simmons, 2012). Only 2 of 18 families of bats (New and Old World fruit bats) exploit plant resources, but each has explosively radiated to harvest pollen, nectar, and fruit, becoming vital partners in the life histories of many tropical plants (Lobova *et al.*, 2009).

Many groups of mammals swim and/or dive (Berta *et al.*, 2005). The buoyancy of water releases mammals from gravitational constraints, but poses challenges involving thermoregulation, locomotion, respiration, and pressure, especially for deep-diving species. The high thermal conductivity of water means most aquatic and marine mammals are larger than land-living relatives and smaller species are restricted to freshwater, near-shore or tropical waters (Williams *et al.*, 1998). Marine species commonly supplement the favorable surface-to-volume relationship of large body size with layers of insulating blubber and counter-current heat exchangers in their extremities. The body is typically streamlined (**Figure 8**) and propulsion may be from the forelimbs (platypus, some fissipeds), hind limbs (water opossum, other fissipeds and rodents), or modifications

Figure 7 Straw-colored bats (*Eidolon helvum*), family Pteropodidae (Chiroptera), taking flight from a tree roost. Flight offers rapid, efficient and long-distance movements that underlie both the explosive diversification of bats and the migratory habits of this species. Photographed by B.D. Patterson in Mbale, Kenya (© 2012).

Figure 8 Afro-Australian fur seals (*Arctocephalus pusillus*), family Otariidae (Carnivora), resting on a beach. Although recently derived from terrestrial carnivores, eared seals exhibit the fusiform bodies and flattened flippers of most marine mammals, as well as minute ears. The powerful pectoral fins are used by members of this family for propulsion. Photographed by B.D. Patterson in Walvis Bay, Namibia (© 2005).

of the tail (some rodents, manatees, and cetaceans). In different groups, each of these extremities may be involved in steering. Breathing is accomplished through dorsally positioned nostrils. Diving species are able to remain submerged for extended periods, up to 2 h in sperm whales, by virtue of efficient extraction of oxygen, elevated number of erythrocytes, and much higher concentrations of myoglobin in the tissues; because their lungs collapse completely during deep dives, they can avoid decompression sickness.

The Age of Mammals

The Cenozoic Era, extending from the Mesozoic to the present, is commonly called 'The Age of Mammals' because of the explosive radiations of mammals during this period. Recently, in recognition of the massive and irreversible changes wrought by an ever-expanding human population, many have suggested that we have entered a new era, the Anthropocene (Steffen *et al.*, 2011). Human activities threaten many mammal groups, both directly through habitat loss, fragmentation, and direct persecution for many terrestrial mammals, or pollution and accidental mortality, especially among marine mammals (Schipper *et al.*, 2008). It is a testament to the adaptability of mammals that a host of species (Brush-tailed possums, European rats and house mice, gray squirrels, house cats, rabbits, mink – all considered invasive or pest species) have been successful in exploiting this ever-changing landscape, often despite efforts by humans to control them.

See also: Amniotes, Diversification of. Biogeography of Vertebrates. Mammalian Diversification. Mammals, Origin of

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Mammals, Origin of

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Introduction

Mammals are not the most speciose group of living vertebrates. Actually, far from it; among tetrapods alone, mammals are one of the groups with less living species, with approximately 5500 species (Wilson and Reeder, 2005), about 25% of all diversity of tetrapods, well behind Lissamphibians (~6600 spp), Lepidosaurians (~9000 spp), and Archosaurians (including birds and crocodilians; ~10 000 spp.) (Pough *et al.*, 2012). However, mammals are certainly the most disparate group of vertebrates, with a wide range of different kinds of adaptations including, but not restricted to, fossoriality, powered flight, and aquatic adaptations. They vary widely in shape and size, and the largest animal to ever exist is a mammal – the blue whale (*Balaenoptera musculus*). Mammals are also very conspicuous elements in many modern ecosystems and have a huge impact in shaping ecological landscapes; most large modern animals are mammals. We ourselves are mammals, so understanding mammalian evolution is also to understand a little about our own history.

Mammalian Characteristics

Independent of size and shape, all mammals share a unique set of characteristics both from soft tissue and the skeleton. Mammals' hearts have a single aortic arc, the right one, and enucleated red blood cells (**Figure 1**). Mammals usually have a very glandular skin, with different kinds of glands as sweat and sebaceous glands, which exercise many different functions as lubrication of the fur and thermoregulation (through evaporation of sweat, for example). However, the most characteristic and unique of the skin glands of mammals are the mammary glands, which give the group its name. Mammary glands are a very important aspect of mammalian life history, and allow mammals to provide nutrients and feed their young independently of the availability of food in the environment (Oftedal, 2002). All mammals suckle their young, and consequently all mammals show some degree of parental care (**Figure 2**).

Most mammals are viviparous, giving birth to live young, although there are exceptions. Monotremes, a group of mammals that is today restricted to Australia and New Guinea and

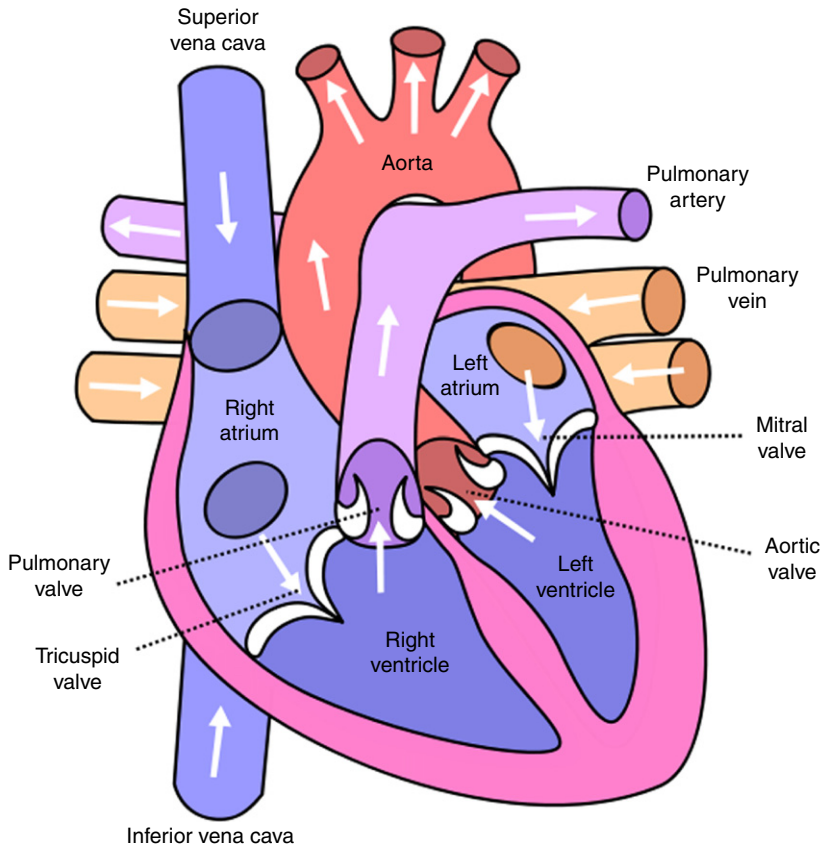

Figure 1 A diagram showing the mammalian heart. Creative Commons (https://commons.wikimedia.org/wiki/File:Diagram_of_the_human_heart_(cropped).svg).

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include platypuses and echidnas, are the only modern mammals that lay eggs. Oviparity is a primitive condition for mammals, and probably was present in the non-mammalian ancestors of the group (Hopson, 1973). The remaining groups of mammals, the marsupials and placentals, share the derived condition of viviparity, despite the differences in reproductive mode that we find today between them. Endothermy is also frequently associated to mammals, although it is not an exclusive characteristic of them, having evolved independently in birds (Pough *et al.*, 2012). However, it is very likely that the evolution of endothermy may have had a big role in shaping the evolution of many of the other mammalian characteristics (McNab, 1978; Kemp, 2006). For example, the evolution of fur is probably related to the evolution of endothermy. Fur exert numerous functions, as defense (think about quills of porcupines or scales of pangolins), orientation (vibirissae), camouflage, and communication, but its primary function is thermal insulation, i.e., to avoid loss of body heat to the environment (Ji *et al.*, 2006).

Figure 2 All mammalians suckle their young. Here is a photograph of a sculpture illustrating the Rome's foundation myth in which a she-wolf suckles the founders of Rome. Creative Commons (https://commons.wikimedia.org/wiki/File:La_Louve_(349313603).jpg).

Mammals are also heterodont, which means that they have highly differentiated teeth, the incisors, canines, premolars, and molars. These differentiated teeth exert different functions during apprehension and mastication of the food, and contribute to better efficiency in processing and assimilation of the nutrients. Mammals have especially complex molars that have a wide contact surface between upper and lowers molars called the occlusal surface, which enhance the efficiency of mastication (Hillson, 2005). Living mammals, in almost all their totality, have tribosphenic molars, a kind of molar that primitively has three main cusps. Most of the varied and different dental morphologies that we see in today's mammals are modified from primitive tribosphenic molars (Kielan-Jaworowska *et al.*, 2004; Luo, 2007).

Mammals also have a bony secondary palate, a structure that isolate respiratory and swallowing functions, and allow feeding and breathing at the same time, which was probably crucial in the evolution of breastfeeding (Pough *et al.*, 2012). Breathing in mammals is also enhanced by the presence of a muscular diaphragm, a muscle that helps in the contraction and expansion of the thoracic cage (Vaughan *et al.*, 2013). The presence of the diaphragm is reflected on the skeleton: mammals usually have reduction of the neck and lumbar ribs, keeping only the ribs on the thoracic cage, creating a clear division between thoracic and lumbar portions of the vertebral column. This division corresponds approximately to the localization of the diaphragm (**Figure 3**).

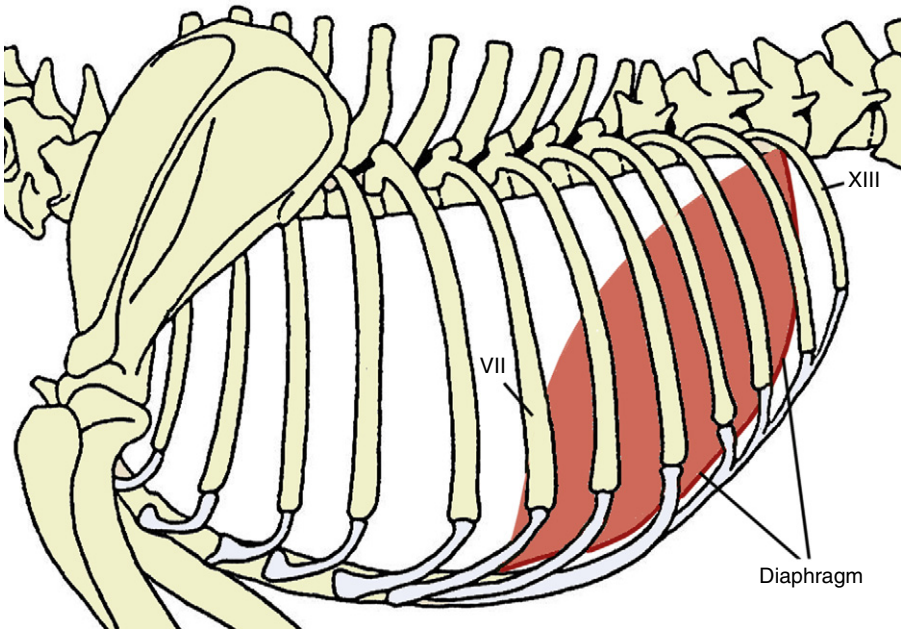

Figure 3 Illustration showing the diaphragm in the thorax of a dog. Creative Commons (https://commons.wikimedia.org/wiki/File:Thorax-withdiaphragm-dog.png).

However, the defining characters of mammals are the structures associated with the ear, jaw, and the temporomandibular articulation of the skull (Kermack and Kermack, 1984; Rowe, 1988). Mammals are the only vertebrates to have the middle ear composed by three ossicles: the incus, malleus, and stapes. Mammals also have the mandible formed by a single bone, the dentary, while all other vertebrates have the mandible composed by many different bones having, besides the dentary, many post-dentary bones. The dentary of mammals also participate in the articulation of the mandible with the squamosal bone of the skull. In most other vertebrates, this connection is formed by different bones, the articular in the mandible and the quadrate in the skull (Kemp, 2005).

To understand how those unique mammalian characteristics evolved, we have to understand the evolutionary processes by which the different lineages of Synapsida went through in the last 300 Ma.

Early Evolution of Synapsids

Mammals are the only living survivors of an ancient lineage of amniotes, the Synapsida. Mammals themselves are known from the Triassic, at approximately 205 Ma, but the first synapsids date from the Carboniferous, more than 320 Ma (Reisz, 1972). Synapsida are one of the oldest known groups of Amniota. They can be readily distinguished from other amniotes by the presence of a single temporal opening on the temporal region of the skull. Today, most amniotes are part of Diapsida, who share two temporal openings on the skull, and the mammals are the only surviving group of synapsids, not sharing a close relationship with any of the other living amniotes (Cracraft and Donoghue, 2004). Mammals are the last in a series of successive radiations of synapsids throughout the end of the Paleozoic and the beginning of the Mesozoic. Extinct species of non-mammalian synapsids are frequently known collectively as 'mammal-like reptiles'. The name, however, is misleading, since these organisms are neither reptiles nor mammals. 'Mammal-like reptiles' is not a natural group, since it excludes the whole of the mammalian radiation. Thus, it is a paraphyletic group in relationship to mammals. Reptiles themselves are paraphyletic, for they exclude both birds and mammals. To be considered a natural group, it must include all descendents from a common ancestor, that is, it should be a monophyletic group.

Synapsids were the first lineage of amniotes to radiate extensively in terrestrial ecosystems, and during the Permian and the first half of the Triassic, non-mammalian synapsids were the predominant group of large vertebrates (Kemp, 2005). The more primitive synapsids and the first group to irradiate are the 'reptile-looking' 'pelycosaurs' (**Figures 4–7**). They are also a paraphyletic group, that is, they are constituted of a sequence of successive radiations before the radiation of the more recent synapsids and do not form a natural group. They were the predominant group of terrestrial vertebrates during the lower Permian (300–250 Ma) (Kemp, 1982).

'Pelycosaurs' had a generalized body plan, with sprawling stance and heavy tails. In these aspects, pelycosaurs recall what most people would identify as a 'reptile' (**Figures 4–7**). Most early Permian ecosystems were dominated by pelycosaurs, which included very small and insectivore species as well as large animals, some attaining 3 m or more in length, and including both herbivore and carnivore species (Kemp, 1982). Herbivores typically had small skulls, large barrel-shaped bodies, and spatulated teeth, adapted to acquire and process vegetable matter (Sues and Reisz, 1998). Carnivores, on the

Figure 4 A skeleton of *Dimetrodon*, a pelycosaur at the National Museum of Natural History in Washington, DC (https://commons.wikimedia.org/wiki/File:Dimetrodon_skeleton.jpg).

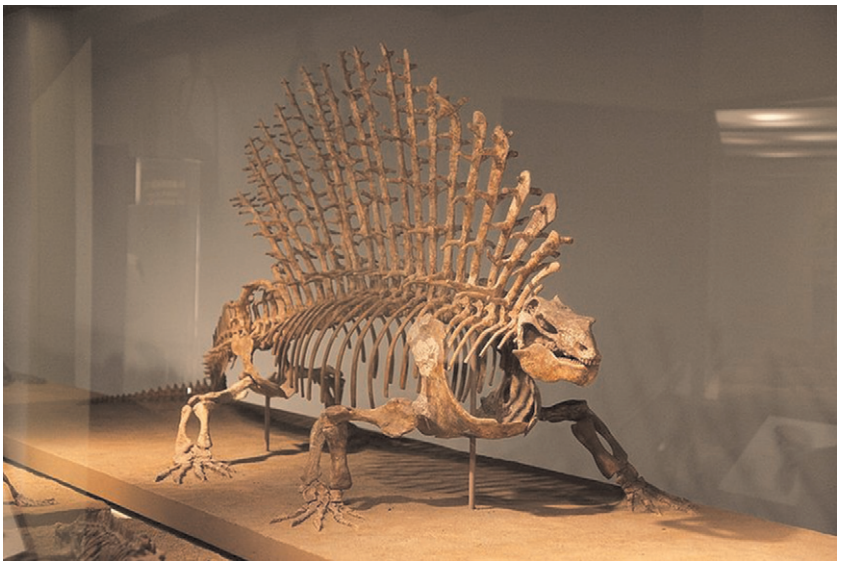

Figure 5 A skeleton of *Edaphosaurus pogonias*, a pelycosaur, at the Field Museum of Natural History in Chicago (https://commons.wikimedia.org/wiki/File:Edaphosaurus.jpg).

Figure 6 A skeleton of *Ophiacodon mirus*, a pelycosaur, at the Field Museum of Natural History in Chicago (https://commons.wikimedia.org/wiki/File:Ophiacodon_mirus_fm.jpg).

other hand, usually had big heads with dagger-like teeth to subjugate and dismember prey. Widely known species as *Dimetrodon* are frequently associated to dinosaurs, but they are actually pelycosaurs (from the family Sphenacodontidae), being more closely related to mammals than to dinosaurs (Angielczyk, 2009). Different species of pelycosaurs, including the carnivorous *Dimetrodon* and the herbivorous *Edaphosaurus*, evolved independently extremely elongated neural spines of the vertebrae (Romer and Price, 1940; **Figures 4** and **5**). These spines probably were covered in living tissue in life, forming a kind of 'sail'. It is thought that these 'sails' may have functioned during thermoregulation – they were restricted to large species, and were probably rich in blood vessels. When the animal would want to warm themselves, they would turn the 'sail' perpendicularly to the sun. Since the sail has a low surface–volume ratio, it allowed the animal to warm faster than other species (Kemp, 1982). Likewise, cooling was also enhanced by the presence of the 'sail.' If confirmed its role in thermoregulation, it is interesting to observe the evolution of early mechanisms of controlling the temperature in the lineage of synapsids.

Despite their generalized appearance, some pelycosaurs already showed the early stages of a series of characteristics that we will find in more advanced condition in later synapsids and mammals (Kemp, 1982; Kermack and Kermack, 1984). Those characteristics were especially developed in derived families as the Sphenacodontidae. Some of these derived pelycosaurs had a beginning of dental differentiation, with some carnivore species displaying clearly elongated caniniform teeth, and showing some differentiation between

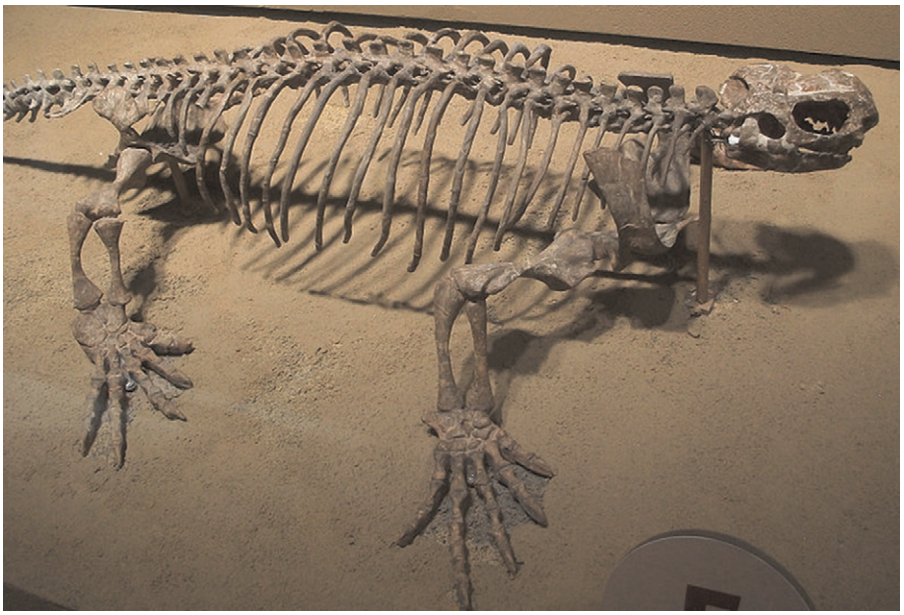

Figure 7 A skeleton of *Casea broilii*, a pelycosaur, at the Field Museum of Natural History in Chicago (https://commons.wikimedia.org/wiki/File:Casea_broilii.jpg).

pre-caniniform teeth (incisiviforms) and post-caniniform teeth. They had a typical primitive synapsid skull, but derived groups showed expansion of the dentary on the mandible at expense of post-dentary bones and an archeated palate, which may presage the development of a secondary bony palate (Kemp, 2005).

The next radiation of synapsids is the group known as Therapsida. Therapsids mostly replaced pelycosaurs from the middle until the end of the Permian as the dominant group of terrestrial vertebrates. As pelycosaurs, therapsids are a paraphyletic group, unless you include mammals in it (Kemp, 2005). There are many groups of non-mammalian therapsids that show characteristics progressively more mammalian. Non-mammalian therapsids irradiated throughout most of the upper Permian and lower Triassic, and extinguished around the upper Jurassic, but by then their diversity was much diminished.

Even some of the more primitive therapsids already show some characteristics that evoke the condition we see in mammals. Many of those characteristics are possibly associated with higher metabolic rates and higher levels of activity when compared to pelycosaurs (Hopson, 2012). There's a tendency for the enlargement of the primitive temporal fenestra of the synapsids, associated with the expansion of the masticatory musculature (Kemp, 2005). There is also further differentiation between the different kinds of dentition, with clearly defined caniniforms, incisiforms, and anterior and posterior post-caniniform teeth (Kermack and Kermack, 1984). The coanae (internal apertures of the respiratory tract) are connected to the external nares by a sulcus on the palate, and the dentary are much larger than in pelycosaurs, further usurping the function of the post-dentary bones. These characteristics are not restricted to the skull, and the post-cranial skeleton shows a simplified pelvic girdle with a more forwarding pointing ilium and less sprawling limbs, which suggests a semi-erect position, at least in the more derived therapsids (Rubidge and Sidor, 2001).

Therapsids are known from the upper Permian ($\sim$260 Ma) and irradiated extensively throughout the Upper Permian, effectively replacing the pelycosaurs in most of the niches available to large terrestrial vertebrates, evolving large specialized herbivores as the Dinocephalia, and large carnivores as the Gorgonopsida (Kemp, 2005), but also small insectivores and the first arboreal synapsids known (Fröbisch and Reisz, 2009; **Figures 8–12**). Some of the groups of therapsids were highly successful in terms of diversity and sheer numbers of individuals.

The dicynodonts, one of the longest living groups of Synapsida, spanned from the upper Permian, survived the great extinction at the end of this period and flourished in the Triassic, and perhaps, but controversially, extending into the Early Cretaceous (Thulborn and Turner, 2003), which, if confirmed encompass an astonishing long period of time. Dicynodonts included mostly herbivore forms, from small and fossorial to very large and semi-aquatic. They were one of the most widespread and diverse large herbivores from the Upper Permian and the Lower Triassic (Kemp, 1982). Dicynodonts had reduced dentition and the anterior part of the jaw was covered with a horny beak like those of turtles (Cluver and King, 1983; Kammerer and Angielczyk, 2009), being somehow different from the idea we have of a vertebrate from the lineage of mammals. The therapsids also included a wide range of carnivore forms, and during the Upper Permian most of the larger carnivores belonged to this group (Kemp, 2005).

Despite its diversity, by the end of the Permian most of the lineages of therapsids went extinct, associated with a mass extinction that occurred during this period. The extinction of the end of the Permian was actually the largest extinction known in the history of the Earth, when about 84% of

Figure 8 A skeleton of *Biarmosuchus tener*, a biarmosuchian therapsid at the Dinosaurium exhibition, in Prague (https://commons.wikimedia.org/wiki/File:Dinosaurium,_Biarmosuchus_tener_1.jpg).

Figure 9 A fossil skeleton of *Inostrancevia alexandri*, a gorgonopsid therapsid (https://commons.wikimedia.org/wiki/File:Inostrancevia_alexandri.JPG).

the genera and 90% of the species then known went extinct (Benton, 2005). The causes of this extinction are still debatable, including extraterrestrial impacts, climate change, increased volcanic activity, etc. Beyond the Permian/Triassic frontier, we have practically only two groups of surviving synapsids: the already mentioned Dicynodonts and another group, the Cynodonts (Smith and Ward, 2001), from which probably emerged the ancestor of modern day mammals.

The first record of cynodonts date from the Upper Permian, and they are one of the few groups of synapsids to survive the extinction at the end of the Permian, radiating extensively during the Lower Triassic (Kemp, 2005). Most of cynodonts were small vertebrates, mainly carnivores or insectivores, with a strong tendency for miniaturization in the lineage, especially in the Upper Triassic. Although in the beginning of the Triassic some cynodonts, like *Cynognathus* (with a snout-vent length of about 1 m), were relatively large, by the end of the Triassic no cynodont was larger than 200 mm (Ruta *et al.*, 2013; **Figures 13–15**). They were the most mammal-like of the non-mammalian therapsids, with high degree of dental differentiation, with post-caniniform teeth frequently multicuspidated and showing dental occlusion, development of a maxillary fossae on the dentary for the insertion of masticatory muscles, enlargement of the infraorbital foramen (Hopson and Kitching, 2001), which may indicate the presence of vibrissae, and evidence of the presence of turbinal bones (Hillenius, 1994). The

Figure 10 A skeleton of *Lycaenops ornatus*, a gorgonopsid therapsid, at the Museo di Storia Naturale di Milano (https://commons.wikimedia.org/wiki/File:Lycaenops_ornatus_1.JPG).

Figure 11 A skeleton of *Moschops capensis*, a dinocephalian therapsid at the American Museum of Natural History, in New York (https://commons.wikimedia.org/wiki/File:Moschops_capensis_-_AMNH_-_DSC06321.JPG).

dentary was also very large, and a tall coronoid process has evolved, while most post-dentary bones were small and relatively unimportant (Kemp, 2005).

Evolution of Mammalian Characteristics

Since we are close to the origin of mammals, it may be useful to review the evolutionary tendencies that we observe along the lineage of synapsida leading to mammals. Synapsids were one of the earliest lineages of amniotes to diverge, and they remained as one of the most significative components of terrestrial faunas until they were mainly displaced by Diapsid reptiles by the end of the Triassic. Groups of Synapsida tend to show characteristics progressively more mammalian during the course of its evolution, and they are possibly associated to metabolic changes associated to endothermy and a better processing of the food (Sidor and Hopson, 1998). The exact time of evolution of endothermy and which groups of synapsids had any level of control of its temperature are still controversial. Cynodonts, in special, show so many mammalian characteristics that it seems pretty likely that they already were endothermic. Pelycosaurs, on the other hand, almost certainly were ectothermic, with a metabolism similar to modern lizards and crocodiles (Kemp, 2006). However, many of the characters that would strongly imply endothermic metabolism (as the presence of fur) only under exceptional circumstances fossilize (Ji *et al.*, 2006), so we usually have to extrapolate from the indirect evidence of the skeleton.

Among the synapsids there is a tendency for the enlargement of the temporal fenestra originally present in the ancestor of synapsids. This enlargement is associated to the increase of the cranial capacity, the enlargement of the brain, and mainly, with the development of the masticatory musculature. The enlargement of the temporal fenestrae is accompanied by the outward projection of the inferior bar

Figure 12 A skeleton of *Placerias*, a dicynodont therapsid at the Rainbow Forest Museum in Holbrook, Arizona (https://commons.wikimedia.org/wiki/File:Placerias_at_the_Rainbow_Forest_Museum.jpg).

Figure 13 A skull of *Chiniquodon theotonicus*, a cynodont therapsid at the Museum of Paleontology, Tuebingen (https://commons.wikimedia.org/wiki/File:Chiniquodon_theotonicus_skull_43.JPG).

Figure 14 A skull of *Cynognathus*, a cynodont therapsid at Museum Mensch und Natur, Munich (https://commons.wikimedia.org/wiki/File:Cynognathus.JPG).

of the fenestra, which in mammals forms the zygomatic arch (Kemp, 2009). Pelycosaurs didn't have a secondary palate, but some advanced forms did have the palate slightly archeated.

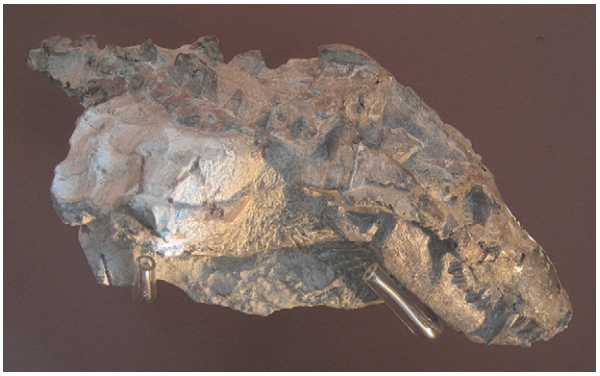

Figure 15 A skull of *Thrinaxodon liorhinus*, a cynodont therapsid at Cosmocaixa, Barcelona (https://commons.wikimedia.org/wiki/File:Thrinaxodon_liorhinus.jpg).

Some advanced non-cynodont therapsids had an incomplete secondary palate, i.e., the palate curved inwards almost closing in itself, and in life they probably were completely closed by soft tissue. Most cynodonts already had evolved a complete secondary palate (Angielczyk, 2009).

The postcranial skeleton also went through drastic changes during the evolution of synapsids. There's a tendency to the progressive positioning of the limbs under the body, in contrast with the primitive amniote condition, where the limbs were positioned perpendicularly in relation to the central axis of the body, resulting in a sprawling stance. The changes in the positioning of the limbs are also reflected in the limb musculature insertions, and consequently, in the structure of pelvic and scapular girdles (Kemp, 1982). Mammals have a reduced pubis when compared with its pelycosaurs precursors, and the ileum projects forward, reflecting the restructuring of the locomotor muscles (Jenkins and Parrington, 1976). There is also a tendency for the reduction of the scapular elements, like the interclavicule and choracoid bones, that are absent in most mammals, but present in non-mammalian synapsids (Jenkins

and Parrington, 1976; Ji *et al.*, 1999). The phalanges of mammals are also shortened when compared specially with pelycosaurs, as a consequence of the limbs being positioned under the body and shift in gait. In non-mammalian amniotes, the astragalus is positioned laterally to the calcaneous, while in mammals, the position of the astragalus is changed, being positioned over the calcaneus, restricting lateral movements, but emphasizing parasagittal movements (Ji *et al.*, 1999). Some primitive mammals (like monotremes) have the astragalus only partially superimposed over the calcaneus. Finally, there is the formation of a projection of the calcaneum for the insertion of the lower limb musculature (Kemp, 2005).

However, the most important changes are related to the evolution of the middle ear and the temporomandibular articulation. The dentition of the synapsid becomes more and more complex, starting from a homodont condition, where all the teeth share the same basic morphology and evolve toward dentary differentiation and dentary occlusion (Kermack and Kermack, 1984). The dentary expands progressively, taking the functions of the post-dentary bones, until those are greatly reduced. In most of the amniotes, the temporomandibular articulation is formed by the articular bone in the mandible and the quadrate bone in the skull. In mammals, the articulation is formed by the squamosal and the dentary. As the dentary expands, the articular and quadrate gradually loses the function of articulating the mandible with the skull. As those bones lose function, they are co-opted to the middle ear, and give origin to the ossicles of the ear of mammals. The articular then is homologous to the maleus and the quadrate is homologous to the incus. The other bone of the middle ear of mammals is the stapes, that is homologous to the single bone of non-mammalian amniotes, the columela. The angular bone of the mandible of non-mammalian synapsids is homologous to the ectotympanic bone, a ring-shaped bone which supports the tympanic membrane (Luo, 2011).

Some derived cynodonts and basal mammals had a double (mixed) articulation, where both the dentary and the articular participated in the articulation with the squamosal and quadrate, so these bones are not yet incorporated in the middle ear (Kermack and Kermack, 1984; Crompton, 1970). Interestingly, during the embryonic development, some mammals initiate the development with the middle ear ossicle and the ectotympanic connected to the mandible in the corresponding position of the post-dentary bones of synapsids, and only later in the development they migrate to the middle ear (Rowe, 1996).

Mammalian Evolution

By the end of the Triassic and the beginning of the Jurassic, non-mammalian therapsids were virtually extinct, and most of the niches for large terrestrial vertebrates were occupied by Diapsids, mainly dinosaurs. The only group of synapsids to flourish in this period was the mammals. The oldest mammals were from the Upper Triassic ($\sim$200 Ma) (Kielan-Jaworowska *et al.*, 2004). There is some debate if these early representatives of the group should be really called Mammalia. Some authors prefer to restrict the name to modern species and their fossil relatives, and those early mammals would be part of the more inclusive group Mammaliformes (Rowe, 1988). However, since they show the fundamental characteristics of mammals, as the articulation of the dentary with the squamosal, and probably were fully mammalian in their biology, we should use Mammalia in this more inclusive meaning, and consider those early representatives as mammals.

The earliest mammals like *Morganucodon* and *Megazoostrodon* show a continuation of the trend we observe in the cynodonts and were very small animals, with less than 100 g, and were probably nocturnal insectivores (Kermack and Kermack, 1984; Kielan-Jaworowska *et al.*, 2004). From these early forms, mammals irradiated extensively during the Mesozoic. The Mesozoic correspond to two-thirds of the history of mammals (Kielan-Jaworowska *et al.*, 2004). For a long time, Mesozoic mammals were thought to be relatively little diverse, being predominantly little scurrying insectivores. However, in the last decade, new discoveries showed a greater diversity of adaptive types among Mesozoic mammals than previously suspected, including species adapted for swimming, digging, and gliding (Luo, 2007). Despite this variety, the Mesozoic radiation of mammals was still greatly limited when compared to more modern groups, lacking the ecological equivalents of whales, bats, and antelopes.

The diversification of Mesozoic mammals occurred through successive radiations (Luo, 2007). The first one occurred in the Jurassic and Lower Cretaceous, involving mainly archaic groups that, in their majority, did not leave living descendents. The second radiation occurred mainly in the Middle and Upper Cretaceous and involved more derived forms, including the ancestors of today marsupials and placentals. The oldest metatherian (*Sinodelphys*) is from the Lower Cretaceous ($\sim$125 Ma) (Luo *et al.*, 2003) and the oldest eutherian (*Juramaia*) is from the Upper Jurassic ($\sim$160 Ma) (Luo *et al.*, 2011). Both are from China, and show that the divergence between those two groups occurred at least at the end of the Jurassic.

By the end of the Cretaceous there was a new mass extinction that, while less devastating than the extinction of the end of the Permian, extinguished most of the non-avian dinosaurs and many of the archaic groups of mammals. However, the ancestors of living placentals and marsupiais survived and thrived, irradiating extensively during the Cenozoic. As in the case of the extinction of the end of the Permian, the causes of the end of the Cretaceous extinction are still debatable, although there is some agreement that a large asteroid hit the Earth around this time (Schulte *et al.*, 2010). During the beginning of the Cenozoic, in the period known as Paleogene, the climate was hot and forests similar to today's tropical forests spanned to above the Arctic Circle (Blois and Hadly, 2009). During this time, many archaic groups of mammals evolved, as well as the first representatives of modern orders of mammals. Many unique types of mammals were known then, and the fauna would look unfamiliar to modern eyes. From the Oligocene onwards, the planet temperature started falling, leading to the formation of polar caps and restricting tropical-type forests to the equatorial regions. Consequently, there was an increase in the aridity of the planet and large plains covered in grasses started to spread. Many of the archaic groups of mammals became extinct during this time, opening avenues for new fauna to flourish with a more modern aspect (Rose, 2006).

See also: Mammalian Diversification. Mammals Everywhere

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Mate Choice and Sexually Selected Traits

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Glossary

Genetic correlation or linkage disequilibrium A statistical association between alleles at two or more loci, where specific alleles at each locus are found together in a genome more often than expected by random assortment.

Indirect selection Selection that acts at a locus as a result of its genetic correlation with another locus that is the primary target of selection. During sexual selection, this often arises when a preference or choosiness allele spreads because it is in linkage disequilibrium with alleles that are favored by natural or sexual selection.

Marginal cost of display The cost of producing a unit level of display. This may also be thought of as the inverse of the efficiency of producing a mating display. For example, in the epistatic indicator mechanism, low quality males pay a higher cost for making a given amount (size/intensity) of a display compared to the cost paid by high quality males for producing the same display, thus resulting in higher marginal costs for low quality males.

Mate choice In the most general sense, a process that results in nonrandom mating, in which one sex, usually the female, determines her mate. Two components of mate choice are 'preference,' which determines the identity of the male trait toward which matings are biased, and 'choosiness,' which determines the strength of the bias. These two components may or may not be governed by the same genetic loci.

Mating system A description of the relative variation in mating success of males and females in a population or species. This can also include a description of whether individuals change mates over their lifetime.

Monogamy A mating system in which males and females form pair bonds. In genetic monogamy mating only occurs within the pair and both sexes hence have equal mating success (if there is social but not genetic monogamy, extra-pair copulations may take place, and mating success between the sexes may differ; there may also be serial monogamy if pairings change across breeding seasons).

Polygyny A mating system where females have low variation in the number of mates, but males exhibit a large amount of variation in their mating success. Usually, but not always, associated with low promiscuity in females and high promiscuity in males.

Speciation The process by which one species splits into two species via the cessation of gene flow between them.

Introduction

Elaborate male mating displays are common in a wide variety of taxa (Andersson, 1994). Courtship dances, powerful or complex song, pheromones, and showy visual features, ranging from ornate plumage in birds to colorful dewlaps in lizards, are just a few examples of traits that seem unnecessary for survival, but have another important function (Figure 1). Specifically, these traits can give males an advantage through the process of sexual selection if they increase male mating success. This benefit can arise if the trait improves success in male–male contests or if females prefer larger or more elaborate display traits in their mates. An important question in evolutionary biology is to understand the evolution of such male traits and female mate choice favoring them.

Although female mating displays do occur in some systems, males are generally the showier sex (but see Section 'Display traits in females'). In a polygynous mating system, the most common mating system in nature, there is little variation in female mating success, but males can vary widely in the number of mates that they acquire. In such cases, females have the opportunity to choose mates from the more numerous courting males. Male display traits can often form the basis for this mate choice. The fact that these traits can increase mating success is necessary to explain their evolution; many displays generally reduce the viability of males that produce them, and thus their evolution cannot be explained by natural selection alone. Given heritable variation, the evolution of male mating displays is then fairly straight-forward to understand; the advantage of gaining more mates simply needs to be large enough to compensate for the viability costs of producing the display. Why females evolve to favor certain male traits is often a more complex question.

Female Preferences for Male Traits

The intricacies of evolution through sexual selection due to mate choice largely focus on the selection that favors the evolution of preferences in females. Although the relative availability of males and females during polygyny gives females the opportunity for choice, it is likely that in most cases this choice is costly. For instance, searching for specific types of mates invariably involves spending energy and time. Females may also expose themselves to predation, or may not be able to start reproducing early. The maintenance of neural mechanisms to evaluate different aspects of a male display may also be costly. Given that mate choice may reduce female viability in these ways, some form of selection must overcome these costs for female mate choice to evolve.

Although often discussed as a single female characteristic, female mate choice can have two components that can potentially originate and evolve independently: (1) female mating preference, and (2) female choosiness (Jennions and

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Figure 1 Types of male displays – (clockwise from top-left) a calling male Houston toad, a sharp-tailed grouse performing a courtship dance, a long-tailed widowbird with ornate, long tail feathers, a Honduran giant anole displaying brightly colored dewlap, and a male stalk-eyed fly with exaggerated eye-stalks (*Teleopsis dalmanni*).

Petrie, 1997). A mating preference describes the identity of the male trait that females prefer. For example, female mating preference could describe what sound frequency of a male call or what particular plumage color is preferred by females. Choosiness is the degree to which females express their preference. For example, all females may prefer the same type of call, but choosier females may sample a larger number of males or wait longer to find their ideal mate before mating. Thus, a preference is 'which' male trait is favored by females, while choosiness is 'how much' they favor it. The distinction between these components of female mate choice is important, because preference and choosiness may be controlled by different loci and face different costs. The cost of a mating preference may be restricted in some cases to the cost of maintaining neural mechanisms to evaluate different aspects of male displays (but see below). The cost of choosiness, on the other hand, would be influenced by factors such as availability of preferred mates or the cost of mate searching.

Certain properties of male traits may be important in determining the nature of selection that may act on female preferences. First, male traits may influence the cost of mate choice. Displays that are easier to evaluate may reduce the amount of time and energy that females have to spend on mate choice. The display traits themselves may also impose costs on females if aggressive displays reduce female viability or attract predators. Second, displays may influence the potential benefits that females gain through mate choice. By mating with attractive males, females, by default, are more likely to have more attractive sons, provided that male attractiveness is heritable. But females may also gain other benefits if male displays are correlated with the ability of a male to provide viability or fecundity advantages to females or their offspring.

These potential benefits and other explanations for the origin and elaboration of female mating preferences eluded Darwin when he first proposed the idea of sexual selection (Darwin, 1871), but have been the focus of extensive research on sexual selection in the past several decades. Below we first discuss how mating preferences may originate and then explore the factors that shape the elaboration of preexisting preferences (i.e., the evolution of preference for more extreme displays or the evolution of higher choosiness).

The Origin of Female Mating Preferences: Sensory Bias

In spite of the important role of mating preferences in the reproductive biology of a species, they may originate in contexts unrelated to mating. New mutations that produce mating preferences can, of course, spread in a population through the process of drift. More interestingly, selection on females in nonreproductive contexts can give rise to mating preferences. Evolution of the female neural system in response to the numerous sources of natural selection can result in females being more sensitive to certain external stimuli over others. For example, selection for more efficient foraging may make females more likely to notice colors that correspond to food. Even without this form of natural selection, the physical properties of the sensory system may result in sensitivity peaks for certain stimuli (e.g., the structure of the auditory system may cause higher sensitivity to a particular sound frequency). The evolution of the female sensory system in non-mating contexts can thus lead to sensory biases. Males that possess traits that match such sensory biases in females may be more easily noticed, potentially giving them a mating advantage. Evolution of male traits to exploit such preexisting sensory biases in females can result in these biases gaining the secondary 'function' of a mating preference. At this initial stage these preferences may not be very strong, but they can now be acted upon by sexual selection in addition to the natural selection that gave rise to them. Below we discuss how preexisting sensory biases may become exaggerated by sexual selection beyond their natural selection optimum.

Elaboration of Female Mating Preferences

A number of mechanisms have been proposed to explain how female mating preferences become more elaborate once variation in preferences and male traits is established. The different mechanisms discussed below are not mutually exclusive, and it is likely that multiple mechanisms shape the evolution of female mate choice even within a singe system. However, it is useful to consider these mechanisms separately for heuristic purposes.

Fisherian Process

Fisher (1930) was the first to provide a verbal explanation for the evolution of female mating preferences, which was later mathematically formalized by Lande (1981) and Kirkpatrick (1982). The Fisherian process (sometimes also called the Lande–Kirkpatrick model) is based on the fact that the mere existence of heritable variation in a female preference and male trait will inevitably build up a statistical correlation between them. Any selection, including both natural and sexual selection, acting on the male trait then results in indirect selection on the female preference. Because female preferences themselves place sexual selection on male traits, this results in a positive feedback loop. This may theoretically lead to a 'runaway' with both preference and trait becoming highly exaggerated. But even in the absence of a runaway, indirect selection on female preferences due to the Fisherian process is an inevitable component of all cases of sexual selection where heritable variation exists for a female preference and male trait. The Fisherian process (or Lande–Kirkpatrick model) has therefore been proposed as the null model for sexual selection through mate choice (Prum, 2010).

Direct Benefits of Mate Choice

In many cases, females may gain more than sperm from males; they may gain resources, territory, protection, or parental care, all of which can directly improve female viability or fecundity. If the amount of such 'direct benefits' provided by males is correlated with the size or quality of a display, females that preferentially mate with males with bigger or better displays would gain more direct benefits. When females can gain direct benefits from males, mating preferences may evolve due to the resulting direct selection even in the absence of heritable variation in male traits (e.g., when male displays and the benefits provided by males are completely determined by environmental factors). In such cases the Fisherian process would be absent. However, it is important to realize that as long as heritable variation exists in displays and preferences, female mating preferences would evolve due to both the direct benefits and the Fisherian process.

Good Genes

Females may gain benefits through mate choice even when males provide only sperm. If male display traits are correlated with any heritable component of viability, such as, for example, better resistance to parasites (Hamilton and Zuk, 1982) or higher survivability (Zahavi, 1975), females that preferentially mate with more ornamented males would sire offspring that are more likely to gain these genes for higher viability. Females can thus gain an indirect benefit of acquiring 'good genes' for their offspring through mate choice. The correlation between female preferences and alleles for higher viability that would result from this process can lead to the evolution of more exaggerated female preferences. The Fisherian process, which works through a correlation between female mating preferences and male display, still remains a part of the good genes process. The good genes benefit can thus only add to the Fisherian process.

For females to be able to gain indirect viability benefits through mate choice, it is necessary that male displays be phenotypically correlated with male genetic quality. That is, male displays must be honest indicators of male quality. Three mechanisms, which may work simultaneously in any given system, have been proposed to enforce honesty and cause the build-up of such a display–quality correlation (**Figure 2**). These mechanisms differ in how male genetic quality influences the production of the display.

Condition-Dependent Indicators

If high quality males are capable of producing a display that is larger or more elaborate than the displays that can be produced by low quality males, females may be able to use display size as an indicator of male genetic quality. The ability to produce a large display may be restricted to high quality males because they have more resources available to invest in costly display production. It may also arise due to physiological constraints that may enforce a dependency of display on male condition (e.g., the growth of horns in rhinoceros beetles, Emlen *et al.*, 2012). Because low quality males produce a smaller display, they pay a proportionally smaller cost; their mating benefit, however, is also lower because females do not prefer them (**Figure 2(a)**).

Revealing Indicators

It is possible that male genetic quality influences not the size of the male display, but the quality of the display that males are capable of maintaining. For example, male birds sensitive to feather mites may have their plumage deteriorate, while resistant males may be able to maintain a higher quality plumage. The displays thus produced would bear equal cost for all males, but would 'reveal' male genetic quality through the display quality. Revealing indicators thus differ from condition-dependent indicators only in that in this mechanism, low quality males pay the full cost of display production and yet do not get a proportional mating advantage (**Figure 2(b)**).

Pure Epistatic Indicators

The pure epistatic indicator mechanism can give rise to a phenotypic correlation between male display and male genetic quality because males of different quality pay different 'marginal costs' of display production. If low quality males bear a higher cost for producing displays compared to high quality males, males with elaborate displays are more likely to be the high quality males that were able to survive to the time of mating. Zahavi (1975) originally proposed this mechanism in the extreme form where low quality males simply do not survive when they produce a display. But a correlation between display and genetic quality may arise even with smaller differences in the viability costs of the display between males of different quality (**Figure 2(c)**).

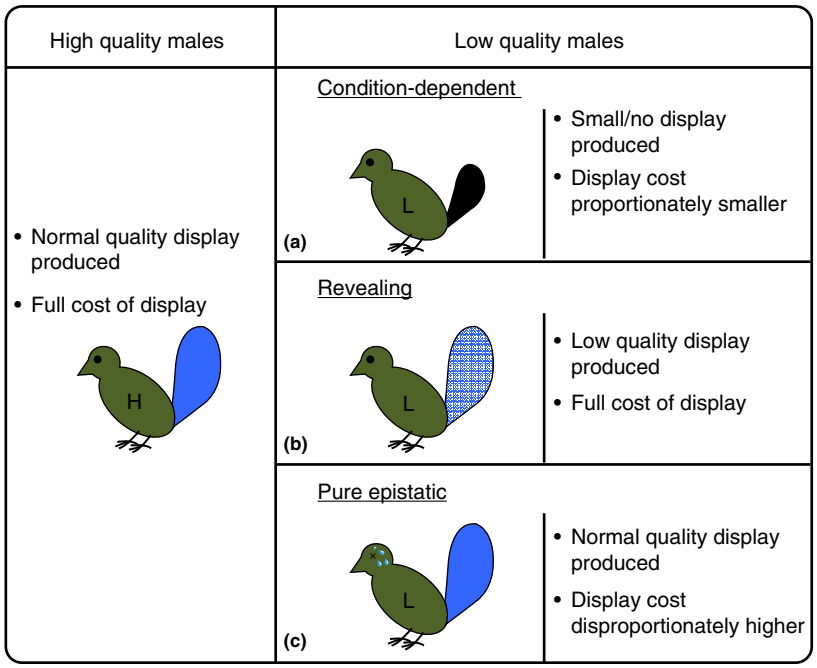

Figure 2 The three indicator mechanisms that can result in honest indication of male quality through a display differ in what gives rise to the correlation between male display and male quality – display size, quality, or cost. The left panel shows a high quality male that can produce a normal display. The three rows on the right show the three classic indicator mechanisms discussed in the text. High and low quality males are indicated by the letters 'H' and 'L' respectively.

The relative feasibility of these three good genes mechanisms and their relative importance in the evolution of female mate choice and male displays is highly debated. Part of the debate is focused around the theoretical feasibility of the different mechanism, with most mathematical models of these processes deeming pure epistatic indicators unlikely to evolve. Empirical evidence for good genes processes in general (i.e., a correlation between display and male quality) has been found in several systems (see Prokop *et al.*, 2012). Empirically distinguishing between the three specific good genes mechanisms above is, however, difficult. In particular, demonstrating that low quality males pay a 'disproportionately' higher cost for a display is especially challenging.

Heritability of Display Traits

The evolution of display traits, as of any phenotype, requires the trait to have a heritable basis. Yet there are many cases in which preferred trait phenotypes may be largely, or in some cases entirely, determined by the biotic or abiotic environment. Red plumage ornamentation, for example, can be affected by the availability of carotenoids in the diet. In this case, mating preferences that may exist for redder coloration could potentially have an evolutionary effect both on the ability of males to express red coloration given carotenoids in the diet, and on dietary preferences for foods with these carotenoids. Ultimately, however, phenotypic expression of the trait will still be highly dependent on dietary carotenoid availability.

Other traits that can influence mate choice, such as the song of oscine passerines, may be learned. Depending on the form of learning, these traits may still have a heritable component, even if this component is not genetic. Song learned from parents or siblings, for example, mimics genetically inherited phenotypic traits in that offspring phenotypes are correlated with those of their parents. Cultural evolution of such 'vertically transmitted' song traits is thus expected to proceed, in many cases, in a similar manner to that of genetic evolution of non-learned traits; in these two cases sexual selection may have very similar effects on phenotypic changes in traits over time. In other cases, birdsong or other culturally attractive behaviors may be learned from unrelated individuals in the population. Such traits have no heritable component, and so these traits are not necessarily expected to be similar between generations.

The Importance of Mating System

As discussed above, in a polygynous mating system, where the mating success of females is equal but that of males can differ, female preferences will impose sexual selection on male traits, leading to display trait evolution. Female mating preferences may not have the same effect, however, in other types of mating systems. In a mating system that is both socially and genetically monogamous, the mating success of both males and females should be equal. In other words, there would be no sexual selection on male (or female) traits, even if there were mating preferences. Preferences in this case would lead to genetic correlations between loci controlling the preferences and traits, but would not, by themselves, affect the frequencies of alleles at these loci. Mathematical models of monogamy show that additional effects, such as high fecundity of preferential matings or of preferred males, must be present in order

for showy male traits to evolve in such a system (O'Donald, 1980; Kirkpatrick *et al.*, 1990). Male traits can also evolve in some cases during monogamy, as during polygyny, if they are honest indicators of genetic quality (Andersson, 1986).

Display Traits in Females

The ability of ornamental traits to evolve in females, as in males, is highly dependent on the mating system. In sex-role reversed species (i.e., in a polyandrous mating system) such as the red-necked phalarope (Figure 3), there are fewer males available to mate than females at any one time, and females may have differential mating success. It is therefore expected that in such species, mate choice imposed by male preferences upon female traits may operate very similarly to mate choice imposed by female preferences upon male traits in a polygynous mating system. Female traits may then evolve by sexual selection in a manner very similar to that discussed for male traits above.

Mathematical models indicate that the situation is expected to be very different, however, if the mating system is changed. Female and male roles are expected to be indistinguishable during strict social and genetic monogamy; therefore, as discussed for male traits above, factors such as fecundity selection or condition dependence may have to be present to allow the evolution of showy female traits in monogamous systems. During strict polygyny, where all females have equal mating success, male mating preferences may be expressed as differential courtship by males toward ornamented versus unornamented females. Males that bias their courtship toward ornamented females would place themselves in a situation of many competitors vying for matings with these females, while males with no mating preference instead would often find themselves in a low competition situation, trying to mate with an unornamented female (see Figure 4). Because during polygyny both ornamented and unornamented females are expected to have equal mating success, males that prefer ornamented females would end up with lower mating success than would males that have no preference, due to the fact that the former males face higher mating competition (Servedio and Lande, 2006). The direct selection against male preferences resulting from these competitive effects would make it very difficult for male preferences for strictly ornamental female traits to evolve during polygyny. Furthermore, because females by definition have equal mating success under strict polygyny, male mating preferences during polygyny do not result in differential mating success of females; in other words, there is no sexual selection placed on female traits by male preferences during strict polygyny, so female traits will not evolve simply because of the presence of male preferences.

Despite these theoretical predictions, male preferences for female traits are sometimes found in polygynous mating systems (Amundsen, 2000; Bonduriansky, 2001). In such cases, however, these female traits are generally not strictly ornamental, but are instead direct indicators of high fecundity, such as large body size. In other cases, female traits may evolve due to pleiotropy between the expression of these traits in females and in males, where they are sexually selected.

Figure 3 Females (in the back) of the red-necked phalarope are more brightly colored than the males (in the front).

Trait Evolution during Speciation

Mating traits are thought to have great importance in the context of speciation. Mating preferences for traits that differ between conspecifics and heterospecifics can constitute a potent barrier to the exchange of genes between species. The traits that differentiate species are often very striking in their diversity among closely related taxa.

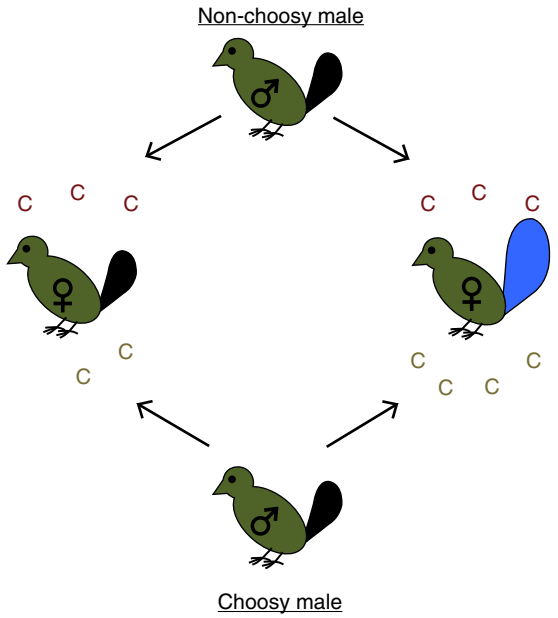

Figure 4 Imagine two males with equal amounts of resources available for courting females (total number of Cs). One male courts females randomly (non-choosy male; red Cs) and the other preferentially courts ornamented females (choosy male; green Cs). If mating success is proportional to the relative courtship effort perceived by a given female, choosy males would gain lower mating success than non-choosy males. In this figure, for instance, choosy males gain a small advantage with ornamented females (4 out of 7 Cs), but non-choosy males have a relatively higher advantage with unornamented females (3 out of 5 Cs).

Both mating preferences and traits are expected to respond, under certain cases, to evolutionary pressures resulting from the speciation process. This evolution is particularly easy to understand in the process of reinforcement, the evolution of characters involved in premating reproductive isolation to avoid hybridization (Dobzhansky, 1937). If partially differentiated species are still capable of successful hybridization, but hybrids have low fitness, this places selection on individuals in each species to mate with conspecifics, rather than heterospecifics. Individuals with mating preferences or traits that are more divergent from those in the other species will be less likely to hybridize, and thus have a selective advantage. This selection leads to the evolution of further differentiation between species in these mating characteristics. Ecologically divergent selection may also allow the evolutionary divergence of mating preferences and traits. Exploration of the evolution of mating preferences and traits during speciation is the subject of a rich literature consisting of both mathematical models and empirical work, but much more work remains to be done in this area before a synthetic consensus between these approaches is reached.

See also: Mating and Parental Sex Roles, Diversity in. Mating Systems, A Brief History of. Sexual Selection, Theory of. Speciation, Sexual Selection and

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Maternal Effects

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Glossary

Maternal effects Parental effects specifically caused by mothers.

Maternal effect coefficient The coefficient that gives the change in an offspring's trait value per unit change in a trait value of the mother, when this maternal trait has a maternal effect.

Parental effects Influences of maternal and/or paternal attributes on the offspring phenotype. The causality of the effects is not via direct genetic transmission of parental alleles.

Phenotypic plasticity The capacity of an organism to have different phenotypic trait values in response to environmental conditions.

Reaction norm A function describing how phenotypic trait values of a given genotype change across a range of values of an environmental variable.

Transgenerational plasticity The plastic change in the phenotype of an organism in response to an environment experienced by its parents and mediated by parental phenotypes.

Introduction

Mothers do not just provide genes to their offspring; they also often determine the phenotypes of eggs and newborn offspring and the environment in which they develop (Badyaev and Uller, 2009; Mousseau and Fox, 1998; Roach and Wulff, 1987; Uller, 2008; Wade, 1998; Wolf and Wade, 2009). Maternal effects are therefore defined as influences of maternal attributes on the offspring phenotype, where the causality of the effects is not via direct genetic transmission of maternal alleles but via maternal phenotypic traits and/or variable environmental properties that mothers experience (Mousseau and Fox, 1998; Wolf and Wade, 2009).

First identified by quantitative geneticists more than 60 years ago (Dickerson, 1947), maternal effects were first perceived to be environmentally determined sources of phenotypic variation that contaminated estimates of heritability (Falconer and Mackay, 1996; Wade, 1998). The substantial impact that maternal effects had on the artificial selection studies of animal breeders and the increasing realization that they might have a (indirect) genetic component, fueled the development of quantitative genetic models designed to understand, predict, and measure evolutionary dynamics when mothers, or more generally parents, directly affect the phenotypes of their offspring (Hadfield, 2012; McGlothlin *et al.*, 2009). We provide a brief overview of these models, focusing on the Kirkpatrick and Lande model (K–L) (Kirkpatrick and Lande, 1989) and its extensions as a framework for understanding how maternal effects can influence evolutionary dynamics. In retrospect, Kirkpatrick and Lande's (1989) paper had a pivotal role in summarizing previous modeling efforts and pointed out several consequences of maternal effects on changes in traits across generations. Empirical literature on the quantitative genetics of maternal effects has been reviewed extensively elsewhere (Cheverud, 1984; Kruuk *et al.*, 2008; Räsänen and Kruuk, 2007; Roach and Wulff, 1987; Shaw and Byers, 1998; Wilson and Reale, 2006). Therefore, we concentrate instead on studies that have expanded the quantitative genetic theory of maternal effects by incorporating coadaptation (Kölliker *et al.*, 2005; Wolf and Brodie III, 1998b) and within- and between-generation phenotypic plasticity (Ezard *et al.*, 2014; Hoyle and Ezard, 2012; Kuijper and Hoyle, 2015). We then discuss quantitative genetic theory in relation to nongenetic inheritance (NGI) (see Bonduriansky and Day, 2009; Danchin *et al.*, 2011; Day and Bonduriansky, 2011; Jablonka and Lamb, 2005 for reviews). We conclude by summarizing our understanding of the impact that maternal effects have on evolutionary dynamics and proposing directions for future research.

The Quantitative Genetics of Maternal Effects

Maternal effects are often conceptualized as the effects of 'maternal performance' (Cheverud and Moore, 1994; Wilham, 1972), a composite maternal character which is not directly observed. It summarizes all the effects that a mother has on an offspring trait and its variance and integrates effects of maternal phenotypic traits and maternal environments. Alternatively, if the maternal traits affecting offspring are known, then slopes of their effects on offspring can be estimated per trait separately (Hadfield, 2012; McGlothlin *et al.*, 2009). The maternal performance approach is useful and popular with plant and animal breeders (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Walsh and Lynch, 2012) and empirical quantitative geneticists (McAdam *et al.*, 2014; Rossiter, 1996). The impact that maternal effects have on offspring trait variance can be calculated in an integrative manner by examining how much phenotypic variance is explained by maternal identity (Hadfield, 2012), if the experiment allows this variance component to be estimated separately. However, the approach tells us nothing about the cause of maternal effects (see Wolf and Wade, 2009) and can be inaccurate in predicting selection responses as discussed below. In contrast,

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the trait-based approach (Kirkpatrick and Lande, 1989; McAdam *et al.*, 2014) assumes that maternal effects are mediated by specific causal maternal traits that can have different effect strengths. Causal effects of maternal environments are kept implicit or environmental properties are recast as 'traits.' Hadfield (2012) and McGlothlin and Galloway (2014) show how performance and trait-based approaches are related. They are comparable in some scenarios (McGlothlin *et al.*, 2009; McGlothlin and Galloway, 2014). McAdam *et al.* (2014) provide a detailed discussion of the pros and cons of using either approach as well as presenting a hybrid strategy where maternal performance and maternal trait regressions are jointly modeled. Here we focus on the trait-based approach.

Models with Fixed Maternal Effect Coefficients

The landmark model for trait-based maternal effects was introduced by Kirkpatrick and Lande (1989, 1992) and generalized previous efforts to model maternal effects (see Hadfield, 2012). It assumes that the phenotype of an individual is the sum of an additive genetic component, the maternal effect contribution, plus an environmental contribution. In this case the phenotypic trait value z_o of an offspring individual can be written as

$$z_o = a_o + mz'_m + e_o \quad [1]$$

where a_o is the additive genetic effect in the (offspring) individual, z'_m is the phenotype of the mother affecting the phenotype of her offspring, and e_o is the environmental component of phenotypic variation in the offspring. We use a prime here to stress that the trait of the mother which has an effect does not need to be equal to the trait considered in an offspring, for example, when gestation time influences offspring birth weight (Kirkpatrick and Lande, 1989). Subscripts 'o' and 'm' indicate on which individual a quantity can be measured. The maternal effect coefficient m is special in this respect as it translates a trait of the mother into a trait component of the offspring. Its magnitude expresses the strength of the maternal effect and the sign can be positive or negative, but its absolute value is taken to be smaller than one (Kirkpatrick and Lande, 1989). The maternal effect is the product mz'_m.

Equation [1] can be extended to account for an arbitrary number of traits (k) by expressing the equation using matrix notation:

$$\boldsymbol{z}_o = \boldsymbol{a}_o + \boldsymbol{M}\boldsymbol{z}_m + \boldsymbol{e}_o \quad [2]$$

where $\boldsymbol{z}_o$ and $\boldsymbol{z}_m$ are now vectors of length k containing all distinct phenotypic traits. Equation [1] can be rewritten in this manner as well, with $\boldsymbol{z}=(z,z')$ and $\boldsymbol{M} = \begin{pmatrix} 0 & 0 \\ 0 & m \end{pmatrix}$. Some traits are potentially only investigated in offspring, others with causal effects are either only observed in mothers or both in offspring and mothers. Each individual is assumed to have values for all k traits, even when the trait is not expressed. Thus subscripts 'o' and 'm' are indicators of the individual where a trait can be measured, or where it is a latent variable. The additive genetic values and environmental components of the traits in offspring are given by $\boldsymbol{a}_o$ and $\boldsymbol{e}_o$, respectively. The matrix $\boldsymbol{M}=[m_{ij}]$ $(i,j=1,\ldots,\ k)$ contains all maternal effect coefficients m_{ij}, each the effect of a unit change in a maternal trait (j) value on a trait (i) in the offspring trait vector. Coefficients m_{ij} will be zero if maternal trait j has no effect on offspring trait i. For the purpose of estimation and in most theoretical modeling, the contribution of the individual environmental effect $\boldsymbol{e}_o$ to each separate trait is assumed to be zero on average.

When we want to follow phenotypic trait values across generations, we need to make additional assumptions about the population dynamics. Generally, it is assumed that generations are discrete and nonoverlapping, that populations modeled are diploid sexual and that mating is random. A few commonly used scenarios to model selection on maternal effects are presented below. If we assume that the maternal and offspring traits are in fact the same phenotypic character (no prime required), then a trait decomposition such as eqn [1] gives the following for the average trait value of offspring in generation $t+1$, before selection

$$\bar{z}(t+1) = \bar{a}(t+1) + m\bar{z}^*(t) \quad [3]$$

with $\bar{z}^*(t)$ denoting the average trait value of mothers in generation t contributing to the next generation ('after selection', Hadfield, 2012). We can drop the 'o' and 'm' subscripts here as generations t and $t+1$ already differentiate between mothers and offspring. Kirkpatrick and Lande (1989) showed for this case that one can derive the change between generations t and $t+1$ in average trait value of individuals before selection as

$$\Delta\bar{z}(t) = C_{az}\beta(t) + m\Delta\bar{z}(t-1) + mP\Delta\beta(t-1) \quad [4]$$

with C_{az} being the covariance between additive genetic value 'a' and phenotype 'z' in the maternal generation t, P the phenotypic variance, $\beta(t)$ the selection gradient on trait z in the maternal generation t and $\Delta\beta(t-1)$ the difference between selection gradients in generations t (maternal) and $t-1$ (grandmaternal). C_{az} and P are assumed constant. The first term accounts for the phenotypic change caused by selection in generation t and mediated by the direct genetic transmission of the trait. Additionally, the change $\Delta\bar{z}(t)$ depends on two factors mediated by maternal effects: the average trait change between the previous generations and the difference between the selection gradients in the parental and grandparental generations. The last term vanishes when the selection gradient does not change between generations t and $t-1$. In comparison to the classical breeders equation, $\Delta\bar{z}(t) = C_{az}\beta(t)$ where C_{az} is equal to the additive genetic variance for z, the covariance C_{az} itself becomes different from the additive genetic variance when maternal effects are present (Kirkpatrick and Lande, 1989).

For the multi-trait case, the population mean values of the traits are similarly updated between generations using the rule

$$\Delta\bar{\boldsymbol{z}}(t) = \mathbf{C}_{az}(t) + \boldsymbol{M}\Delta\bar{\boldsymbol{z}}(t-1) + \boldsymbol{MP}\Delta\boldsymbol{\beta}(t-1) \quad [5]$$

where $\boldsymbol{C}_{az}$ and $\boldsymbol{P}$ are now covariance matrices and β is the selection gradient vector.

Insights from the K–L Model

The K–L model demonstrates that when maternal effects are present, selection pressures in the maternal and grandmaternal generations affect the trait changes between maternal and

offspring generations. This generates time lags in the response to selection. With several traits involved (eqn [5]), the evolution of traits under selection can depend on maternal effects of traits that are not under selection. Moreover, in the case where n traits are coupled by maternal effects, lagged responses can occur up to n generations after a single generation of selection (Kirkpatrick and Lande, 1989). If we decide to subsume all n traits causing the maternal effects in a single maternal performance trait, then this will introduce imprecisions in the predictions of lagged selection responses.

Understanding how the maternal effect coefficient alters the resemblance of parents and offspring is critical. If selection starts from a population at equilibrium, a positive coefficient (e.g., larger mothers produce larger offspring or smaller mothers produce smaller offspring) will speed up a response to selection, whereas a negative maternal effect coefficient (e.g., larger mothers produce smaller offspring or smaller mothers produce larger offspring) can slow down or even reverse a response to selection temporarily (Falconer and Mackay, 1996; Hoyle and Ezard, 2012; Kirkpatrick and Lande, 1989; Kuijper and Hoyle, 2015; Räsänen and Kruuk, 2007).

Moreover, 'evolutionary momentum' can be generated where trait means continue to change when the directional selection pressure is removed. This occurs at a constantly decreasing rate and requires that a trait involved in a maternal effect alters the same trait in offspring or that the effects lead back to the original character after several generations (Kirkpatrick and Lande, 1989). When the absence of genetic variation for any of the traits in $\boldsymbol{z}$ would cause $\boldsymbol{C}_{az}$ to become zero, traits can still change through maternal effects rather than additive genetic variation in the traits directly under selection, but the selection gradient needs to continually change or the response will gradually decay due to evolutionary momentum. This is fundamentally different from exclusively Mendelian inheritance where characters without genetic variation cannot respond to selection (Badyaev, 2005, 2008; Bonduriansky and Day, 2009; Jablonka and Lamb, 2005).

Trait Covariance and Coadaptation

The K–L model demonstrates that a trait can respond to selection via two pathways: via the transmission of alleles between mothers and offspring (additive genetic variance) or via the maternal trait effects. However, Kirkpatrick and Lande (1989) did not make any predictions regarding the joint distribution of genotypic values of maternal and offspring traits and assumed it to be fixed. Wolf and Brodie III (1998b) relaxed that assumption and found in a two-trait model with stabilizing selection on offspring phenotype alone, that the sign of the genetic correlation evolves to match the pattern of coadaptation favored by selection. Maternal and additive genetic effects evolve negative genetic correlations, which reduces the variance in average fitness. With directional selection and a particular model where the maternal effect coefficient is in fact the additive genetic value times a constant ($m = n\ a_o$), they found that the genetic correlation between maternal effect and additive genetic effect evolves to obtain the same sign as the directional selection gradient. Here, this pattern allows a faster selection response. Kölliker *et al.* (2005) considered how begging by offspring and provisioning by parents coevolve in a model with two traits having reciprocal maternal effects, finding that the sign of the genetic correlation between begging and provisioning depended on whether selection was primarily on the offspring or on the parental trait.

Empirical Validation

While it is clear that maternal effects are ubiquitous (Mousseau and Fox, 1998; Mousseau *et al.*, 2009) and may affect the evolutionary dynamics of traits (Falconer and Mackay, 1996; Hadfield, 2012; Hoyle and Ezard, 2012; Kirkpatrick and Lande, 1989), the impact that they actually have depends on the magnitude and sign of all the relevant model parameters. Räsänen and Kruuk (2007) reviewed the available data and found abundant evidence for maternal genetic effects in domestic and wild populations, suggesting that maternal effects cannot be left implicit as an environmental source of phenotypic variation. This is confirmed by studies such as Wolf and Cheverud (2012) that combined genotype information from parents and offspring with experimental cross-fostering in order to identify maternal effect quantitative trait loci. Räsänen and Kruuk (2007) also found evidence for positive and negative offspring–parent trait covariances and maternal effect coefficients but too few reliable estimates were available to draw conclusions about the conditions responsible for the magnitude and direction of specific parameters.

Evolving Maternal Effect Coefficients

Maternal effects have been seen as adaptations for a long time (Mousseau and Fox, 1998) and genetic variation for maternal effect coefficients has been demonstrated repeatedly (Hall and Ebert, 2012; Räsänen and Kruuk, 2007; Stjernman and Little, 2011; Wilson and Reale, 2006; Wilson *et al.*, 2005). But models have only recently begun to focus on how or why the strengths and directions of maternal effects evolve. We focus here on models that are closest to the K–L model of maternal effects and briefly convey their main general conclusions. Kuijper *et al.* (2014) and Kuijper and Hoyle (2015) investigated a single-trait model and a two-trait model. The general intuition from exploring a number of different stationary and cyclically varying selection regimes is that maternal effects evolve signs and magnitudes that make the best use of maternal phenotypes to predict and adjust offspring phenotypes to coming selective conditions when these are only partially predictable (**Figure 1**). In a scenario where the selection optimum shifts abruptly to a novel value, transient positive maternal effects evolve (**Figure 2**). Mothers that survive have phenotypes that are closer to the new optimum, but then also produce offspring that are closer, speeding up adaptation. In a constant selection regime, with selection favoring a fixed optimal trait combination, negative maternal effect coefficients evolve which minimize phenotypic variance (canalization) and therefore maximize mean population fitness. Kuijper *et al.*'s (2014) results indicate that intricate dynamic phenotypic effects can occur when each offspring trait is affected by more than one maternal trait. The maternal effect coefficients

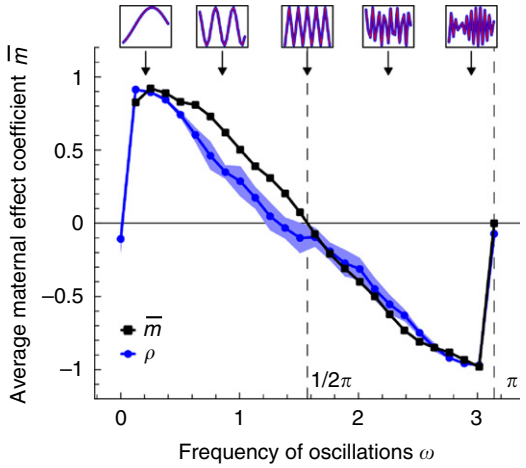

Figure 1 The evolution of maternal effect coefficients when the selection optimum for a trait shared by mothers and offspring cycles periodically. Parameter ω controls the frequency (speed) of oscillations. Each dot represents the average evolved maternal effect coefficient over 10 replicate simulations. The shaded areas indicate the corresponding standard deviations. Boxes above the graph show the cycles in the selective optimum over 20 generations. The black line gives the autocorrelation ρ of selective conditions between the parental and offspring generations. Note that the maternal effect coefficient evolves to match the autocorrelation between maternal and offspring selective environments. Adapted from Figure 2 of Kuijper, B., Johnstone, R.A., Townley, S., 2014. The evolution of multivariate maternal effects. PLoS Computational Biology 10(4), e1003550.

can then evolve in such a way that offspring phenotypes change periodically to track changes in environmental conditions over more than a single generation, for example, to make offspring phenotypes very different from those of their grandparents (Kuijper *et al.*, 2014).

Phenotypic Plasticity

In eqns [1] and [2] above, all responses to environmental variables other than maternal phenotypes are summarized by a single term e_o, which is on average zero by assumption. The dependence of offspring phenotype on specific environmental variables can be made explicit, similar to when we replace maternal performance by maternal trait effects. Consistent changes in phenotype when environmental variables are changed are called phenotypic plasticity. With plasticity, an offspring trait can be modeled as:

$$z_o = a_o + mz'_m + b\varepsilon_o + e_o \quad [6]$$

Compare eqn [6] with eqn [1]. It additionally includes a term $b\varepsilon_o$ which represents the effect of the value ε_o of an environmental variable on the phenotype. The strength of the effect is expressed by slope parameter b, which is often genetically variable. Maternal effects can be considered to be a kind of transgenerational plasticity (Agrawal, 2001; Mousseau and Fox, 1998) both by analogy, since the offspring phenotype depends explicitly on the maternal phenotype, which itself can be considered part of the offspring's environment (Badyaev and Uller, 2009; Day and Bonduriansky, 2011; Jablonka and Lamb, 2005; West-Eberhard, 2003), and also because the

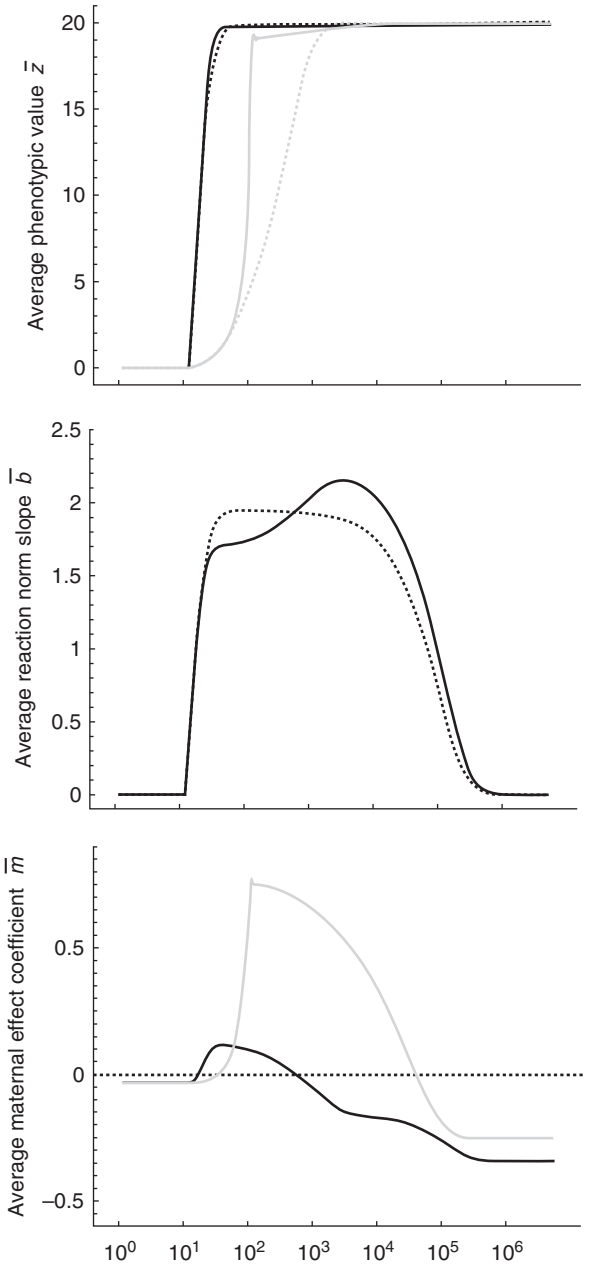

Figure 2 Adaptation to a sudden shift in the environment which occurs at generation $t=10$ and where the optimal phenotypic trait value shifts from 0 to 20. Populations differ in the presence/absence of plasticity or maternal effects, while the genotypic value a is always allowed to evolve. Solid black lines: within-generational plasticity and maternal effect coefficients are allowed to evolve. Solid gray line: only maternal effects evolve. Dashed black line: only plasticity evolves. Dashed gray line: neither maternal effects nor plasticity evolve. Top: Average phenotype per generation. Middle: Average reaction norm slope per generation. Bottom: Average maternal effect coefficient per generation. Adapted from Figure 2 of Kuijper, B., Hoyle, R.B., 2015. When to rely on maternal effects and when on phenotypic plasticity? Evolution 69, 950–968.

maternal phenotype can include a plastic response to the environment experienced by the mother, which thus indirectly affects the offspring. There are interesting parallels between

maternal effects and phenotypic plasticity, because the latter is also believed to use environmental variables to predict coming selective conditions. In that respect, the correlation between the state of the environment sensed and the properties of coming selection are important for plasticity evolution as well. Hoyle and Ezard (2012) and Ezard *et al.* (2014) explored effects of a fixed maternal effect coefficient in the presence of evolving plasticity. Kuijper and Hoyle (2015) allowed both to evolve simultaneously to study the interplay between additive genetic variance, within-generation plasticity (phenotypic plasticity) and between-generation plasticity (maternal effects) when adapting to constant, periodically cycling, or novel environments. In comparison to models with evolving maternal effects but without evolving plasticity, evolving phenotypic plasticity often removes the selective advantage of maternal effects and maternal effect coefficients evolve to smaller magnitudes (**Figure 2**). It often seems more advantageous for the offspring to observe the environment and adjust directly, instead of having to rely on maternal phenotypes. When the evolution of plasticity is constrained, either through costs or informational time-lags, larger maternal effects evolve (Kuijper and Hoyle, 2015). These predictions support the outcome of a recent meta-analysis that concluded that there is only weak support for adaptive transgenerational plasticity (Uller *et al.*, 2013).

Extensions

If we generalize the previous equations slightly to multivariate environments and phenotypes, we obtain

$$\boldsymbol{z}_{\mathrm{o}} = \boldsymbol{a}_{\mathrm{o}} + \boldsymbol{M}\boldsymbol{z}'_{\mathrm{m}} + \boldsymbol{B}\boldsymbol{\varepsilon}_{\mathrm{o}} + \boldsymbol{e}_{\mathrm{o}} \qquad [7]$$

where we allow matrices **M** and **B** to contain coefficients that can evolve, i.e., that are quantitative genetic traits themselves, and whose components and variability we can try to determine experimentally. Such studies of course involve more complex interactions between phenotypes and environments. If the maternal effect coefficient is an offspring trait itself, such as in models where these coefficients evolve (Kuijper and Hoyle, 2015), then it could also depend on environmental variables experienced by offspring. Maternal effect coefficients can be plastic in response to changes in the maternal environment (Marshall and Uller, 2007; Räsänen and Kruuk, 2007) and maternal state (Plaistow *et al.*, 2007, 2015) meaning that the maternal effect coefficient might itself be better modeled as a reaction norm rather than a fixed value. Empirical studies have also demonstrated that maternal effects are highly context-dependent influencing different traits in different offspring environments (Bernardo, 1996; Berven, 1990; Czesak and Fox, 2003; Gliwicz and Guisande, 1992; Lardies *et al.*, 2004; Parichy and Kaplan, 1992; Räsänen *et al.*, 2005), the fitness consequences of which probably depend on the coadaptation of mothers and offspring (Badyaev and Uller, 2009; Kölliker *et al.*, 2005; Wolf and Brodie III, 1998b) across these different environments.

Heritability Extended: Nongenetic Inheritance

Quantitative genetic models assume that genes are the sole basis of inheritance. However, there is an increasing realization that offspring inherit more than just DNA from their parents. NGI can be defined as any effect on offspring phenotype brought about by the transmission of factors other than DNA sequences from parents or more remote ancestors (Bonduriansky and Day, 2009; Danchin *et al.*, 2011; Day and Bonduriansky, 2011; Hallsson *et al.*, 2012). NGI mechanisms that operate alongside Mendelian-genetic inheritance are diverse and include transgenerational epigenetic inheritance and genomic imprinting, transmission of diverse cytoplasmic and somatic factors, nutrients, elements of the extra-organismal environment, and the transmission of behavioral variation through learning (Bonduriansky and Day, 2009). Some of these mechanisms are clearly covered already by current approaches to maternal effects, while others such as genomic imprinting are not seen to be maternal effects (Wolf and Wade, 2009). Day and Bonduriansky (2011) have worked out a unified approach to investigate NGI and selection across generations, which is in essence by separately following a genetic value and a nongenetic heritable value in individuals across generations and not just individual phenotypes or genetic values. Such an approach corresponds to our statement above that maternal effect coefficients or maternal effects can be seen as separate traits, which can be followed across generations explicitly and separately. The question then becomes: whose trait is it? As maternal effects can be instances of indirect genetic effects, where the environment is genetically variable (McAdam *et al.*, 2014; Wolf *et al.*, 1998a), the answer will be case-specific.

To conclude, we want to stress that maternal effects are often studied in behavioral ecology and elsewhere in biology using methods other than strict quantitative genetics. Intuition and predictions arising from these methods seem generally in line with what we have stated here (English *et al.*, 2015; Leimar and McNamara, 2015). The studies suggest further avenues to extend quantitative genetic modeling of maternal effects: for example, including maternal effects on offspring variance (Crean and Marshall, 2009) or taking more explicit account of developmental processes in the individual (Atchley *et al.*, 1994). There is a long history of developmental models of quantitative genetic variation (see references in Wolf *et al.*, 2001). These have in common that the progress of development is modeled by a chain of events where earlier traits and environmental inputs determine properties of later developmental processes and the subsequent development of new traits. Most traits that quantitative genetics focuses on are thus composite mosaics of earlier developmental traits (Wolf *et al.*, 2001) that may all be influenced by integrated genetic, nongenetic, and environmental cues. Such models that include maternal effects are required to better align current theoretical and empirical findings, to obtain a better understanding of how phenotypes integrate different sources of information and ultimately to understand how maternal effects influence the evolutionary trajectories of populations.

See also: Developmental Plasticity and Phenotypic Evolution. Ecological Evolutionary Developmental Biology. Quantitative Genetic Variation

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Mating and Parental Sex Roles, Diversity in

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Glossary

Anisogamy Unequal investment in gametes between the sexes.
Armaments Morphological characters used to gain an advantage over rivals in contests for access to mates.
Bateman gradient The slope of the regression of reproductive success on mate number for a given sex.
Direct benefits (material benefits) Processes that directly improve the fitness of an animal as a result of an additional mating event or a particular mate choice; direct benefits include nutritional resources provided by a mating partner, access to a territory or refuge, or parental care.
Indirect benefits (genetic benefits) Processes that improve the genetic quality of an animal's offspring (either their viability, attractiveness, or diversity) as a result of an additional mating event or a particular choice of mate.
Mate choice A preference for some phenotypic classes of mate over others.
Monogamy A mating system in which an individual mates with only one partner.
Ornament Morphological character used to attract the opposite sex during episodes of mate choice.
Operational sex ratio The ratio of sexually receptive males to females; usually expressed as a proportion (sexually receptive males/(sexually receptive males + sexually receptive females)).
Parental investment Investment in an offspring at a cost of investing in other components of parental fitness.
Parental care Parental investment that increases an offspring's chance of survival after fertilization.
Polyandry A mating system in which females mate with multiple partners.
Sex role reversal A controversial designation of deviation from the conventional sex roles (see Box 1).
Sex roles Aspects of mating receptivity, mate choice, and parental care that generally differ between males and females; typically masculine sex roles involve high levels of sexual receptivity and low levels of choice and care, while feminine sex roles are less receptive to mating, and have high levels of choice and care.
Social selection Differences in survival or reproductive success linked to variation in success in social competition for resources; sexual selection is a subset of social selection, in the form of social competition for mates.

Introduction

The sexes usually differ in morphology and behavior, but the degree of difference varies dramatically across species. Males typically compete for access to female mates, whereas females rarely compete for males. Instead, females tend to invest considerably more in offspring and choosing mates than males do. The sex difference in contest intensity over mates has selected for weapons and extravagant ornaments in males of many species, while females are rarely armed or adorned in this way. General differences in mating receptivity, mate choice, and parental care are collectively known as 'sex roles,' and species that deviate markedly from the general patterns (e.g., when females compete for mates, and males are choosy or provide care), are often described as 'sex role reversed' (a controversial designation; see Box 1).

Here, we focus primarily on animals with separate sexes (sexual selection theory has fascinating implications for hermaphroditic organisms, but these are beyond the scope of our article). We will explore the causes of sexual differences in both mating and parental behavior, and illustrate how some unusual systems deviate from conventional sex roles. We also highlight how many of these unusual systems provide strong tests of sexual selection theory, and suggest some directions for future work that may help clarify unresolved questions about the diversity of sex roles among animals.

Box 1 Sex Role Reversal

Ah-King and Ahnesjö (2013) critique the use of the term 'sex role reversal' in part because it reduces variation in behavior and morphology into two discrete categories, which obscures the tremendous variation in natural sex roles (including variation within species across different traits and ecological contexts – see section on Variation in Mating and Parental Roles). These problems have particular resonance for an evolutionary perspective that is motivated to explain diversity. The phrase is unlikely to disappear entirely, in part because its concise form is useful for instantly evoking in an audience something unconventional about a focal mating system. Nevertheless, we agree that it evokes different ideas in different audience members, and therefore endorse Ah-King and Ahnesjö's (2013) recommendation that authors should provide operational descriptions of the specific phenotypic features that are being referred to in any focal case.

What Causes Sexual Differences in Mating and Parental Roles?

There are behavioral and morphological traits that distinguish the sexes in many animal species. Typical sex differences are thought to have evolved because the sexes experience disparate

forms and intensities of selection. For most species this sex difference in selection is ultimately a consequence of unequal 'parental investment' in gametes ('anisogamy'); spermatozoa in males are small and relatively plentiful while eggs in females are large and relatively few (Bateman, 1948; Trivers, 1972; Williams, 1975). Females are typically constrained from producing more eggs because of the substantial cost of each of them, and this constraint has important consequences for sex differences in both mating and parental behavior.

Mating Roles

One consequence of greater female parental investment is that males can potentially produce many more offspring than females by parasitizing the substantial investment in gametes of many mates. A male's fitness is therefore often directly related to his ability to secure mates. By contrast, females sometimes gain little fitness by remating, especially if a male's only contribution during mating is sperm. The fact that males can gain much from remating, while females gain relatively little, means that selection for acquiring mates tends to be stronger on males. This contrast in how mating success covaries with fitness is central to sexual selection theory. The empirical measurement of the regression of fitness on mating success is known as the 'Bateman gradient,' acknowledging Angus Bateman's (1948) work on *Drosophila* that first highlighted the sex differences in relative fitness gains from mating (**Figure 1**).

Although males may gain more fitness by remating than females, the two sexes must on average mate equally frequently, because every mating requires an individual of each sex. Consequently, there are often many more sexually receptive males than females (if female fitness does not covary strongly with mating success, females should prefer to spend most of their time in activities other than mate seeking, such as foraging or caring for young). This resulting bias in the operational sex ratio (Emlen and Oring, 1977) often selects for investment in secondary sexual characters in males (Enders, 1993; Jirotkul, 1999) that help them to find, win, and guard mates from current rivals, and to displace ejaculates of previous rivals stored within females; such contests are responsible for the impressive array of male 'armaments,' fighting (Emlen, 2008) and, during copulation, penile traits that remove sperm (Simmons, 2001). When there is an excess in the number of sexually receptive males, females typically improve their reproductive success by carefully selecting from many willing potential partners ('mate choice') rather than by mating more frequently. The preferred mate can improve a female's fitness in several ways (Jennions and Petrie, 1997). The careful attention of choosy females in turn selects for the expression of numerous 'ornaments' that appeal to discriminating females (Houde, 2001).

Parental Roles

In addition to the general sex differences in mating roles described above, there are also typical parental roles involving the provision of post-zygotic care: in most animals the female invests more than the male in 'parental care.' The explanation rests in both the costs and benefits of deserting (i.e., failing to care for) the current brood (Trivers, 1972). The considerable investment required by females to produce ova prior to mating means that a female who abandons her current brood (e.g., to seek a better mate) may be unable to replace the brood using her metabolic reserves: she may be better off caring for the current brood (even if she cares alone, and even if the offspring are of below-average quality) than trying to start all over again with a new partner. In contrast, males can usually easily start over with another mate, because the cost of the initial contribution to mating is so much less, and therefore more easily reproduced from metabolic reserves.

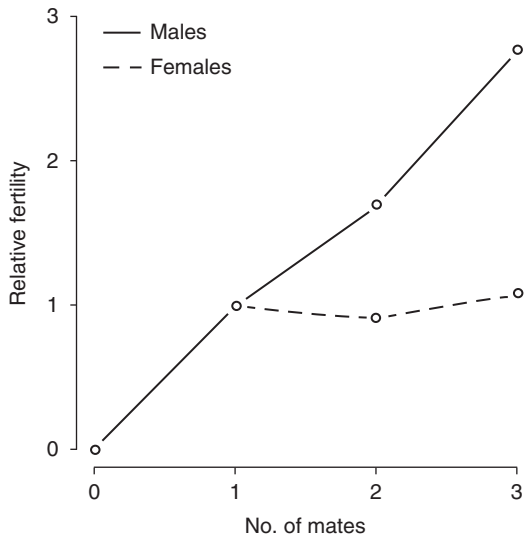

Figure 1 Bateman's (1948) *Drosophila* experiments first highlighted that the relationship between fitness (measured as 'relative fertility') and mating success differed between the sexes, with males gaining more fitness by remating than females. Here we reproduce his most famous figure of series 5 and 6 combined. Males are represented by a solid line, and females are represented by a dashed line. Adapted from Bateman, A.J., 1948. Intra-sexual selection in *Drosophila*. Heredity 2 (Pt.3) pp. 349–368, with permission from Macmillan Publishers Ltd.

Moreover, relative to females, males can usually gain substantially by deserting and acquiring further mates. First, males typically gain more by deserting due to an inherent risk of cuckoldry (where an individual provides care in another individual's offspring); male confidence of parentage is lower than that of females (especially in species with internal fertilization). With few exceptions, females have strong confidence that the offspring they invest in are their own. As the risk of cuckoldry increases, so should selection favoring male abandonment of offspring in favor of new mating opportunities, such as in the brood-rearing bluegill sunfish (*Lepomis macrochirus*) (Neff and Gross, 2001; but see Alonzo and Klug, 2012 for discussion). In addition, investing time and effort in male care comes at a cost to other opportunities to gain fitness, such as by pursuing further mates.

Variation in Mating and Parental Roles

There are many exceptions to the typical patterns of sex differences in behavior and morphology that we have described.

Box 2

When males limit the reproductive success of females, females can compete with each other for males. Gwynne and Simmons used an experimental approach to illustrate how environmental food availability altered the mating roles for *Kawanaphila nartee* bushcrickets (Gwynne and Simmons, 1990). Like many bushcrickets (Orthoptera: Tettigoniidae), *K. nartee* males provide nutritious spermatophylax gifts attached to their sperm packets (**Figure 2**). In *K. nartee*, these gifts comprise 10% of a male's weight and take 5 days to produce (Vahed, 2007). There is a reversal in the mating roles in nature when hungry females compete sexually for matings (male gifts). In experiments with *Kawanaphila* and other tettigoniid species, decreasing proteinaceous food causes a relative increase in male parental investment (gift nutrients in eggs increases), decreases the number of males able to mate (produce gifts) and (in field enclosures) causes a reversal in the mating roles by greatly increasing male choice and female–female competition for mates (Gwynne, 2001). Furthermore, there was even evidence of context-dependent sexual selection on female morphology: the heaviest females had a mating advantage in the control treatments but not food-supplemented treatments. Experimental role reversal also affects other aspects of life history apart from mating itself: as relative male parental investment increases, so does male immune activity, whereas sexually competing females have reduced immune function as paternal investment increases (Vincent and Gwynne, 2014).

Exceptional taxa showing unusual mating and/or parental roles provide fascinating examples of the diversity of natural systems. These exceptions have also provided strong tests of the putative causes of sexual differences highlighted above. Although most of the species mentioned below feature rather dramatic departures from the conventional sex roles described above, we note that sexual selection theory predicts (and empirical research supports) continuous variation in mating and parental behavior, with many taxa occupying the middle ground between the conventional stereotypes above and the departures listed below (Jones and Hunter, 1999; South and Arnqvist, 2008; South *et al.*, 2009).

Most studies of unusual sex roles have supported the links between relative investment by the sexes, the operational sex ratio, sexual selection, and sexual differences. In a few cases there is plasticity in mating roles that allow tests of factors controlling sexual differences (**Box 2**). For example, in certain environments males provide large material benefits to females in the form of nutritious 'nuptial gifts.' The cost of providing these material donations may constrain male ability to remate, while simultaneously increasing the value of remating for females. If male investment is sufficiently important to female fitness, females may be under so much selection to acquire male donations that the operational sex ratio becomes female-biased. Males in turn may then become more choosy than females about their mates (see **Box 2**). Unusual mating roles are not always directly driven by parental investment, however: in one exceptional case (in a butterfly) a female bias in mate availability is caused by extremely elevated male mortality (due to *Wolbachia* infection), which skews the sex ratio to such a degree that females form mating leks (Jiggins *et al.*, 2000).

Figure 2 *Kawanaphila nartee* bushcricket female eating a spermatophore gift. Photo by Darryl Gwynne.

Polyandrous Females

As we have seen, the relationship between mate number and fitness is usually strongest in males, whereas females often gain much less by remating (Bateman, 1948). The explanation for this sex difference lies both in the costs and the benefits of remating for females. Female mating costs include mate search and mate-assessment costs. Mating may also heighten the risk of predation (Arnqvist, 1989), or decrease lifespan, for example, due to injuries sustained during coupling (Crudgington and Siva-Jothy, 2000) or to damaging effects of seminal fluid (Chapman *et al.*, 1995). While males may also suffer costs such as mate searching, female costs appear to be generally greater. For example, females rarely transfer secretions to males that are costly to the partner's reproductive physiology (female Zeus bugs produce a glandular secretion thought to reduce costs of kleptoparasitism by males, but whether this secretion decreases or improves male fitness is unclear; see Arnqvist *et al.*, 2006).

Given these costs, what do females gain by remating? Most empirical studies point to 'direct benefits' of multiple mating, to replenish sperm supplies, acquire goods and services from males, or to avoid the costs of resisting harassment in male mating attempts. In contrast, there is substantially less evidence for 'indirect benefits,' including the acquisition of genetically superior or more compatible sperm, or more diverse offspring (Arnqvist and Nilsson, 2000; Slatyer *et al.*, 2012). The benefits of multiple mating for males always involve the direct benefits of fertilizing female ova. Consequently, even when sexually receptive females outnumber males, the covariance between male mating success and fitness will usually be positive. In such cases, male fitness may be constrained more by other aspects of their biology than by access to mates (e.g., if each mating requires substantial male investment).

Armed and Ornamented Females

When females are so eager to mate that they outnumber sexually receptive males, we expect females to compete sexually, but even in cases of unusual mating roles, females rarely use weaponry to compete or ornaments to attract mates. Examples of female armaments that function in mate competition are virtually unknown. One explanation is that the

cost of expressing weaponry includes structural and metabolic costs as well as the risk of injury during fights; any substantial investment in weaponry could therefore come at a direct cost to a female's own fecundity, and undermine the benefits of winning competition for mates (Berglund, 2013). In fact, in most examples, female armaments appear to have evolved in the context of direct competition with other females for resources rather than mates. Because female reproductive fitness is closely related to maximizing their own fecundity (even in systems in which females are relatively polyandrous), females are more likely than males to compete for resources that will benefit the development and production of their offspring (Clutton-Brock, 2007, 2009). The intensity of the reproductive competition between females, and the development of secondary sexual characters, is therefore closely associated with such resource acquisition. In acknowledging the importance of female competition for material resources essential to reproduction (including competition between potential reproductives (queens) in eusocial animals), some authors have advocated developing a broader perspective of 'social selection' (which emphasizes competition for all resources, not just mates, in social interactions) to facilitate comparisons of secondary sexual traits that arose in differing contexts (West-Eberhard, 1979, 2014; Tobias *et al.*, 2012; but see discussion by Shuker, 2010 and Clutton-Brock and Huchard, 2013).

Examples of female ornaments that function in attracting mates crop up in fishes, birds, and some insects (Tobias *et al.*, 2012). As with female weapons, investment in ornaments may come at a direct cost to a female's own fecundity, which may explain their rarity in spite of the fact that sexual selection on females is relatively common (Bonduriansky, 2001). Ornament evolution is also constrained because it is mediated by male preferences. Any potential trade-off between investment in ornaments and offspring is unlikely to be favored by choosy males, who would presumably prefer an unadorned but highly fecund mate (Fitzpatrick *et al.*, 1995). Furthermore, selection for mate attraction by females could be self-limiting in another respect: seductive females who attract more mates probably provide smaller paternity shares for focal males than relatively unpopular rivals that offer a lower risk or intensity of sperm competition (Wheeler *et al.*, 2012).

Choosy Males

Male choice is generally constrained by the opportunity cost of mate searching and assessment: if males can best gain fitness by acquiring additional mates instead of choosing among them, then choice seems unlikely to evolve. However, if sexually receptive females outnumber receptive males, female quality is variable (e.g., if many receptive females are not yet gravid) and males invest heavily in each mating, males tend to favor traits in females that directly increase their fertilization success (Bonduriansky, 2001). In taxa where female egg number varies substantially (e.g., invertebrates and fish), males generally prefer traits that signal high fecundity, such as large body size. For taxa where females have less variable fecundity (e.g., mammals and birds), males instead tend to focus on traits that signal reduced sperm competition, such as female mating status or age (Bonduriansky, 2001).

Caring Males

For male care to evolve, the benefit to the fitness of his offspring should be greater than the cost incurred by lost mating opportunities (Clutton-Brock, 1991). Relative parental investment is central to sexual selection theory because investment in offspring is both the ultimate cause of sex differences and one of its consequences (by shaping the respective care strategies for males and females). However, discerning cause and consequence can be difficult, as Trivers (1972) first noted (but see experiments in **Box 2**).

Although the circumstances described in the section on parental roles, above, suggest general sex differences in the likelihood of investing in care, male care is not uncommon. It may be that low certainty of paternity limits care, or alternatively care can enhance paternity (Kvarnemo, 2006). Whatever the cause of the association, high paternity confidence should usually be assured in species with male care (Smith, 1979; Møller and Birkhead, 1993). For example, in the water bug, *Abedus herberti*, males brood eggs that gravid females oviposit on their backs. Although females of this species can store sperm, males reduce the risk associated with uncertain paternity by copulating frequently with the female (approximately every second egg laid) during oviposition, which can last up to 2 days (Smith, 1979). A second factor selecting for male care is that care per se attracts additional mates as in certain fishes. Thus caring males can achieve higher mating success than non-caring males (Tallamy, 2000). This is more likely to enhance male than female fitness, because reproductive rate is less limited in males (Smiseth, 2014). In some systems, such female preferences lead to competition between females for males that can provide the best care (Petrie, 1983; Owens *et al.*, 1994).

Unresolved Questions about Sex Roles

The detailed causes of differences in sexual selection are not yet clear. The complex relationships between investment costs and mating opportunities make distinguishing between causes and effects difficult. Empirical tests (such as Gwynne and Simmons, 1990) have shown that varying parental investment can reverse mating roles. However, determining general drivers of patterns across diverse species is complicated (Borg *et al.*, 2002) because we still do not know the extent to which differences in parental care are a cause of the general sex role syndromes (the fact that males can avoid care may be what allows them to maximize fitness by mating repeatedly), or rather an ultimate consequence of differences in sexual selection (males may generally avoid care because they gain more by pursuing more mates than by improving the fitness of their existing offspring) (Kokko and Jennions, 2008). Similarly, there is ongoing controversy concerning whether the ratio of sexually receptive males and females determines sexual trait expression by controlling sexual selection intensity, or whether it emerges as a consequence of differences in sexual selection intensity (Kokko *et al.*, 2014).

These questions are compounded by ongoing debates about how to compare the conditions that affect mating and parental roles across sexes and species. For example,

Kokko *et al.* (2014) suggest that while operational sex ratios and Bateman gradients typically covary, they can provide different but complementary information on fitness benefits of investing in secondary sexual characters. For example, if obtaining new mates becomes more difficult for males, and fitness benefits of mate seeking decrease, there may be selection for paternal care, which affirms Trivers' (1972) insight that parental investment is both a cause and a consequence of differences in selection on the sexes. There have been similar debates about the best metrics for sexual selection intensity between the sexes and across species (measuring selection accurately is a prerequisite for explaining diversity in mating systems). Some authors contend that metrics based on variance in reproductive and mating success (such as the Bateman gradient and the opportunity for sexual selection) (Wade, 1979) are better predictors of sex differences in behavior and morphology across species than trait-specific measures of sexual selection (e.g., the covariance between reproductive success and body size) (Fritzsche and Arnqvist, 2013). One reason for using measures unrelated to phenotypic traits is that traits under strong directional selection can have depleted genetic variance (Prokuda and Roff, 2014), which can lead to lower trait-based estimates of sexual selection. Other authors have demonstrated that variance based measures are only good predictors of sexual selection intensity in very limited circumstances, such as when the potential for mate monopolization is high (Klug *et al.*, 2010; Jennions *et al.*, 2012).

Although what ultimately causes sexual differences, and how best to measure them, remains unresolved, there are some promising research directions involving species with unusual mating systems. We have already noted how such taxa have been instrumental in testing some of the key predictions of sexual selection theory, and they promise new insights thanks to some as yet understudied aspects of their biology. For example, one of the major ongoing questions in sexual selection concerns the relative importance of direct and indirect benefits in driving the evolution of mate choice. Species in which males provide substantial nutritional investment have already been deployed to study this problem (e.g., Fedorka and Mousseau, 2002; Iyengar and Eisner, 2004). Species with unusual sex roles may also shed light on general questions concerning life history, because the life history consequences of investing in costly weapons or ornaments are rather different for males than for females (Houslay and Bussière, 2012). Although the theoretical reasons for this rarity have been compellingly documented, there still remain some unexploited opportunities to test these arguments among taxa that possess impressive female armaments and ornaments. Rare examples of such work include that on female-specific armaments in *Onthophagus sagittarius* (**Figure 3**; Simmons and Emlen, 2008) and female-specific ornaments in *Rhamphomyia longicauda* (**Figure 4**; Funk and Tallamy, 2000). Such tests continue the tradition established by early work on species with unconventional mating systems of using exceptions to prove the rules.

Figure 3 Female-specific armaments in *Onthophagus sagittarius*. Females are armored with a pronotal horn absent in males. Photo by Doug Emlen.

Figure 4 Female-specific ornaments in *Rhamphomyia longicauda*. Females are adorned with pinnate scales on their legs and inflatable abdominal sacs to exaggerate their size in mating swarms. Photo by Dave Funk.

See also: Mate Choice and Sexually Selected Traits. Polyandry and Female Postcopulatory Choice

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Mating Systems, A Brief History of

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Glossary

Breeding system A description of mating and parental care behavior by males and females.

Mating success The number of social and/or genetic mates that an individual has during some specified period of time or its entire life.

Mating system The way that individuals are grouped in relation to mating and the way in which matings are achieved through time in a population.

Parental care Parental behavior that (1) occurs post-fertilization (or after the production of daughter cells if reproduction is asexual), (2) is directed at offspring, and (3) appears likely to increase offspring lifetime reproductive success.

Parental investment Any parental expenditure (time, energy, or other resources) that benefits the fitness of offspring but reduces the ability of a parent to invest in other components of fitness.

Reproductive success The number of viable and surviving offspring that an individual produces during some specified period of time or its entire life.

Sexual selection Covariation among a phenotypic trait and mating success.

Mating Dynamics Are Highly Diverse

Mating dynamics are highly variable and diverse. In many animals, such as the elephant seal (*Mirounga angustirostris* and *Mirounga leonina*), males compete intensely for access to female mates, and both sexes mate multiply (Hoelzel *et al.*, 1999). In other cases, such as the pipefish *Syngnathus typhle* females compete for access to males, males are choosy with respect to who they mate with, and males provide parental care to offspring (Berglund *et al.*, 1986). In some relatively rare cases, such as in the spotted sandpiper (*Actitis macularia*) females compete for and mate with multiple males, leaving each of those males to care for their offspring (Oring *et al.*, 1994).

Scientists who study mating systems are interested in explaining why such variation in mating dynamics exists. One thing that has become clear in recent decades is that mating dynamics, including mate competition and fertilization, are heavily influenced by sexual selection, parental investment, and ecological factors. The focus of mating system research tends to be twofold: First, researchers aim to categorize mating systems. Indeed, a great deal of work since the 1970s has focused on developing comprehensive qualitative and quantitative descriptors of mating systems, a challenging task given the huge variation in mating dynamics that exists in nature. Second, researchers aim to understand and explain variation in mating dynamics. For example, a large body of work has focused on understanding variation in the number of mates that each sex has in a given population or species, why sex-specific patterns of parental care vary across systems and how these patterns are linked to mating dynamics, and how resources required for mating can influence patterns of mating behavior.

Below, an overview of the history of mating system research is provided. The article begins with classic definitions and classification systems of mating systems. Then it describes the factors that have historically and/or are currently thought to have strong influences on mating systems. Finally, future directions in mating system research are briefly discussed.

History of a Name: Defining Mating Systems

The term mating system refers to the way that individuals are grouped in relation to mating and the way in which matings are achieved through time in a population (reviewed in Emlen and Oring, 1977; Davies, 1991; Shuster and Wade, 2003; Klug, 2011). Most simply, mating systems are classified by the number of mates that a given individual has during a specified period of time or throughout their entire lifetime (Emlen and Oring, 1977). For example, monogamy describes a mating system in which each individual has a single mate for some specified period of time (e.g., one or more breeding seasons) or their entire life; polygyny refers to the mating system in which females have a single mate during some specified period of time or their entire life and males mate with multiple females; polyandry describes a mating system in which males have a single mate during some specified period of time or their entire life and females mate with multiple males; and polygamy or multiple mating describes a system in which both sexes mate multiple times (reviewed in Emlen and Oring, 1977; **Table 1**). While these definitions of mating systems have been widely used, some researchers have argued that the way in which we categorize mating systems needs to be refined. For example, some authors have expanded simple mating system classifications to account for how individuals acquire mates (e.g., Emlen and Oring, 1977; Shuster and Wade, 2003), the pair bond duration between males and females (Davies, 1991; Shuster and Wade, 2003), details of parental care (e.g., Reynolds, 1996; Shuster and Wade, 2003), and other specific details of mating behavior, such as coercion (Shuster and Wade, 2003).

Shuster and Wade (2003), for instance, have suggested that variation in mating success is a key feature of mating systems and should therefore be accounted for in our categorization of mating systems. In addition to the classic mating systems of monogamy, polyandry, and polygyny described above, they provide a more detailed classification system for scenarios in

Table 1 A description of classic mating systems. Mating system categorizations have been expanded, and additional mating systems are described in the text

Mating system	*Description*
Monogamy	Both males and females have only one mate for their entire lifetime or some specified period of time
Polyandry	Females mate with multiple males and males have only a single mate over the course of their entire lifetime or some specified period of time
Polygyny	Males mate with multiple females and females have only a single mate over the course of their entire lifetime or some specified period of time
Polygamy or multiple mating	Both males and females mate with multiple opposite-sex individuals

which both sexes engage in multiple mating. According to their classification system, polygamy occurs when both sexes mate multiply and average male and female mating success is approximately equal, polygynandry occurs when both sexes mate multiply and there is greater variation in female mating success in comparison to male mating success, and polyandrogyny is characterized by both sexes mating multiply and greater variation in male mating success relative to that of female mating success (Shuster and Wade, 2003). Because there can be a large amount of variation in any mating system category, Shuster and Wade (2003) have also advocated quantitative measures of mating system that explicitly account for the variation in mating success in males and females (**Box 1**).

Reynolds (1996) additionally suggested that understanding parental care is key to understanding mating dynamics, and proposed the term 'breeding system' (rather than 'mating system') to more explicitly account for the role that care plays in mating dynamics. Reynolds (1996) defined breeding systems as a description of mating and parental care behavior by males and females, and argued that breeding system classifications should encompass the form and duration of parental care, the form and duration of pair bonds, the number of genetic and social mates that each sex has, forms of courtship and mate competition, details of resource that are required for mating, and the extent of mate choice, including sperm choice (Reynolds, 1996). Indeed, all of these factors can interact to influence mating dynamics, and there can be co-evolutionary feedback among sexual selection and parental care (discussed below and reviewed in Reynolds, 1996; Kokko and Jennions, 2008; Alonzo, 2010).

Developing a comprehensive system to categorize mating systems has been a key focus in mating system research. More broadly, though, mating system researchers are interested in understanding why there is so much diversity in the mating systems that exist in nature. Below, the factors that are thought to influence mating systems are discussed, and when applicable, an overview of how these factors have been incorporated into our classification of mating systems is provided.

Box 1 A quantitative descriptor of mating systems

Categorizing mating systems qualitatively (e.g., monogamy, polyandry, polygyny, polygamy) provides insight into the general mating dynamics that are occurring in a given population or species. However, qualitative descriptors of mating systems have been criticized as being too vague (e.g., Shuster and Wade, 2003). For example, when classifying a mating system, it is often unclear what time scale should be focused on, and empirically, there is frequently inconsistency in how mating systems are categorized. Additionally, with respect to mating systems in which one or both sex mates multiply, qualitative descriptors of mating systems provide little or no information about the extent of variation in mating success that each sex experiences.

Because of the limitations of qualitative mating system descriptors, Shuster and Wade (2003) have argued that quantitative descriptors of mating systems are needed. Specifically, they have suggested that measuring the variance in relative fitness (e.g., mating and/or reproductive success) for males and females will provide strong insight into the mating system that a population experiences. The opportunity for selection, (if reproductive success is the variable of interest), and the opportunity for sexual selection, I_s (if mating success is the variable of interest), are dimensionless measures of the variance in relative fitness for a given sex. Each measure (I and I_s) is calculated as the variance in mating or reproductive success divided by the mean mating or reproductive success squared (described in detail in Shuster and Wade, 2003). The opportunity for selection and the opportunity for sexual selection reflect the maximum possible selection that can be occurring during a particular episode or multiple episodes of selection. Importantly, however, these measures do not always correlate well with the actual strength of sexual selection (reviewed in Klug *et al.*, 2010), and should therefore not be used as a measure of the strength of sexual selection. Despite this limitation, I and I_s provide insight into the variation in mating dynamics that are occurring at a given point in time in a given population. Given that a major goal in mating system research is to categorize the mating dynamics in a given system, I and I_s can be useful for quantifying and understanding the variation in mating and reproductive success that occurs within and across different mating systems (Shuster and Wade, 2003). Once variation in mating and reproductive success is quantified using these measures, an obvious next step is to attempt to understand why such variation exists.

Mate Monopolization Matters

Emlen and Oring (1977) viewed mate monopolization as a defining and driving feature of mating systems and incorporated mate monopolization into their detailed classification system of mating systems. They argued that mating system will be affected by mate monopolization, and that mate monopolization will largely be determined by the spatial and temporal distribution of resources and mates that utilize those

resources. If, for example, females are the choosier sex and resources are evenly distributed in space, females who utilize those resources will be spread out over space, and, it potentially becomes more difficult for a single male to monopolize multiple females. Likewise, if females become sexually receptive in unison, it will be relatively difficult for a single male to monopolize multiple females (Emlen and Oring, 1977). In such cases, a polygynous mating system would be unlikely to occur. On the other hand, if resources, and hence females, are clumped in space and/or if females are sexually receptive at different points in time, then it becomes more likely that a single male can monopolize and defend multiple mates, and polygyny becomes more likely (Emlen and Oring, 1977).

Emlen and Oring (1977) expanded the classification of mating systems to account for the important role that resources can play in driving mating dynamics, and their classification system also accounts for the specific way in which mates are monopolized. For example, with respect to polygyny, Emlen and Oring (1977) identified three general scenarios: (1) resource defense polygyny, in which males indirectly control access to females by monopolizing resources; (2) female defense polygyny, in which males directly control access to multiple females; and (3) male dominance polygyny, in which males aggregate during the breeding season and females select mates from those aggregations. With respect to polyandry, they identified two general scenarios: (1) resource defense polyandry, in which females control access to males by monopolizing resources; and (2) female access polyandry, in which females limit and control access to mates.

In addition to expanding the way in which we classify mating systems, Emlen and Oring (1977) developed a conceptual framework to explain the way in which male and female mate availability, resources, and sexual selection interact to influence mating systems. In particular, they thought that the sex ratio of individuals currently prepared to mate would have strong effects on mating dynamics, and this topic is discussed in more detail in the following section.

The Link between Operational Sex Ratio, Sexual Selection, and Mating System

As mentioned above, Emlen and Oring (1977) suggested that there is a clear link between mate monopolization, resources, and mating systems. They argued that mate monopolization is likely influenced by the operational sex ratio, which is the ratio of males to females prepared to mate at a given time and in a given location (OSR; Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996). In general, they predicted that a relatively small number of the mate-limited sex individuals will monopolize the more common sex when the OSR is biased (see also Klug *et al.*, 2010 for criticisms of this argument). Specifically, Emlen and Oring (1977) predicted that a male-biased OSR will lead to polygyny, and a female-biased OSR will lead to polyandry. Emlen and Oring (1977) additionally predicted that the strength of sexual selection on the mate-limited sex (males in most cases) will increase as the OSR becomes more biased and as mate monopolization increases (Emlen and Oring, 1977), because when mate monopolization is high, traits that allow a small number of individuals to monopolize mates will be strongly selected for. This view has recently been challenged (Shuster and Wade, 2003; Klug *et al.*, 2010; Jennions *et al.*, 2012), as there is no logical reason to assume a priori that mate monopolization, and hence the strength of sexual selection and polyandry or polygyny, will increase as the OSR becomes more biased toward one sex. Indeed, as OSR becomes more biased and there are more same-sex competitors, it is equally plausible that mate monopolization will become more difficult (e.g., due to interference among members of one sex) (**Figure 1**; Klug *et al.*, 2010).

Indeed, OSR is not a reliable predictor of mate monopolization, mating system, or sexual selection across many biologically-realistic scenarios (Shuster and Wade, 2003; Klug *et al.*, 2010; Jennions *et al.*, 2011). Mate monopolization, on the other hand, is a reliable predictor of the strength of sexual selection (**Figure 1**), and the strength of sexual selection will increase as mate monopolization increases if one or more phenotypic traits are under sexual selection (Emlen and Oring, 1977; Klug *et al.*, 2010). In addition to identifying the link between mate monopolization and sexual selection, Emlen and Oring (1977) recognized that mating behavior, parental care, and sexual selection are intimately linked and will have interactive effects on mating systems.

Parental Investment and Sexual Selection Shape Mating Systems

Emlen and Oring (1977) suggested that polygamy is more likely when one sex does not provide parental care and/or when parental care requirements are relatively minimal. They additionally suggested that monogamy is more likely to occur when the potential for monopolizing multiple mates is low and when fitness is maximized by providing care to current offspring (Emlen and Oring, 1977). These ideas have been expanded upon in recent years, and we now have a more complete understanding of the complex relationship between care, mate monopolization, sexual selection, and mating systems.

In many animals, one sex tends to be the choosier sex and the other sex is mate limited and engages in mate competition and/or attraction (Darwin, 1871; reviewed in Andersson, 1994). As mentioned in the previous section, the mate limited sex will experience relatively strong sexual selection if a small number of individuals possess traits that allow them to monopolize many opposite-sex mates (Emlen and Oring, 1977; reviewed recently in Klug *et al.*, 2010). Sexual selection can thus have strong influences on mating dynamics and the resulting mating system. For example, sexual selection can favor traits that increase mating success and mate monopolization, such as fighting ability, increased body size, traits that are preferred by the opposite sex, and traits that increase fertilization success (reviewed in Andersson, 1994). In general, males tend to be mate limited and experience greater sexual selection and females tend to be the choosier sex and experience relatively weaker sexual selection (reviewed in Clutton-Brock and Parker, 1992), although in some sex-role reversed systems, males are the choosier sex and females are mate limited.

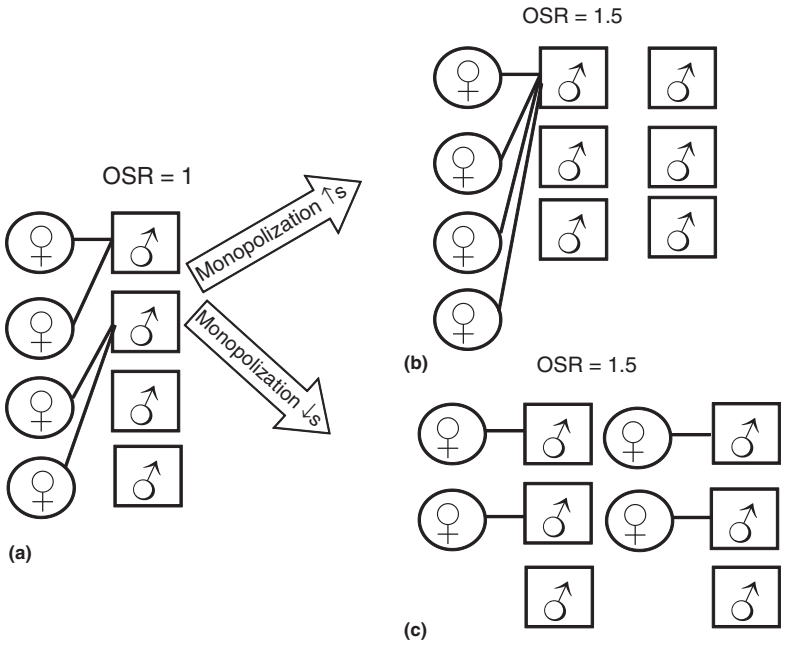

Figure 1 The complex relationship between operational sex ratio (OSR), mate monopolization, and sexual selection. Emlen and Oring (1977) suggested that mate monopolization will increase as the OSR becomes more biased toward one sex. Recent research has demonstrated that there is no a priori reason to assume that this will occur. For example, imagine an unbiased OSR in which there are four females and four males (a). In our scenario (a), if each of two males monopolizes two female mates, there will be a moderate degree of mate monopolization, and two males will remain unmated. If the operational sex ratio changes, it is possible that mate monopolization will also change. For example, if sex ratio becomes male-biased, such that we now have four females and six males ((b) and (c)), it is possible that a single male will now be able to monopolize all female mates, in which case mate monopolization would increase (b). Such a pattern would be consistent with the predictions of Emlen and Oring (1977). Alternatively, though, it is possible that the addition of two new males will make mate monopolization more difficult (e.g., if there is interference among males). In such a scenario, it is possible that mate monopolization will decrease as the OSR becomes more biased (c). Such a pattern is not predicted by classic theory (Emlen and Oring, 1977), but there is no reason to assume that either one of our scenarios ((a) → (b) or (a) → (c)) will be more likely. Mate monopolization is a key component of a mating system and affects sexual selection. If mate monopolization increases because one or a small number of males possess a trait that allows them to monopolize multiple mates, that trait will be favored by sexual selection. As the degree of mate monopolization increases, sexual selection on the trait that allows for monopolization will also increase. Thus, in our scenarios ((a), (b), and (c)), if mate monopolization occurs because of a trait, we would expect that trait to experience the greatest sexual selection in scenario (b). Modified from Klug, H., Heuschele, J., Jennions, M.D., Kokko, H., 2010. The mismeasurement of sexual selection. *Journal of Evolutionary Biology* 23, 447–462.

Historically, sex differences in parental investment have been used to explain why males tend to be mate limited and females choosy (Trivers, 1972). Trivers (1972) noted that females, by definition, produce larger gametes than males, and as a result have greater pre-mating parental investment than males. Trivers (1972) argued that females will be more likely to have greater post-mating parental investment than males because of their relatively high pre-mating parental investment. Additionally, because females produce larger gametes, males are expected to return to the mating pool faster than females after mating. As a result, the relatively high potential reproductive rate (PRR; Clutton-Brock and Parker, 1992) of males will create a male-biased OSR, and this in turn has been predicted to lead to greater sexual selection on male traits and a mating system in which some males monopolize and mate with multiple females (Clutton-Brock and Vincent, 1991; Clutton-Brock and Parker, 1992; reviewed in Kokko and Jennions, 2008). Both of these predictions – (1) that females will have greater post-mating investment because they have already invested more into gametes than males and (2) that a relatively large male PRR and male-biased OSR will lead to relatively strong sexual selection on males – have been questioned in recent years.

Dawkins and Carlisle (1976) noted that past investment should not necessarily affect future investment, and the prediction that females will have greater parental investment than males simply because they have already invested more in gametes than males commits the Concorde fallacy (Dawkins and Carlisle, 1976; Queller, 1997). While greater female investment in gametes can affect mate availability and the future reproductive opportunities that each sex experiences, we now know that past investment alone will not drive sex differences in future investment (reviewed in Kokko and Jennions, 2008). Males and females, however, do differ in many ways, and these differences can affect parental investment, sexual selection, and ultimately, mating system. For example, males and females might differ in (1) the costs and/or benefits associated with providing care and/or competing for mates, (2) the ability to provide parental care (e.g., one sex might be unable to provide essential forms of care), (3) certainty of parentage, (4) mate availability, and (5) the costs and/or benefits associated with mate preference (Queller, 1997; Houston and

McNamara, 2002; Kokko and Jennions, 2008; Alonzo, 2010; Klug *et al.*, 2012). Such differences can, in turn, affect mating system dynamics.

In particular, the costs and benefits associated with mate competition versus parental care can strongly influence sex roles and sexual selection (reviewed in Kokko and Jennions, 2008). When competing for mates is associated with higher mortality than providing parental care, the sex that provides less care and competes more for mates (i.e., the earlier deserting sex) will become relatively rare in the population because individuals of that sex will have greater mortality. Such a scenario is expected to favor earlier desertion by the sex that is relatively rare, and this, in turn, is predicted to lead to maternal care and greater male mate competition if females are initially selected to care or paternal care and greater female mate competition if males are initially more likely to care (Kokko and Jennions, 2008). In contrast, if providing parental care is more dangerous than competing for mates, individuals who provide less care and desert offspring earlier will experience reduced mortality, and the earlier deserting sex will become more common in the population. If the earlier deserting sex is more common, individuals of this sex will have difficulty finding mates, and this scenario will select for increased paternal investment by the deserting sex. In this case, selection would be expected to favor increased parental investment by both sexes (Kokko and Jennions, 2008). Additionally, if parental care itself is under sexual selection, such that the choosier sex prefers mates that provide parental care, the more competitive, mate-limited sex would be expected to also provide parental care in some cases (Alonzo, 2012). Indeed, the relationship between parental care, sexual selection, and mating dynamics can be complex.

Females Benefit from Multiple Mating and Paternity is often Uncertain

Two additional complexities of mating dynamics, and issues that have challenged the conventional resource-based and sexual-selection focused view of mating systems, is the fact that (1) females of many species choose to mate multiply even when a single male could fertilize all of their eggs and (2) that males often experience uncertain paternity (reviewed in Reynolds, 1996).

In a now classic study, Bateman (1948) examined the relationship between number of mates and the number of offspring produced in *Drosophila melanogaster*. While male reproductive success increased linearly with increasing mating success, female reproductive success increased only marginally (if at all) with increasing mating success after a single mating. If female reproductive success does not increase substantially with each additional mate, we would expect (1) sexual selection to act more strongly on males than females, and (2) females to avoid actively mating with multiple males if there are costs of additional matings. In contrast to this latter prediction, females of many species actively choose to mate with multiple males (reviewed in Reynolds, 1996; Birkhead *et al.*, 1997). A number of hypotheses have been proposed to explain why females mate multiply. In some cases, females might benefit from mating multiply because they gain indirect benefits (e.g., higher genetic quality in offspring), additional parental care from mates, and/or nutrients or other resources (reviewed in Birkhead *et al.*, 1997; Jennions and Petrie, 2000). Regardless of the specific benefit associated with female multiple mating, when females mate with multiple males during a given reproductive bout, their mates' expected certainty of paternity is expected to decrease. Paternity uncertainty is then expected to influence mating dynamics, parental investment, and sexual selection (Birkhead and Møller, 1998; recently reviewed in Alonzo, 2010).

Within a species, individual males are expected to reduce their parental care in response to decreased paternity if paternity is expected to be greater during future reproductive episodes (Houston *et al.*, 2005; Kokko and Jennions, 2008; Alonzo, 2010). Across species, a positive relationship is expected between paternity certainty and the level of paternal care provided (reviewed in Alonzo, 2010). Surprisingly, the support for these predictions is mixed. Within species, a recent review found that reduced paternity leads to decreased care in less than half of the studies considered (Alonzo, 2010). It is possible that paternity cues simply do not exist in some animals or that individual variation in male condition makes it difficult to detect a relationship between paternity and paternal care (Alonzo, 2010). In general, though, it is often unclear why paternity uncertainty does not appear to influence parental investment. Regardless, it is clear that more work is needed to fully understand how multiple mating and uncertain paternity affect mating systems and parental care.

Conclusions: Understanding Mating Systems Requires a Focus on Co-Evolutionary Dynamics

In the past 20 years, many researchers focused on mating system research have moved away from simplistic ecological classifications of mating systems and focused more explicitly on how sexual selection, ecology, and parental care interact to shape mating dynamics (e.g., Reynolds, 1996; Shuster and Wade, 2003; Kokko and Jennions, 2008; Alonzo, 2010). Nonetheless, we are still far from fully understanding mating dynamics. Indeed, post hoc explanations of mating behavior rather than well-supported a priori predictions are common (Alonzo, 2010). In the future, there are several avenues of research that are key to expanding our understanding of mating dynamics. First, recent theory has highlighted the important co-evolutionary feedback that can occur between parental investment, mate choice, mate competition, and fertilization (e.g., Kokko and Jennions, 2008; Alonzo, 2010). Additional theory is needed to more fully understand such links, and perhaps more importantly, experimental evolution and phylogenetic studies are essential to test predictions of this theory. Likewise, chance (Jennions *et al.*, 2012) and basic life-history differences between the sexes (Klug *et al.*, 2013) can have important roles in shaping mating dynamics, and both of these topics warrant further attention. Indeed, some studies have found large variation in mating success but no evidence of strong sexual selection (e.g., Westneat, 2006), and it is possible that random events have larger effects mating systems than is currently recognized. Finally, in the future, it will be key to give more focus on the genetic basis of mating and parental

behavior. Enhanced understanding of the mechanistic basis of mating behavior will allow us to develop a more complete conceptual framework of mating systems that links variation in genes with variation in populations and species.

See also: Life History Evolution: The Role of Mating Systems. Mating Systems in A Changing Environment. Mating Systems in Flowering Plants. Mating Systems in Plants, Genome Evolution and. Mating Tactics and Mating Strategies

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Mating Systems in A Changing Environment

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Glossary

Alternative reproductive behavior Discrete alternative behaviors involved in the pursuit of mating success.
Effective population size The number of individuals in a population that contribute offspring to the next generation.
Evolutionary trap Traits that have evolved under earlier environmental conditions that become maladaptive under changed environmental conditions. Includes ecological traps where animals make maladaptive habitat choices.
Mating system Describes the manner in which males and females associate during mating in an attempt to maximize their lifetime reproductive success. Animal mating systems are categorized according to the number of mates a given sex is able to monopolize (monogamy, polygamy, and promiscuity). Such categorizations can be based on social bonds (social mating system) or on genetic paternity and maternity of the resultant offspring (genetic mating system).
Operational sex ratio, OSR The average ratio of fertilizable females to sexually active males at any given time.
Potential reproductive rate, PRR The rate at which an individual is able to reproduce if it is not limited by the availability of mates.
Polygamy The situation that arises when members of one sex mate with multiple members of the opposite sex. Polygamy includes polygyny (when male mates with several females), polyandry (when female mates with several males), and polygynandry (when several males mate with several females).
Resource-based mating system Individuals of one sex (usually males) defend a resource that might sustain several members of the opposite sex (usually females).
Sexual conflict The conflict that arises when the reproductive interests of males and females are not aligned, resulting in sex-specific selection on traits.
Sexual selection Selection driven by competition among members of one sex (usually males) for access to the gametes of members of the opposite sex (usually females). Sexual selection can be divided into intrasexual selection, when members of the same sex compete among themselves for access to the opposite sex, and intersexual selection, when members of the same sex compete to be chosen by the opposite sex.

What Determines Mating Systems?

Animal mating systems are spectacularly diverse and exhibit considerable variation both within and between species. In some animals, males and females form lasting pair bonds and exhibit lifelong fidelity to a single mating partner. In others, sexual encounters may be little more than a fleeting tryst involving multiple mating partners or a chance encounter between the sperm and eggs of different individuals released into the surrounding environment. Ultimately, the mating strategy an animal adopts depends on the number of mates it can successfully monopolize as it attempts to maximize its reproductive success relative to others in the population. In this regard, competition among individuals for access to mates can often be taxing. A considerable investment of time and effort, for example, may be needed to successfully acquire and defend resources that are important in searching for, or attracting, mates or looking after offspring.

Not surprisingly, the costs and benefits of any mating strategy is expected to depend on a wide range of demographic and ecological factors, such as the density and distribution of the population, the abundance of resources, the potential reproductive rate of the sexes, the adult sex ratio, and the need for parental care (Emlen and Oring, 1977; Shuster and Wade, 2003; **Figures 1** and **2**). Moreover, the most 'profitable' mating system for one sex might be very different for the other (**Figure 3**).

Why are Mating Systems Sensitive to Environmental Change?

Ecological factors heavily influence animal mating systems. Such factors not only regulate the distribution and density of the population in space and time, but also determine the distribution and accessibility of resources that individuals require to monopolize mates and/or provide for offspring (Emlen and Oring, 1977; Clutton-Brock, 1989). In resource-based mating systems, a patchy distribution of vital resources, such as food, increases the potential for polygamy by allowing certain individuals of a given sex (usually males) to monopolize the resource and, with that, members of the opposite sex (**Figure 4**). In non-resource based mating systems, the distribution of the population itself is expected to be more critical. A spatially clumped distribution of mates (usually females) increases the potential for polygamy, while a temporally clumped distribution results in the opposite pattern.

Ecological factors also influence mating systems by affecting the efficacy of the communication system used to

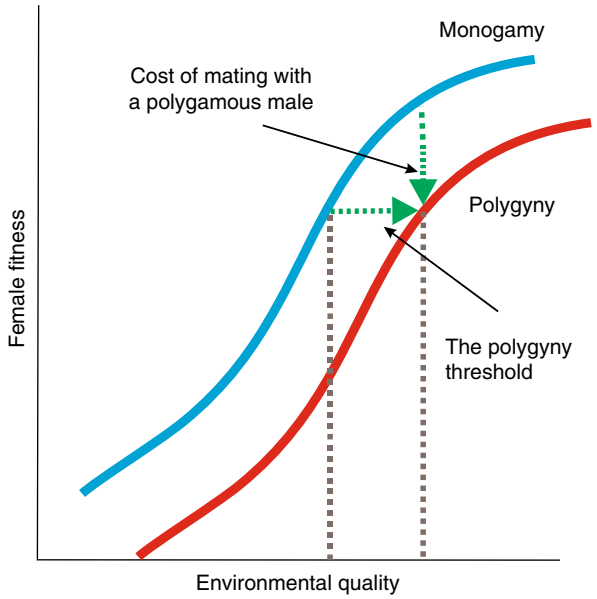

Figure 1 The benefit to a female of mating with a male that already has a mate depends on environmental quality, such as the availability of food that can be divided among the females. The switch-point when the benefit exceeds the cost is indicated by the polygyny threshold. As the environment improves, the net benefit of mating with a polygamous male increases and eventually becomes as high as mating with a monogamous male in the old environment. Reproduced from Orians, G.H., 1969. On evolution of mating systems in birds and mammals. American Naturalist 103, 589–603.

detect and select mates, the need for parental care and the potential reproductive rate of the sexes (Clutton-Brock, 1991).

How Does Environmental Change Influence Mating Systems?

Mating systems are susceptible to changes in the environment during acquisition and defense of resources, when searching for and attracting mates, during mate choice and sperm competition, and when providing for developing offspring (Figure 5). We discuss each of these in detail below.

Resource Acquisition and Defense

The ability of individuals to monopolize members of the opposite sex is sensitive to changes in the amount, quality, and distribution of critical resources needed to obtain and maintain mates (Clutton-Brock, 1989; Figure 4). Similarly, changes in the ability of individuals to defend resources can be altered by environmental change. Californian sea lions (*Zalophus californianus*), for example, defend territories close to the shore in an attempt to monopolize the females that preside within those territories. Higher temperatures, however, are forcing territorial holders to spend more of their time immersed in water, which reduces their ability to effectively guard the females against other males searching for mating opportunities (Bohorquez-Herrera *et al.*, 2014; Figure 6). As a consequence, such changes are likely to increase the incidence of extra-pair

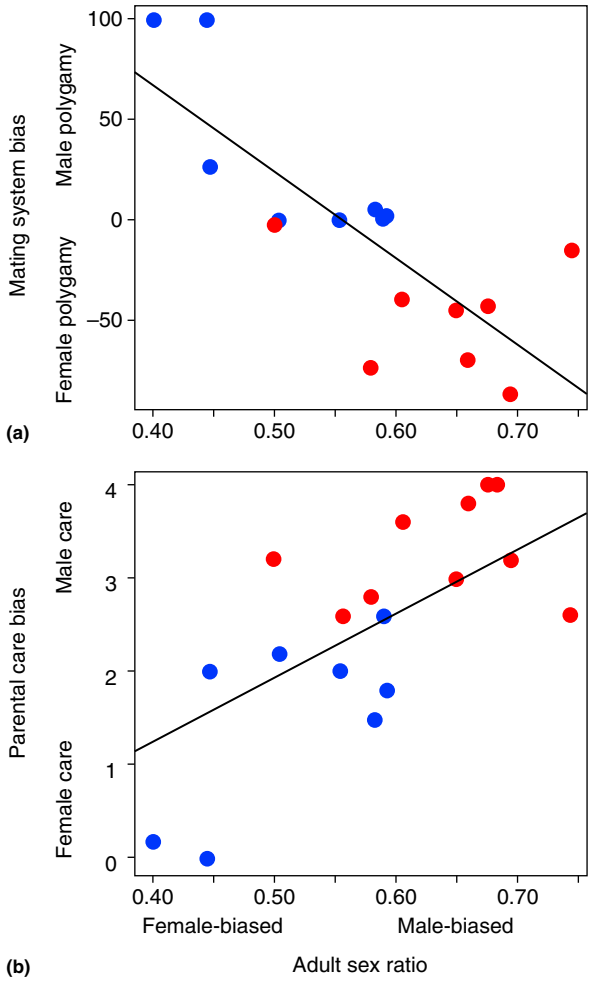

Figure 2 The mating system adopted depends on the adult sex ratio, as does biases between the sexes in parental care. (a) A more male-biased sex ratio lowers the probability that a male will be able to mate with several females, which increases the probability of female polygamy, while a female-biased sex ratio favors male polygamy. (b) A change in sex ratio also alters the sex-bias in parental care effort; when the sex ratio becomes more male-biased, and the potential for male polygamy decreases, males increase their parenting effort in relation to that of females. Blue and red dots are species with conventional and reversed sex roles, respectively. Reproduced from Liker, A., Freckleton, R.P., Szekely, T., 2013. The evolution of sex roles in birds is related to adult sex ratio. Nature Communications 4, 1587. doi: 10.1038/ncomms2600.

matings, reduce polygyny, and decrease the skew in mating success among male sea lions.

Mate Encounter Rate

Mate encounter rate depends not only on the distribution and density of individuals ready to mate, but also the probability that the sexes will be able to successfully encounter each other. Anthropogenic changes, in this regard, can affect encounter rates in a variety of ways. Habitat fragmentation can hinder dispersal (Banks *et al.*, 2007), global warming can alter hatchling sex ratios (Pen *et al.*, 2010), size-selective harvesting can reduce the abundance of the larger sex (Kendall and

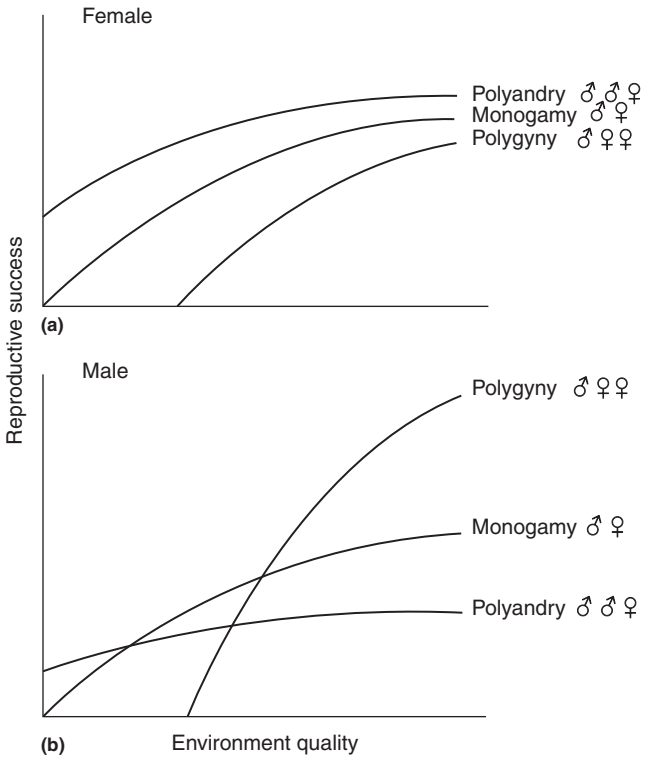

Figure 3 The mating system that is most 'profitable' in each environment varies between the sexes. Females always benefit from a polyandrous mating system, where several males provide for the female and the offspring, while males benefit from a polygynous mating system only in rich environments where enough resources are available to provide for several females and their offspring. This results in sexual conflict. Reproduced from Davies, N.B., 1991. Mating systems. In: Krebs, J.R., Davies, N.B. (Eds.), Behavioural Ecology: An Evolutionary Approach, third ed. Oxford: Blackwell Scientific Publications, pp. 263–294.

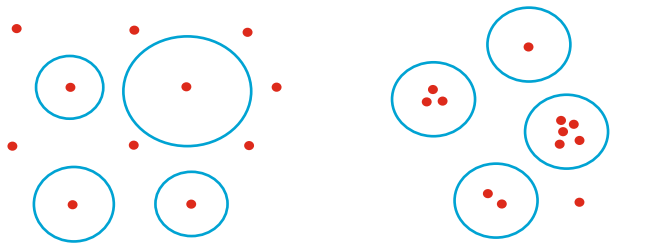

Figure 4 The distribution of resources, such as food or nest sites, determines the ability of individuals to monopolize resources and, hence, the potential for polygamy. The dots are resources and the circles are areas defended by individuals.

Quinn, 2013), and habitat changes can influence the use of signals important in mate detection. For instance, increased noise in urban areas is affecting the vocal signals of song birds (**Figure 7**) and frogs, while pollution of the aquatic environment is affecting the efficacy of chemical signals in fish (**Figure 8**; Fisher *et al.*, 2006; van der Sluijs *et al.*, 2011; Rosenthal and Stuart-Fox, 2012).

Mate Attraction and Mate Choice

Environmental conditions can profoundly affect mate competition and mate choice by altering the amount of effort the competing sex invests into conspicuous sexual displays, as well as the effort the more choosy sex invests in mate selection (Candolin and Wong, 2012). For instance, increased predation risk decreases the number of displays that guppies (*Poecilia reticulata*) perform to attract females, and instead increases the frequency of forced matings (**Figure 9**).

The possibility of making an informed choice about potential mates is hindered by environmental changes that hamper the production, transmission, and reception of signals used in mate choice (Rosenthal and Stuart-Fox, 2012). For instance, female threespine sticklebacks (*Gasterostues aculeatus*) have to spend more time evaluating males under eutrophied conditions where visibility is poor because of dense growth of

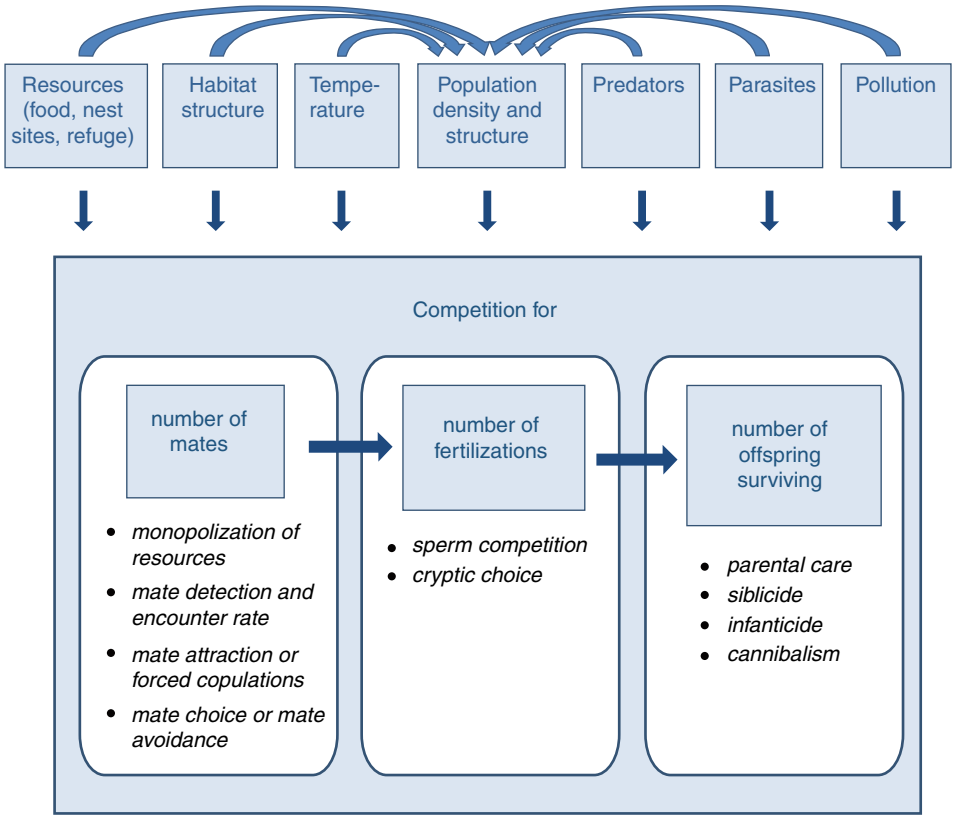

Figure 5 The reproductive success of an individual depends on the number of mates, the number of fertilized eggs the matings result in, and the survival of the ensuing offspring. Changes in the environment can influence any of these stages of reproduction, both directly and indirectly by influencing population demography.

Figure 6 The ability of sea lions to guard their females against intruding males searching for mating opportunities is hampered by the necessity to spend more time immersed in the water when temperature increases. Photo: Jimena Bohórquez- Herrera.

algae. This is true even though males attempt to compensate for the reduced visibility by increasing their courtship activity, a sexually selected trait (Figure 10).

If environmental changes alters the expression of heritable variation among individuals so that the difference in genetic makeup between individuals is altered, then this can

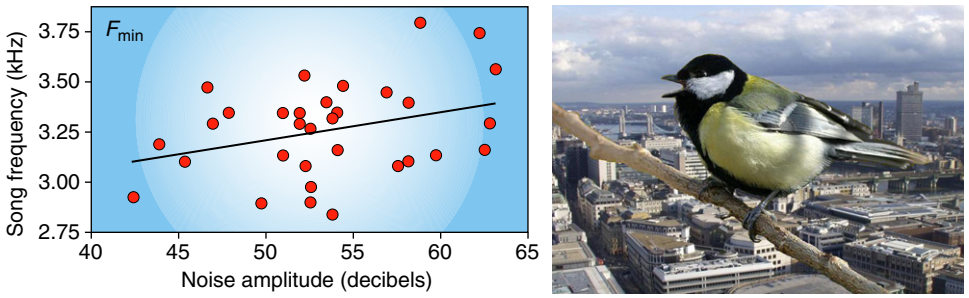

Figure 7 In urban areas where the background noise is louder (higher noise amplitude) great tits elevate the pitch of their songs (higher song frequency) to ensure that their calls can be heard despite the noise. Reproduced from Slabbekoorn, H., Peet, M., 2003. Ecology: Birds sing at a higher pitch in urban noise – Great tits hit the high notes to ensure that their mating calls are heard above the city's din. Nature 424, 267–267.

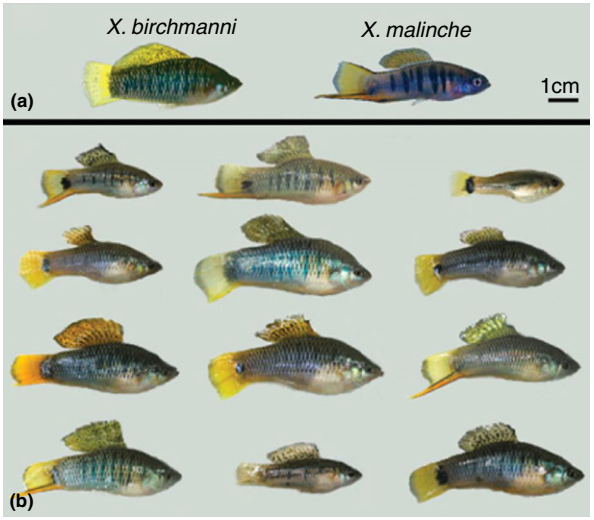

Figure 8 Pollution has been implicated in the disruption of chemical communication in two species of Mexican swordtail fish, leading to the formation of hybrids. (a) Males of the original parental species *Xiphophorus birchmanii* and *X. malinche* and (b) hybrid males (Fisher *et al.*, 2006).

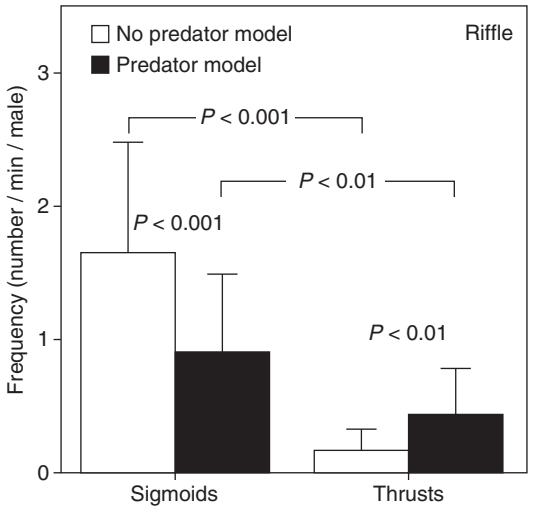

Figure 9 Male guppies reduce sigmoid courtship displays and increase the frequency of an alternative reproductive behavior, gonopodial thrusts, when predation risk increases. During sigmoid displays the male attracts females by arching his body into an S-shape. During gonopodial thrusting, males circumvent female mate preferences by approaching the female surreptitiously from behind and try to inseminate herusing his intromittant organ, the gonopodium. Data are mean + SD. Reproduced from Godin, J.G.J., 1995. Predation risk and alternative mating tactics in male Trinidadian guppies (Poecilia reticulata). Oecologia 103, 224–229.

affect mate choice for individuals that would confer 'good genes' to their offspring (Hoffmann and Merila, 1999).

Fertilization

The probability that mating results in fertilization can be altered by environmental changes that influence fertility, the intensity of sperm competition, and the possibility of adopting alternative reproductive tactics. For instance, environmental contamination by chemical pollutants known as endocrine disruptors can block or mimic the effect of hormones in exposed animals and result in modified reproductive anatomy and reduced fertility (Zala and Penn, 2004).

Some animals, despite being socially monogamous, engage in extra-pair matings, while others partake in 'sneak' fertilization attempts when others are mating. In those species, the benefit of adopting these alternative mating tactics can be altered by environmental change (Westneat and Sherman, 1997). For instance, as discussed above for guppies (**Figure 9**), an increase in the cost of ostentatious traits or behaviors required to attract members of the opposite sex can increase the prevalence of alternative mating tactics, such as forced copulations or sneak fertilizations, that do not rely on costly signals.

Parental Care and Offspring Survival

The amount of time and effort individuals devote to caring for offspring can potentially impinge on their ability to pursue additional mating opportunities (Gross, 2005). Hence, the decision to care for young will be affected by the demands of the current offspring, the willingness of the other parent to increase its investment into offspring care, and future reproductive opportunities. All of these components are sensitive to changes in the environment.

Future reproductive opportunities depend on mate encounter rate and attractiveness, and on the willingness of the

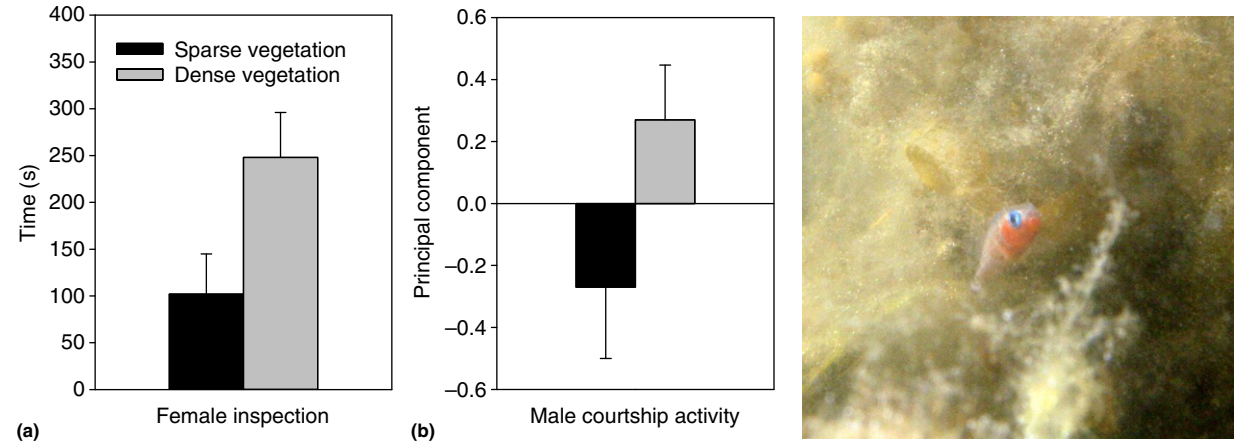

Figure 10 (a) When the density of filamentous algae increases because of human-induced eutrophication, female threespine sticklebacks have to spend more time evaluating males. (b) Males in turn increase their courtship activity, but still the females have a difficult time in making their choice. Data are means + SE. Photo: Jan Heuschele. Reproduced from Candolin, U., Salesto, T., Evers, M., 2007. Changed environmental conditions weaken sexual selection in sticklebacks. Journal of Evolutionary Biology 20, 233–239.

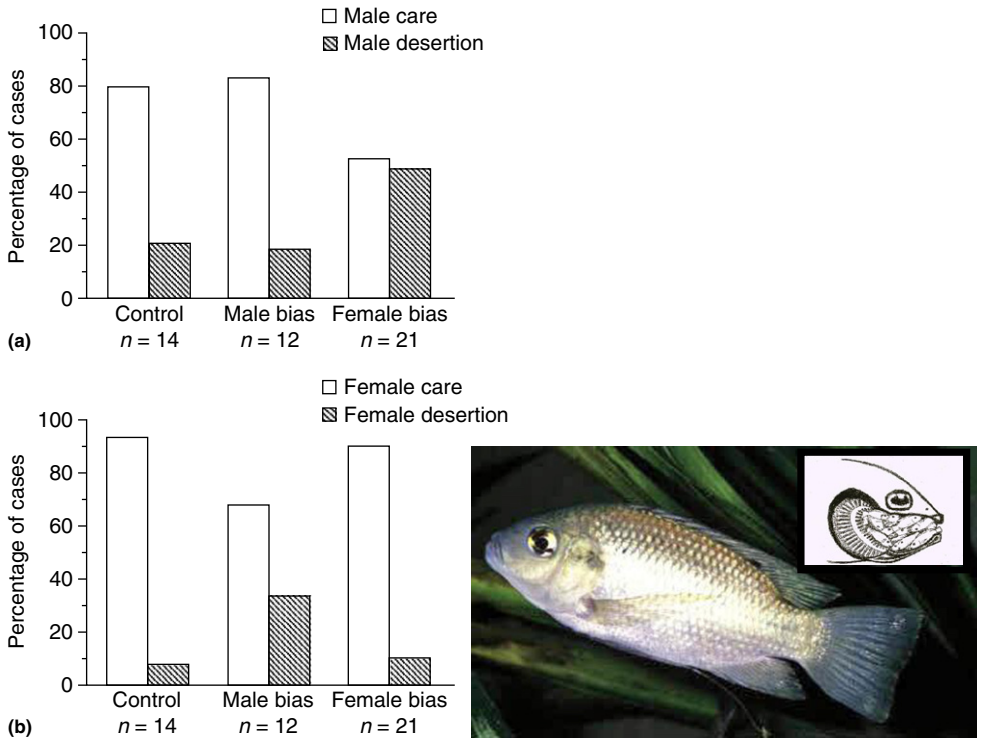

Figure 11 Investment of each sex into parental care depends on the sex ratio and future mating opportunities in the mouthbrooding Galilee St Peter's fish (*Sarotherodon galilaeus*). When the sex ratio is male-biased, females are more likely to desert their mate, and when it is females biased, males are more likely to desert. The control represents equal sex ratios. Photo: Sigal Balshine. Reproduced from Balshine-Earn, S., Earn, D.J.D., 1998. On the evolutionary pathway of parental care in mouth-brooding cichlid fish. Proceedings of the Royal Society B-Biological Sciences 265, 2217–2222.

other parent to increase its investment into parental care. For instance, changes in the operational sex ratio have a profound effect on parental investment in the Galilee St Peter's fish (Figure 11). Indeed, a switch to a male-biased sex ratio can even result in infanticide as has been reported in a group of primates known as langurs (*Presbytis* spp.), with males killing offspring sired by other males to bring females into sexual receptivity so that they, in turn, can reproduce with those females (Sterck, 1998).

Changes in population density and resource availability can also alter the ability of dominant group members to influence the breeding success of subordinates. For example, in the cooperatively breeding banded mongoose (*Mungos mungo*), older females are able to suppress the breeding of

younger females when resources are in short supply by evicting the females from the group (Nichols *et al.*, 2012). This, in turn, causes the expelled females to abort any pregnancies before returning to the group.

Consequences of Altered Mating System?

Mating systems determine the skew in mating success among individuals and, hence, the strength of sexual selection. If the mating system is adaptive, this skew ensures that individuals best adapted to local environmental conditions reproduce and pass on their genes to the following generation, which is important for ensuring a viable population. However, if changes to the mating system distort the correlation between mating success and individual quality, then population viability may be endangered. For instance, as we already have discussed, environmental changes that interfere with animal communication – and the efficacy of sexual signals – can prevent individuals from making adaptive mate choice decisions and result in the production of fewer offspring or those that are less well adapted to the environment.

In particular, environmental change can cause earlier evolved behaviors, signals, and mate preferences to become maladaptive, resulting in so called 'evolutionary traps' (Robertson *et al.*, 2013). An infamous example is seen in male jewel beetles (*Julodimorpha bakewelli*) from Western Australia, which have developed a sexual attraction to discarded beer bottles because the surface of the bottles resemble the look and texture of real females (Gwynne and Rentz, 1983; **Figure 12**). If individuals are not able to quickly adjust their traits and preferences to the altered conditions (or evolve traits that are better suited to the novel environmental conditions) then the reproductive output of the population may drastically decrease and undermine population persistence.

If, on the other hand, mating systems and sexual selection favor traits that increase individual fitness but are detrimental at the population level, then a relaxation of sexual selection could actually improve population viability. For example, male fruit flies (*Drosophila melanogaster*) in an attempt to increase their fertilization success, end up harming females during copulation by reducing their longevity and probability of mating with other males (Chapman *et al.*, 2003). This decreases the fecundity of the females and, hence, the reproductive output of the population. However, both male harm and the capacity for females to resist this harm depend very much on current environmental conditions and the conditions that they have evolved under (Arbuthnott *et al.*, 2014). Hence, a change in the environment because of anthropogenic disturbance that relaxes this sexually antagonistic selection could reduce female harm and, in so doing, have a positive effect on population growth.

Changes in mating system may also influence populations through effects on gene flow, inbreeding, hybridization, and effective population size. In particular, the loss of valuable genetic variation because of habitat fragmentation and inbreeding can reduce the population's capacity to adapt to environmental change (Frankham *et al.*, 2010). Hampered mate choice and altered signals in changing environments can, in turn, lead to the loss of biodiversity by breaking down reproductive barriers and resulting in hybridization between closely-related species. We already discussed the example of hybrids resulting from disruption of chemical communication in Mexican swordtail fish (**Figure 8**). Another example comes from Lake Victoria in eastern Africa where increased water turbidity, because of human-induced eutrophication, has

Figure 12 A male jewel beetle (*Julodimorpha bakewelli*) attempting to mate with a beer bottle. Photo: Darryl Gwynne.

Figure 13 Variation in male nuptial coloration in *Pundamilia* cichlids from Lake Victoria, Africa. The top and bottom images are typical of the coloration of male *P. pundamilia* and *P. nyererei*, respectively. Turbid water hampers species recognition based on male coloration. In clear water, females mate with males of their own species based on differences in coloration. In turbid water, females are not able to distinguish between species based on coloration and hybrids are produced. Photo: Ole Seehausen.

hampered species recognition during mate choice and resulted in hybridizations among cichlid species (**Figure 13**).

Changes in mating systems – because of environmental change – can consequently alter the demography and genetic-make up of populations. This can have serious consequences for the viability and persistence of populations, and, ultimately, for the structure and function of ecosystems. It is therefore vitally important that we consider the consequences that human activities have for mating systems when evaluating the impact of humans on natural animal populations.

See also: Mating Systems, A Brief History of. Responses to Climate Change, Evolution and

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Mating Systems in Flowering Plants

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Glossary

Cosexual The most common sexual system in flowering plants, in which a population is comprised of a single sexual class of hermaphrodites (cosexes) and on average individuals reproduce equally through female and male function.

Dioecy A sexual system in which a population is composed of female and male plants usually differing in secondary sex characters. At equilibrium the sexual morphs are maintained at equal frequency because of negative frequency-dependent selection.

Gender strategies The femaleness and maleness of individuals as reflected in their relative contribution to fitness through maternal and paternal investment.

Gynodioecy and androdioecy Two contrasting polymorphic sexual systems; gynodioecious populations are composed of female and hermaphrodite individuals, a sexual system that is not uncommon; in contrast, androdioecious populations contain male and hermaphrodite individuals, a sexual system that is extremely rare.

Inbreeding depression The reduction in viability and/or fertility of inbred offspring compared to outcrossed offspring as a result of the expression of deleterious recessive alleles in homozygous genotypes. Inbreeding depression is a key factor in determining mating system evolution, can occur throughout the life cycle, and is usually expressed most strongly when inbreeding occurs in a predominantly outcrossing species.

Linked selection Changes in allele frequency due to linkage with positively or negatively selected alleles at other loci, resulting in the loss of genetic diversity across genomic regions.

Modes of self-pollination The different ways that self-pollination can occur in flowering plants; distinguished primarily by the timing of self-pollination, whether pollinators are involved or not, and whether self-pollination occurs within or between flowers on a plant.

Pollen discounting The reduction in outcrossed male siring success caused by self-pollination within and between flowers on a plant.

Pollination The transfer of pollen between the male reproductive organ (stamen) to the receptive area (stigma) of the female organ (pistil). Pollination is necessary for mating but may not result in successful fertilization owing to post-pollination mechanisms, such as self-incompatibility.

Reproductive assurance An increase in seed set caused by self-pollination when conditions for outcrossing are unfavorable as a result of the absence of pollinators or compatible mates; requires that plants are self-compatible and also have the facility for autonomous self-pollination.

Self-incompatibility The inability of a fertile hermaphrodite plant to set abundant seed following self-pollination; the primary mechanism preventing self-fertilization in plants, and the opposite condition to self-compatibility in which plants can self-fertilize.

Sex allocation The parental expenditure of resources to sexual activities, including the relative allocation to female (ovules and seed) and male (pollen) function and also to floral display.

Introduction

Flowering plants (angiosperms) exhibit exceptionally diverse mating systems as a result of several distinctive features of their biology. First, because of their sessile habit plants depend on external agents to transfer male gametes (pollen) between individuals. This reliance has led to the evolution of diverse floral adaptations associated with the three main vectors for pollen dispersal: animals, wind, and water (Harder and Johnston, 2009). Pollination is a key reproductive process as it affects mating opportunities and fitness by determining the quantity and quality of pollen dispersed between flowers. Second, most plants possess hermaphroditic flowers and are therefore susceptible to self-pollination, often at the expense of cross-pollination as a result of pollen discounting (Harder and Barrett, 1995). Diverse morphological and physiological mechanisms have evolved in angiosperms to limit the harmful effects of self-fertilization and promote effective pollen dispersal between flowers on different plants. Finally, the modularity of plants and production of multiple reproductive structures introduces considerable mating complexity as a result of the subdivision of parental reproductive effort. Sexual plasticity and opportunities for combining hermaphroditic and unisexual flowers in different structural and temporal combinations has given rise to diverse gender strategies (Lloyd, 1979). As a result, the distribution of gametes within and between plants in a population is far more complicated than in animals, and reproduction can be highly promiscuous with individuals mating with numerous related and unrelated partners during their lifetimes, as well as themselves.

The term mating system is often used in different ways by reproductive biologists, depending on the organismal group. Perhaps the broadest definition, used primarily by researchers working with organisms with separate sexes, is simply "who mates with whom and how often." Biologists working with hermaphroditic groups (such as plants, worms, snails, slugs, or corals) often use a more restricted definition that simply involves the average frequency of cross- and self-fertilization in a

population. This definition of maternal mating success largely ignores information on male reproductive function (paternity or male outcrossed siring success) but has nevertheless provided important insights on the ecology and evolution of populations. The use of genetic markers over the past few decades has enabled quantitative measurements of selfing rates (s), or its complement the outcrossing rate ($t = 1 - s$), for several hundred angiosperm species representing diverse families, pollination systems, and life histories (Barrett and Eckert, 1990). This body of data has been valuable for testing theoretical models and for gaining insights on the ecological and demographic factors influencing the evolution of mating patterns.

In this article, we begin by considering mating patterns in plant populations with hermaphroditic sex expression (cosexuality), a condition that predominates among angiosperm taxa and is the ancestral state for most plant lineages. We focus on the causes and consequences of evolutionary transitions from outcrossing to predominant selfing, as this is the most common mating system transition among angiosperm families and has attracted considerable attention since Charles Darwin's seminal work on the topic. We then look at plant species with unisexual flowers in which populations are composed of various combinations of female, male, and hermaphrodite individuals. We review models and empirical evidence concerning how these polymorphic sexual systems are thought to have evolved from hermaphroditism and pay particular attention to the evolution of separate sexes from combined sexes.

Evolution of Mating Systems

Among life-history traits, reproductive characters that influence mating are of profound adaptive significance because they govern the character of genetic transmission between generations, the fitness of offspring and the amounts and distribution of genetic diversity in populations. The diversification of many angiosperm families (e.g., the orchid and phlox families) has been attributed to adaptive radiation of pollination and mating systems, often accompanying changes in the ecology of populations. Reproductive versatility is therefore a hallmark of angiosperm evolution and this is manifested by considerable inter- and intra-specific variation in mating systems. This variation implies frequent evolutionary transitions between mating systems, which are usually associated with changes to floral characters, pollination systems, and sex allocation in concert with life-history evolution. Ecological factors play a key role in driving the evolution of reproductive traits and in the maintenance of different mating systems. For example, long-lived tree species of stable plant communities are usually predominantly outcrossing whereas weedy colonists that occupy ephemeral environments are more commonly highly selfing. A major challenge is to determine the specific environmental, demographic, and genetic factors promoting changes in mating system.

The evolution of predominant self-fertilization (autogamy) from outcrossing represents the most important reproductive transition in angiosperms (Stebbins, 1974). There is evidence from numerous herbaceous families (e.g., the mustard and tomato families: Brassicaceae and Solanaceae, respectively) of multiple independent origins of autogamy and these transitions are often associated with the evolutionary breakdown of self-incompatibility, the primary genetic mechanism preventing selfing in plant populations. Investigation of evolutionary transitions from outcrossing to selfing is an active area of research today using comparative, experimental and genomic approaches, and theoretical models (e.g., Lloyd, 1992) on the selective mechanisms governing this transition have been particularly influential in guiding empirical research.

Why has the evolution of selfing from outcrossing been the focus of sustained interest for over a century? First, multiple independent transitions to selfing provide valuable opportunities to study convergent evolution, particularly the genetic and developmental basis of floral traits (e.g., small flowers, reduced floral display, and pollen production) that constitute the selfing syndrome (Sicard and Lenhard, 2011; **Figure 1**). Second, because selfing enables single plants to found colonies following long-distance dispersal, the shift to selfing can have significant biogeographical consequences (**Figure 1**), for example, by facilitating island colonization or migration to range margins where plant density may be low, a phenomenon known as Baker's Law (Baker, 1955). Third, selfing leads to restricted gene flow and reproductive isolation from ancestral outcrossing populations, potentially influencing speciation and lineage diversification (Wright *et al.*, 2013). A persistent theme concerns the extent to which the evolution of selfing is an 'evolutionary dead end' as a result of the ephemerality of selfing lineages compared to those that are outcrossing (Igic and Busch, 2013). Finally, because predominant selfing reduces the effective rate of recombination and effective population size there has been much recent interest on the genomic consequences of transitions to selfing, and the demographic and genetic processes causing genome-wide reductions in diversity in populations (Wright *et al.*, 2008).

Diverse reproductive, demographic, and genetic factors influence mating system evolution in plants. Of particular importance is the relative fitness of progeny that results from cross- versus self-fertilization, a phenomenon known as inbreeding depression. Darwin (1876) conducted controlled pollination studies on 57 species from 30 families and, in virtually all cases, discovered that selfed offspring performed less well compared with outcrossed offspring. Darwin used this observation to explain the function of numerous floral adaptations in angiosperms; they were outcrossing mechanisms limiting the harmful effects of self-fertilization. Today, it is understood that inbreeding depression is near ubiquitous in outcrossing species and largely results from the expression of recessive deleterious alleles in genotypes made homozygous as a result of inbreeding (Charlesworth and Willis, 2009). Inbreeding depression features in most models of mating system evolution and its magnitude plays an important role in helping to explain the observed distribution of outcrossing rates in nature, although explaining the occurrence of a significant number of species with a mixture of outcrossing and selfing (mixed mating) has resulted in considerable debate (Goodwillie *et al.*, 2005). A current challenge is to determine ecologically relevant levels of inbreeding depression under

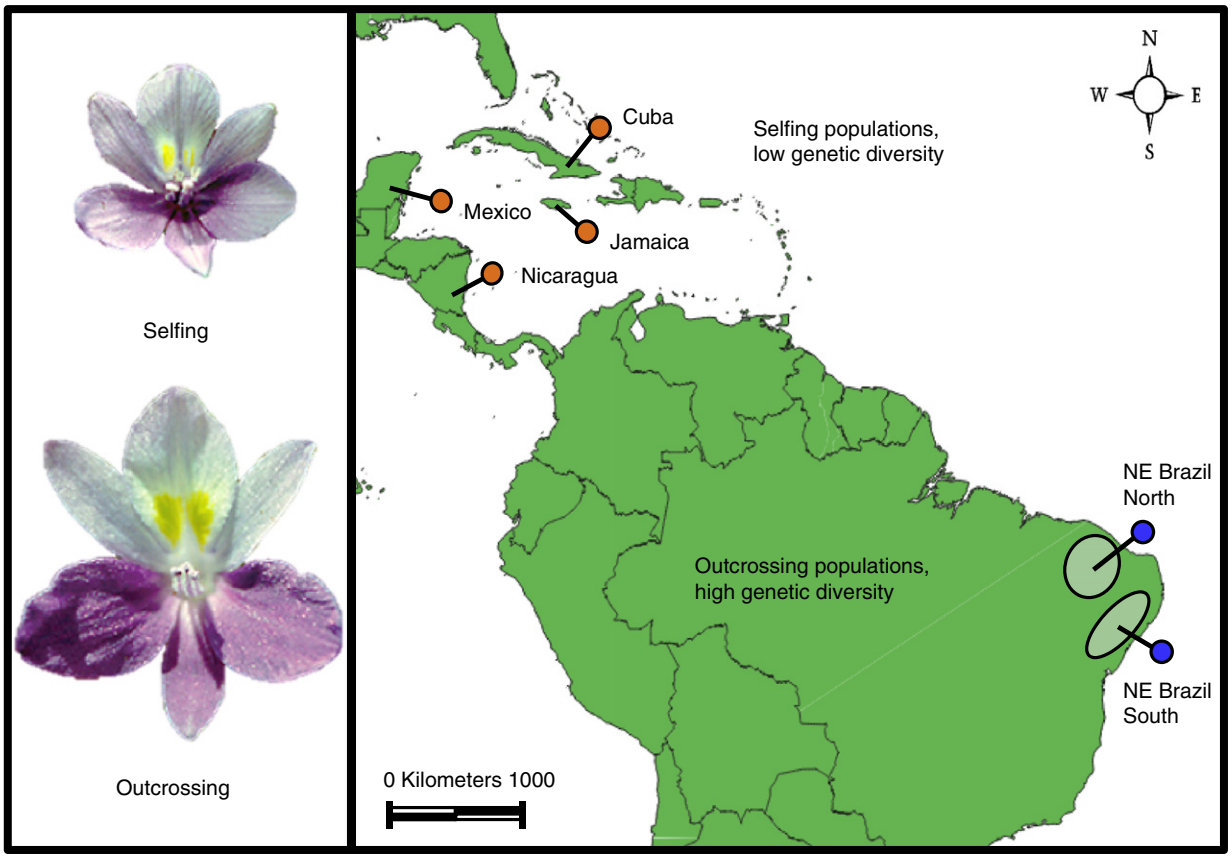

Figure 1 The phenotypic, biogeographical, and genetic consequences of evolutionary transitions from outcrossing to selfing, as exemplified by *Eichhornia paniculata* (Pontederiaceae). In this neotropical species, large-flowered outcrossing populations predominate in NE Brazil (bottom), and small-flowered selfing populations (top) have colonized the Caribbean and Central America. Outcrossing populations maintain high levels of diversity, whereas selfing populations are much less diverse. Further details are provided in Barrett *et al.* (2009).

field conditions as it is clear that comparisons conducted under glasshouse conditions significantly underestimate differences between selfed and outcrossed offspring (Dudash, 1990). Novel methods for estimating inbreeding depression in the field using genetic markers (Koelling *et al.*, 2012) may alleviate this problem, as should recent studies (e.g., Campbell *et al.*, 2013) on the extent to which biotic factors (pests and diseases) differentially influence the fitness of selfed and outcrossed offspring.

Inbreeding depression prevents the evolution of high selfing rates in most outcrossing populations. However, selfing evolves often and approximately 10–15% of flowering plants are predominant selfers, indicating that certain conditions can favor the selection of high selfing rates. The two most general mechanisms to explain the transition to selfing are: (1) The 'reproductive assurance hypothesis,' which traces back to Darwin and is the most frequently invoked explanation for why selfing often evolves under conditions of low density. It proposes that selfing is favored whenever pollen vectors and/or compatible mates are absent, limiting seed set by cross-pollination. (2) The 'automatic selection hypothesis,' which is based on R.A. Fisher's idea that a gene for selfing has a 3:2 transmission advantage when it arises in an outcrossing population. Determining the relative importance of these two hypotheses empirically is not straightforward, particularly if both processes operate during the transition to high selfing rates, as it requires determining the modes of self-fertilization in a population, and whether selection of genetic modifiers of the mating system occurs through pollen and/or seed (Busch and Delph, 2012). As yet, this has not been attempted in any species in which the transition to selfing has been investigated.

It has also been suggested that molecular data might be useful for distinguishing the two main hypotheses for the evolution of selfing. The demographic and genetic processes associated with reproductive assurance may result in a different genomic signature than those associated with automatic selection. This is based on the idea that when selfing evolves by reproductive assurance genetic bottlenecks causing the genome-wide loss of diversity should be common (Schoen *et al.*, 1996). However, recent work demonstrating that linked selection can also reduce genome-wide diversity rapidly following the transition to selfing casts doubt on whether it will be possible to use molecular data to distinguish the two main hypotheses for the evolution of selfing (Barrett *et al.*, 2014). It seems likely that reproductive assurance plays a more important role than automatic selection in initiating the transition to selfing; however, at present the case rests largely on correlative ecological evidence on the geographical distribution of selfing populations and their occurrence under conditions of low density. Experimental evidence on the

selective mechanisms driving the transition to selfing is generally lacking, despite a rich theoretical literature on the topic.

Sexual Systems and Gender Strategies

Most angiosperm species possess flowers with both male and female sexual organs and only ~10% have unisexual flowers (dicliny), with either female or male sex organs. There has been repeated evolution of dicliny across the angiosperm phylogeny, and variation in the temporal and spatial arrangement of unisexual flowers within and between individuals has given rise to the remarkable diversity of sexual systems found in flowering plants (Barrett, 2002). A population producing entirely unisexual flowers can be hermaphroditic if both male and female flowers are present on the same individuals (examples include maize and squash). This sexual system, termed monoecy (**Figure 2(a)**), prevents within-flower self-pollination and allows greater flexibility in the amount, location and timing of female and male investment in response to environmental cues during growth, or as a result of individual condition. Unisexual flowers can also be separated on different individuals, giving rise to male and female sexual morphs (dioecy; **Figures 2(b)** and **2(c)**); examples include kiwifruit and marijuana. However, in some species unisexuals are maintained in populations with hermaphrodite plants, resulting in gynodioecious (females and hermaphrodites; e.g., many members of the mint family, Lamiaceae) and androdioecious (males and hermaphrodites; e.g., annual mercury, *Mercurialis annua*) sexual systems. Finally, all three sex phenotypes can sometimes coexist within a population (subdioecy; e.g., broadleaf arrowhead, *Sagittaria latifolia*), although the extent to which this sexual system is stable over evolutionary time is unclear. Subdioecy is most often associated with evolutionary transitions from gynnodioecy to dioecy, as discussed further below (and see **Figure 3**).

From the perspective of mating patterns it is often more informative to consider the gender strategy of a population, an approach that involves a functional as opposed to morphological definition of plant sexual systems. The term gender strategy concerns the genetic contribution that each plant in a population makes to the next generation through maternal versus paternal expenditure and hence their 'femaleness' or 'maleness' (Lloyd, 1979). Although it is challenging to measure the true functional gender of individuals using genetic markers, many workers obtain a rough approximation of female and male mating success by describing the phenotypic gender of populations, which involves estimating allocation to alternate sex functions. Using this approach it is evident that all plant sexual systems can be divided into two fundamentally distinct strategies involving either gender monomorphism (**Figure 2(d)**) or gender dimorphism (**Figure 2(e)**).

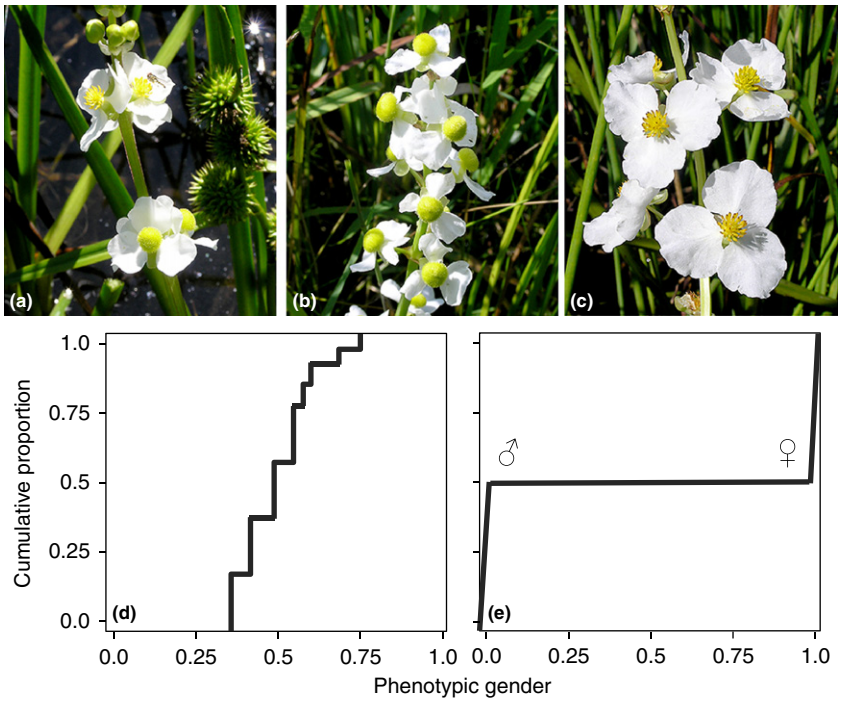

Figure 2 Intraspecific variation in sexual systems in a plant species: in *Sagittaria latifolia* (Alismatacae) three sexual phenotypes occur, with the majority of populations containing either hermaphroditic plants with separate female and male flowers (monoecy) as in (a), or unisexual plants (dioecy) that are either female (b) or male (c). (d) and (e) Illustrate phenotypic gender variation within and among populations of *S. latifolia*, based on allocation to female and male flowers. In (d) gender is monomorphic with continuous variation, whereas in (e) the distribution is dimorphic with approximately equal numbers of male and female plants. In some parts of the range populations with females, males and hermaphrodites also occur (subdioecy; Yakimowski and Barrett, 2014). See Lloyd (1979) for further details on the measurement of gender in plant populations and Sarkissian *et al.* (2001) for details of gender variation in *S. latifolia*.

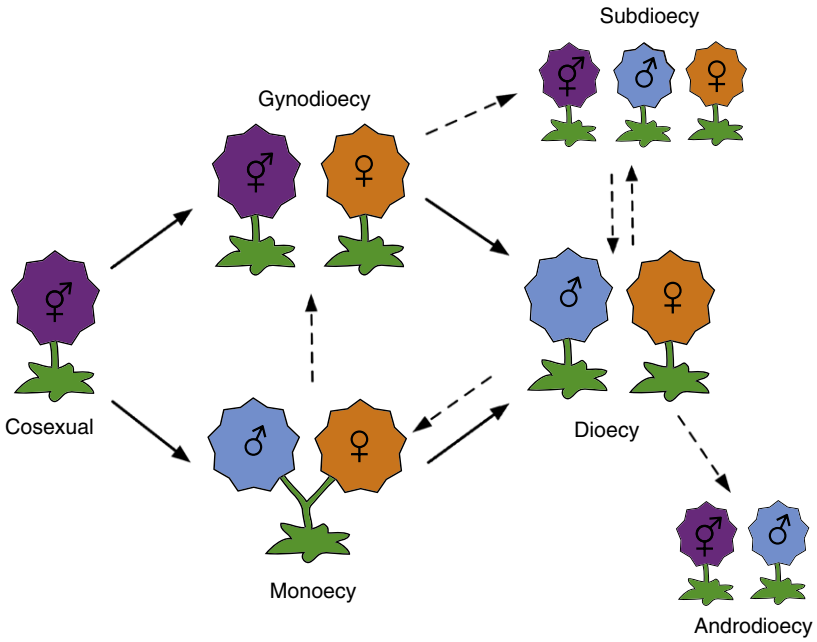

Figure 3 The primary evolutionary pathways by which polymorphic sexual systems originate from cosexuality in flowering plants. In the gynodioecy pathway (above), the first step is the invasion of females into hermaphrodite populations resulting in gynodioecy. This is followed by subsequent loss of female function in hermaphrodites giving males. This second step may often involve an intermediate stage in which hermaphrodites coexist with both females and males (subdioecy), although this condition can also arise from dioecy. The monoecy pathway (below) commences with increased specialization of female or male function by the origin of female and male flowers on hermaphroditic plants (monoecy). This is subsequently followed by gender specialization through gradual alteration in floral sex ratios and the evolution of unisexual plants (females and males). The monoecy pathway may occasionally involve an intermediate gynodioecious stage in which females invade monoecious populations, and occasional reversions from dioecy to monoecy are also known. Androdioecy is most commonly associated with the breakdown of dioecy, with females regaining male function and coexisting with males. For further details of the evolution of sexual system diversity see Barrett (2002).

In populations with gender monomorphism there is continuous (quantitative) variation in gender, with the average individual contributing genes equally through female and male function. All hermaphroditic plant species exhibit gender monomorphism. In contrast, in populations with gender dimorphism there is a bimodal distribution of gender, with a clear distinction between the gender of individuals belonging to different sexual morphs. Clearly, gender dimorphism occurs in dioecious populations, where male and female plants achieve all fitness through their particular gender and on average females and males contribute equally to the next generation, as every seed has both a mother and a father. However, gender dimorphism also occurs in gynodioecious and androdioecious populations, because the presence of unisexual individuals results in hermaphrodites contributing genes to the next generation mostly through the opposite gender to the particular unisexual morph in the population, rendering them functionally more male (gynodioecy) or female (androdioecy). Thinking about plant sexual systems in terms of gender strategies highlights the flexible and frequency-dependent nature of plant mating, with hermaphrodites acting 'more male' or 'more female' depending on the frequency of sexual phenotypes in the population, and this approach provides a functional framework for understanding the mating biology of plants.

The evolution of gender dimorphism from monomorphism requires the invasion of either males or females into a population of hermaphrodites, leading to androdioecy or gynodioecy, respectively. The frequency of these two dimorphic sexual systems is remarkably different among angiosperms families: gynodioecy occurs in ~7% of species and in many families, whereas only a handful of examples of true androdioecy are known. Furthermore, most cases of androdioecy involve a reversion from dioecy via the reacquisition of male function in females (**Figure 3**), rather than through male invasion of hermaphrodite populations, although this pathway does appear to occur rarely (Pannell, 2002).

Why then is gynodioecy much more common than androdioecy? This question can be addressed by considering the conditions that allow a unisexual individual to invade a hermaphrodite population, and how these conditions differ between the sexes. Intuitively, for a female or male individual to invade a population of hermaphrodites they must be at least twice as fit as hermaphrodites through female or male function, respectively (Lloyd, 1975). An important component in models for the evolution of gynodioecy is therefore the presence of inbreeding depression lowering the fitness of hermaphrodites. If hermaphrodites are self-compatible and through selfing suffer inbreeding depression this will lower the threshold over which a female can invade, because all offspring from females must necessarily be outcrossed and will therefore avoid inbreeding depression. However, in the case of androdioecy in a partially selfing population there will be

fewer ovules available for males to fertilize, which limits the potential siring success of invading males. Theoretical models indicate that with some degree of inbreeding depression females are not required to produce twice the number of ovules in order to invade a population of hermaphrodites, whereas males always have to produce at least twice the amount of pollen (Charlesworth and Charlesworth, 1978). Another constraint that reduces the frequency of male invasion relative to female invasion arises from the genetic mechanism causing the loss of male or female function. Cytoplasmic male sterility, where the sterility mutation occurs in either plastid or mitochondrial DNA as opposed to nuclear DNA, is a common phenomenon in plants, and is used in the production of hybrid seed in many crops. Unlike nuclear mutations, these mutations are inherited solely through the maternal line, making it unavailable as a mechanism of female sterility, as required for androdioecy. Furthermore, if male sterility is determined this way, only a slight advantage in female fertility is required for females to increase in frequency in the population (Lewis, 1941). Altogether, the conditions under which females can invade a hermaphrodite population are much less stringent than for males, explaining why gynodioecy is considerably more common than androdioecy in flowering plants.

Only ~6–7% of flowering plant species have separate sexes (Renner and Ricklefs, 1995). Despite being relatively uncommon, dioecy has a scattered distribution across the angiosperm phylogeny occurring in ~40% of all angiosperm families. This indicates that separate sexes have evolved independently many times from hermaphroditism, in concert with a suite of ecological and life history correlates (Vamosi *et al.*, 2003). The common occurrence of dioecy on the tips of phylogenetic trees and the apparent lower species richness of dioecious clades compared to their sister groups has led to the idea that dioecy, like predominant selfing, is an 'evolutionary dead end' either due to the high extinction rates or low speciation rates of dioecious clades (Heilbuth, 2000). However, this idea has recently been challenged based on comparative evidence that dioecious clades actually have a slightly higher net diversification rate than non-dioecious clades, and the phylogenetic distribution is instead a result of a combination of low transition rates to dioecy, as well as frequent losses (Käfer *et al.*, 2014).

The evolution of separate sexes requires two independent sterility mutations affecting female and male fertility, making it improbable that dioecy would arise directly from a hermaphrodite population without any intermediate stages. There has therefore been considerable interest focused on the evolutionary pathways that give rise to separate sexes, with the two most frequently invoked routes being the gynodioecy and monoecy pathways (**Figure 3**). In the gynodioecy pathway, females invade a hermaphroditic population leading to gynodioecy and subsequent selection on hermaphrodite sex allocation increases their 'maleness,' giving rise to an intermediate and often unstable stage of subdioecy and ultimately the evolution of dioecy (Spigler and Ashman, 2012), as has been shown in *Fragaria*, the strawberry genus (Liston *et al.*, 2014).

In contrast, the sequence of changes involved in the monoecy pathway is less well understood. It has often been suggested that disruptive selection on quantitative genetic variation in floral sex ratios within monoecious populations drives the evolution of different degrees of femaleness and maleness and ultimately results in completely separate-sexed plants (**Figure 3**). However, other evidence suggests that dioecy can sometimes evolve from monoecy via the gynodioecy pathway owing to large effect male sterility mutations and female invasion (e.g., broadleaf arrowhead, *Sagittaria latifolia*; Dorken and Barrett, 2004). Although the gynodioecy pathway has been well studied both theoretically and empirically, much less is known about the monoecy pathway, despite the strong correlation between dioecy and monoecy within clades, which has led to the suggestion that most transitions to dioecy follow this route (Renner and Ricklefs, 1995). However, there are two caveats that need to be considered in accepting this interpretation. First, in many clades the ancestral state is not clear and the polarity could therefore sometimes be reversed, with monoecy being derived from dioecy (e.g., in *Momordica*, the bitter gourd genus; Schaefer and Renner, 2010). Second, it is also possible that similar conditions promote the evolution of both monoecy and dioecy, resulting in a correlation independent of any particular transition sequence. What is clear is that more work is needed before a clear picture emerges of the evolution of dioecy via the monoecy pathway.

See also: Angiosperm Phylogeny and Diversification. Mating Systems in Plants, Genome Evolution and

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Mating Systems in Plants, Genome Evolution and

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Glossary

π_N/π_S The ratio of non-synonymous to synonymous polymorphism rate. Similar to the K_a/K_s ratio, π_N/π_S captures the intensity and form of selection acting on protein sequences, but in polymorphism data and hence at a shorter time scale.

Androdioecy A breeding system characterized by the co-occurrence of hermaphroditic and male individuals in the same species.

Apomixis or asexuality or clonality Asexual species only reproduce via mitosis, daughters are genetically identical to mothers unless a mutation occurs. In apomixis a seed develops from an unfertilized ovule.

B chromosomes Supernumerary and facultative chromosomes that do not obey the ordinary Mendelian laws of inheritance.

Dioecy In dioecious species, sexes are separated, females produce only ovules and males produce only pollen.

Genetic drift The random sampling of genetic variants in a finite population that leads to the random loss of some variants.

Gonochorism Separated sexes in animals, individuals are either females (producing only ovules) or males (producing only sperm).

Gynodioecy A breeding system characterized by the co-occurrence of hermaphroditic and female individuals in the same species, often as the result of nucleo-cytoplasmic interactions.

Hermaphroditism In hermaphroditic species, individuals carry both male and female reproductive organs and produce both ovules and pollen grains.

Heterostyly In heterostylous species, obligate outcrossing is enforced by morphological differences in the length or shape of the pistil and stamen. Two or three flower morphs coexist in a species and each individual expresses only one morph for all its flowers. The pollen from a given morph cannot fertilize a flower of the same morph. Is opposed to homostyly.

K_a/K_s The ratio of non-synonymous to synonymous substitution rates. Because selection acts primarily on proteins and not DNA sequences, synonymous changes are often treated as selectively neutral. K_a/K_s ratio is a proxy of the intensity and form of selection acting on protein sequences (≈ 1 for neutral evolution, >1 for positive selection, and <1 for negative selection).

Linkage disequilibrium The non-random association of alleles along a portion of chromosome in a population or species.

Outcrossing Outcrossing species reproduce through the fusion of gametes from distinct individuals at each generation.

Recombination The process by which two DNA molecules exchange genetic information, resulting in the production of a new combination of alleles.

Selection The process by which genetic variants become more or less frequent in a population according to their effect on fitness. Positive selection favors the fixation of advantageous alleles. Purifying selection favors the elimination of deleterious alleles. Balancing selection maintains diversity at a locus.

Selection on codon usage In some species, natural selection may favor some synonymous codons over others for encoding an amino acid. The preferred codons confer greater efficacy and/or accuracy of translation. This may generate a biased use of synonymous codons (i.e., codon usage bias).

Self-incompatibility A genetic system that prevents self-fertilization in hermaphrodites through recognition and rejection of pollen expressing the same allelic specificity as that expressed in the pistils, is opposed to self-compatibility.

Selfing Selfing species are sexual and undergo meiosis, but (in the case of pure selfing) fertilization only occurs between gametes produced by the same hermaphroditic individual.

Sexuality Sexual species reproduce through the alternation of meiosis and syngamy (fusion of gametes). During meiosis, chromosomes recombine and then segregate in different gametes.

Synonymous It refers to a sequence modification that does not result in a change at the protein level, it is opposed to non-synonymous.

Introduction

Breeding systems exhibit the most extraordinary variety in plants, and particularly in angiosperms where most of our knowledge comes from (but see Bainard *et al.*, 2013). Animals are comparatively very uniform with gonochorism being the general rule ($\sim$95%). Plants can either reproduce sexually or asexually or combine both strategies (Neiman *et al.*, 2014). Sexually reproducing plants can either outcross, self-fertilize, or have mixed mating systems. A large diversity of mechanisms have evolved in plants that promote either outcrossing or selfing. As selfing is possible only in hermaphrodites, dioecy is an obvious way of avoiding it. Outcrossing is also obligatory for females in gynodioecious species and for males in androdioecious species. Other mechanisms prevent selfing from happening in hermaphroditic species, such as heterostyly in which individuals have different flower morphologies, or self-incompatibility in which self-pollen is rejected.

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Table 1 Percentages of the breeding systems discussed in this article in angiosperms

Breeding system	*Percentage of angiosperms*
Dioecy	~6
Autogamy	~27
Apomixis	~0.2

Breeding systems are highly labile in plants and transitions from one to another are common. See **Table 1** for the percentages of the breeding systems discussed in this article in angiosperms (Hojsgaard *et al.*, 2014; Igic and Kohn, 2006; Renner and Ricklefs, 1995; Richards, 1997).

In this article, we will focus on the consequences of breeding systems on genome evolution in plants, including their effects on nucleotide diversity (both neutral and selective), genome base composition, genome structure, and genome size. We will focus on the nuclear genome.

Changes in Breeding Systems and Their Effects on Population Genetics Parameters

Effects on Heterozygosity Levels

When selfing evolves from outcrossing, homozygosity is almost complete after a few generations (F_{IS}, the inbreeding coefficient, is close to one). This reveals recessive mutations, whose effects were hidden in heterozygous outcrossers ($F_{IS} \sim 0$). In asexuals, due to clonal transmission of the genome, any new mutation appearing in a genotype endlessly remains at the heterozygous state, unless the exact same mutation occurs on the homologous chromosome (Meselson effect). Heterozygosity is thus high in asexuals ($F_{IS} \sim -1$) (Glémin and Galtier, 2012).

Effects on Effective Recombination

The efficacy of recombination depends on both crossing over and outcrossing rates (Glémin and Galtier, 2012). In asexuals, there are no crossovers so that the whole genome is non-recombining. In selfers, due to high homozygosity levels, homologous chromosomes are virtually identical inside an individual. Consequently, crossovers cannot break linkage between alleles in a selfing population and recombination is mostly inefficient (Glémin and Galtier, 2012; Nordborg, 2000).

Effects on Effective Population Size

With pure selfing, the effective population size, N_e, is decreased by half. Full homozygosity reduces the coalescent process to sampling diploid individuals (n) instead of sampling alleles ($2n$), since in selfers, two copies of the same allele are found in an individual. With partial selfing, $N_e = 2N/(1+F)$ with N the population size and F the expected equilibrium inbreeding coefficient (Nordborg and Donnelly, 1997; Pollak, 1987). N_e is also decreased by half in asexuals due to a similar process of genotype sampling during the coalescent process (Haag and Roze, 2007).

N_e is further reduced in selfers and asexuals due to inefficient or absent recombination, which results in selective interference among genetically linked loci (reviewed in Charlesworth, 2009; Gordo and Charlesworth, 2001). For instance, a strongly deleterious mutation will be removed by selection along with the mutations genetically linked to it, even if these are slightly advantageous ('ruby in the rubbish,' Charlesworth *et al.*, 1993; Peck, 1994). Similarly, mutations on a haplotype carrying a strongly advantageous mutation will reach fixation by genetic hitch-hiking, even though they are slightly deleterious (Maynard Smith and Haigh, 1974). These processes increase genetic drift and decrease genetic variation since more alleles are lost than would be without linkage, resulting in a reduced N_e. In small selfing and asexual populations, genomes without mutations cannot be regenerated by recombination of two genomes carrying different mutations and genomes tend to accumulate mutations until they are removed by selection, reducing N_e further (Muller's ratchet, Muller, 1964; Heller and Maynard Smith, 1978).

The ability of selfers to found new populations from a single seed (Baker 1955) combined with their reduced pollen migration (due to reduced allocation to male function) leads to small and isolated populations where drift is important and recurrent bottleneck events take place, which further reduces N_e (metapopulation dynamics, Ingvarsson, 2002; Pannell and Charlesworth, 2000).

In angiosperms, it has been suggested that dioecy is an evolutionary dead-end (Heilbuth, 2000). Dioecy may bring some handicaps. Only females produce seeds, which may cause less efficient dispersal of individuals and might lead to isolated populations with potentially reduced N_e (the seed-shadow handicap, Heilbuth *et al.*, 2001). Also, fertilization could be reduced: sexual selection drives male flowers to attract pollinators as much as possible, to the detriment of females if pollinators happen to be rare, which might decrease N_e further (Vamosi and Otto, 2002). This reduced N_e is expected to lead to the accumulation of more deleterious mutations in dioecious species compared to non-dioecious species, which could eventually cause their extinction. However, these theories were recently challenged by a study, which found higher (and not lower) diversification rates in dioecious versus non-dioecious clades (Käfer *et al.*, 2014).

Effects on the Efficacy of Selection

The outcome of natural selection depends on the product $N_e s$, where s is the selection coefficient that measures the fitness of a mutation (s is negative for deleterious mutations, positive for advantageous mutations, and equal to zero for neutral mutations) (Kimura, 1962). When N_e is reduced, mutations with low absolute values of s will behave almost neutrally. Weakly deleterious alleles may be fixed while weakly advantageous ones may be lost because of genetic drift overcoming selection. Because of reduced N_e, selfers, asexuals and perhaps dioecious species are expected to experience a reduced efficacy of selection assuming that many newly arisen mutations have low selection coefficients.

The next sections review the real data that tested these theoretical expectations (summarized in **Figure 1**).

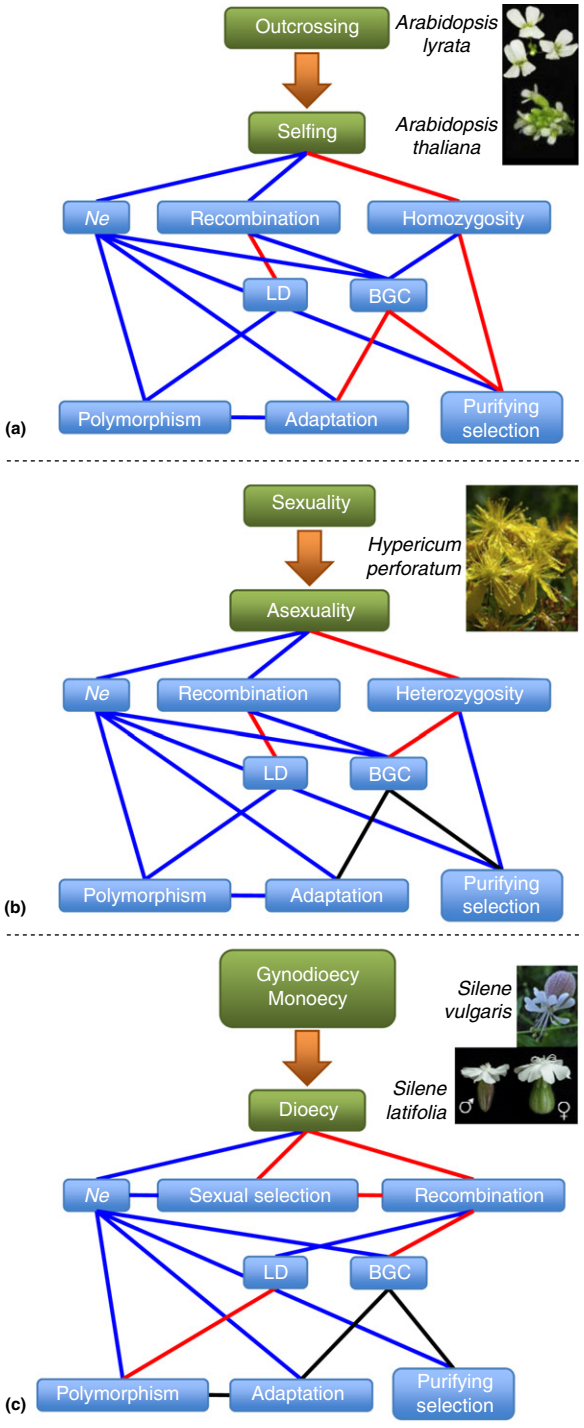

Figure 1 Summary of the theoretical expectations for the effects of three breeding systems on genome evolution in plants. Transitions to (a) selfing, (b) asexuality, and (c) dioecy. Blue lines indicate a negative effect, red lines a positive effect, and black lines an unclear effect. Examples of plant taxa with the studied transitions are shown. *Arabidopsis thaliana* flowers have been magnified three times compared to *Arabidopsis lyrata* ones. *Hypericum perforatum* includes both apomictic and sexual forms. BGC, biased gene conversion; LD, linkage disequilibrium; N_e, effective population size.

Breeding Systems and Within-Species Neutral Diversity

Neutral nucleotide diversity is directly linked to the effective population size ($\pi_s = 4N_e\mu$, where π_s is the population synonymous nucleotide diversity, assumed to reflect neutral diversity, and μ is the mutation rate per locus per generation) so that higher levels of within-population neutral diversity are expected in outcrossers compared to selfers and in sexuals compared to asexuals (reviewed in Charlesworth and Wright, 2001; Glémin and Galtier, 2012). No studies have compared dioecious and non-dioecious species diversity so far.

Selfing, Asexuality and Within-Species Neutral Diversity

Consistent with the theoretical expectations, selfing populations were found to be less diverse than closely related outcrossing populations and asexual species were found to have a lower diversity compared to their sexual counterparts (Table 2). Heterozygosity is, on the other hand, high in asexual individuals as discussed previously (Meselson effect). Selfing reduces within-population diversity while within-species diversity is not affected or is slightly increased because selfing increases population differentiation through reduced pollen gene flow (Charlesworth *et al.*, 1997). Extinction–recolonization dynamics, however, can reduce species-wide N_e in selfers with structured populations (Ingvarsson, 2002). The levels of neutral diversity in selfing and asexual species also depend on whether the change in breeding system has a single or multiple origin (Ness *et al.*, 2010) and whether partial sexuality is maintained in asexuals (Eckert and Barrett, 1993; Pappert *et al.*, 2000).

Self-Incompatibility and the S-Locus Neutral Diversity

In self-incompatible species, self-pollen is recognized and rejected by the pistil through protein interactions. The 'pollen' and 'pistil' genes are found in the tightly linked S-locus, which is under balancing selection: pollen carrying rare alleles are rejected at lower rates compared to pollen carrying common alleles, which prevents the loss of rare alleles through drift. The S-locus is indeed highly diverse (Castric and Vekemans, 2004).

Breeding Systems and the Global Efficacy of Natural Selection

When selection is reduced, species are expected to accumulate more deleterious mutations. Assuming that deleterious mutations are much more frequent than beneficial ones, both π_N/π_S and K_a/K_s should be higher in selfers compared to outcrossers, asexuals compared to sexuals, and perhaps in dioecious compared to hermaphroditic species. Codon usage bias (if under selection) should also be weaker (Marais *et al.*, 2004).

The Transition from Outcrossing to Selfing

Studies based on divergence (K_a/K_s) failed to detect reduced selection in selfers compared to outcrossers (Table 3). This could be due to the purging effect of homozygosity that

Table 2 Summary of studies comparing neutral nucleotide diversity between selfing and outcrossing, or asexual and sexual species

Taxonomic group	*Groups compared*	*Dataset*	*Diversity*[a]	*Comment*	*References*
Selfing vs. outcrossing					
Aegilops	5 Selfers/1 outcrosser	RFLP 52 genes	Yes		Dvorák *et al.*, 1998
Angiosperms		Meta-analysis allozyme diversity	Yes		Hamrick and Godt, 1990
Angiosperms		Meta-analysis allozyme diversity	Yes	High variance in species wide diversity levels in selfers	Schoen and Brown, 1991
Angiosperms		Meta-analysis allozyme diversity	Yes		Hamrick and Godt, 1996
Angiosperms	11 Selfers/12 outcrossers	Meta-analysis allozyme, microsatellite	Yes		Charlesworth and Pannell, 2001
Angiosperms	15 Selfers/15 outcrossers	Meta-analysis allozyme diversity	Yes		Charlesworth, 2003
Angiosperms and gymnosperms	10 Selfers/38 outcrossers	DNA markers	Yes	Low diversity within selfing populations but high among population diversity	Nybom, 2004
Angiosperms	29 Selfers/42 outcrossers	Meta-analysis	Yes		Glémin *et al.*, 2006
Arabidopsis	1 Selfer/1 outcrosser	5 nuc. Loci	Yes		Wright *et al.*, 2003
Arabidopsis/*Capsella*	1 Selfer/1 outcrosser	257 and 483 nuc Genes	Yes		Slotte *et al.*, 2010
Arabidopsis/*Capsella*	2 Selfers/2 outcrossers	780, 354/120, 346 Exons and 821/41introns	Yes		Qiu *et al.*, 2011
Capsella	1 Selfer/1 outcrosser	39 nuc genes, 14/20 individuals	Yes		Foxe *et al.*, 2009
Capsella	1 Selfer/1 outcrosser	1 mit. 17 nuc. Genes, 25/7 individuals	Yes		Guo *et al.*, 2009
Capsella	1 Selfer/1outcrosser	Genome/transcriptome	Yes		Slotte *et al.*, 2013
Capsella	1 Selfer/1outcrosser	6/5 Transcriptomes	Yes		Brandvain *et al.*, 2013
Clarkia xantiana	1 Selfer/1 outcrosser	8 nuc. 3 cp. Genes, 80/75 individuals	Yes		Pettengill and Moeller, 2012
Collinsia	1 Selfer/1 outcrosser	17 nuc Genes	Yes		Hazzouri *et al.*, 2013
Eichhornia paniculata	Outcrossing and selfing populations	Allozyme diversity	Yes		Barrett and Husband, 1990
Eichhornia paniculata	Trimorphic, dimorphic, and monomorphic populations	10 EST, 225 individuals	Yes		Ness *et al.*, 2010
Juan Fernandez Archipelago (Chile)	6 Selfers/12 outcrossers	Allozyme diversity	Yes		Crawford *et al.*, 2001
Leavenworthia	1 Outcrosser/4 selfers	Allozyme diversity	Yes		Charlesworth and Yang, 1998
Laevenworthia	2 Selfers, 1 outcrosser, outcrossing and selfing populations	6 Loci	Yes		Liu *et al.*, 1999, 1998
Leavenworthia alabamica	Outcrossing and selfing populations	8 nuc. Genes	Yes		Busch *et al.*, 2011

(*Continued*)

Table 2 Continued

Taxonomic group	*Groups compared*	*Dataset*	*Diversity*[a]	*Comment*	*References*
Lycopersicon pimpinellifolium	Outcrossing and selfing populations	Allozyme diversity	Yes		Rick *et al.*, 1977
Lycopersicon	3 Selfers, 2 mixed, 4 outcrossers	RFLP 36 loci	Yes		Stephan and Langley, 1998
Lycopersicon	2 Selfers/3 outcrossers	5 Loci	Yes		Baudry *et al.*, 2001
Mimulus	1 Selfer/1 outcrosser	2 nuc Loci	Yes		Sweigart and Willis, 2003
Miscanthus	1 Selfer/1 outcrosser	Adh1 Locus	Yes		Chiang *et al.*, 2003
Asexuality vs. sexuality					
Angiosperms	22 Asexuals	Allozyme diversity	Yes	Most genotypes occur in a single population	Ellstrand and Roose, 1987
Angiosperms		Allozyme diversity	Yes		Hamrick and Godt, 1990
Elodea	3 Asexuals	AFLPs	Yes		Lambertini *et al.*, 2010
Grevillea renwickiana	1 Asexual	10 Microsatellite	Yes		James and McDougall, 2014
Zeuxine/Eulophia	1 Apomictic/2 outcrossers	RAPDs	Yes		Sun and Wong, 2001

[a]Yes if neutral diversity lower in selfers compared to outcrossers, or asexuals compared to sexuals. Note that allozyme diversity is not neutral.

Table 3 Summary of studies comparing molecular evolution between selfing and outcrossing, or asexual and sexual species

Taxonomic group	*Groups compared*	*Dataset*	*dN/dS*[a]	π_N/π_S[a]	*Positive selection*[a]	*Codon usage*[a]	*References*
Selfing vs. outcrossing							
Angiosperms	29 Selfers/42 outcrossers	Meta-analysis (polymorphism)		Yes	Unclear		Glémin *et al.*, 2006
Angiosperms	290 Selfers/426 outcrossers	2 Cytoplasmic genes matK, rbcL	No				Glémin and Muyle, 2014
Arabidopsis/ *Drosophila*	1 Selfer/1 outcrosser + sister species each	12/34 Genes		Yes	Yes		Bustamante *et al.*, 2002
Arabidopsis	1 Selfer/1 outcrosser	23 nuc Genes + 1 chloro gene	No			No	Wright *et al.*, 2002
Arabidopsis	1 Selfer/1 outcrosser	675 Loci/62 large exons nuc genes, 13 orthologous genes		Not on 13 orthologs	No		Foxe *et al.*, 2008
Arabidopsis/ *Brassica*	1 Selfer/2 outcrossers	185 and 83 nuc Genes				No	Wright *et al.*, 2007
Arabidopsis/ *Capsella*	1 Selfer/1 outcrosser + sister species each	257 and 483 nuc Genes	No	Yes	Yes		Slotte *et al.*, 2010
Arabidopsis/ *Capsella*	2 Selfers/2 outcrossers	780, 354/120, 346 Exons and 821/41introns				Yes	Qiu *et al.*, 2011
Capsella	1 Selfer/1 outcrosser	Complete genome/11 transcriptomes		Yes			Slotte *et al.*, 2013
Capsella	1 Selfer/1 outcrosser	Complete genome/11 transcriptomes		Yes (after removing shared ancestral polymorphism)			Brandvain *et al.*, 2013
Collinsia	1 Selfer/1 outcrosser	17 nuc Genes + transcriptomes		Not on 17 genes, yes on transcriptomes		No	Hazzouri *et al.*, 2013
Eichhornia	3 Selfers/1 outcrosser	7890 nuc Genes (transcriptomes)		Yes		Yes	Ness *et al.*, 2012
Triticeae	2 Selfers/2 outcrossers	52 nuc Genes + 1 chloro gene	No		Yes	Yes	Haudry *et al.*, 2008
Triticeae	9 Selfers/10 outcrossers	27 nuc Genes	No				Escobar *et al.*, 2010
Asexuality vs. sexuality							
Boechera spatifolia	2 Asexuals/9 mixed /19 sexual	9126 SNPs, 14 SSR, traits			Yes		Lovell *et al.*, 2014
Oenothera	16 Asexuals/16 sexuals	1 nuc Defense gene (chiB), 5 conserved nuc genes	3 Out of 5 genes		Yes for ChiB		Hersch-Green *et al.*, 2012
Ranunculus auricomus	2 Asexuals/3 sexuals	1231 ORFs (transcriptomes)	Not genome wide, yes on sexual reproduction genes				Pellino *et al.*, 2013
Dioecy vs. hermaphroditism							
Silene	5 Myo dioecious and 3 recent dioecious/1 gynodioecious/1 outgroup	16 nuc Genes, 4 chloro genes, 25 ESTs	Yes in 5 Myo dioecious species				Käfer *et al.*, 2013

[a]Yes, if observation fits theoretical expectations (d*N*/d*S* significantly higher, π_N/π_S significantly higher, positive selection significantly weaker and codon usage significantly less optimized in selfing, asexual, or dioecious species); No, otherwise.
Source: Modified from Glémin, S., Muyle, A., 2014. Mating systems and selection efficacy: A test using chloroplastic sequence data in Angiosperms. Journal of Evolutionary Biology 27, 1386–1399. doi:10.1111/jeb.12356.

reveals recessive mutations in selfers and increases the efficacy of purifying selection (Glémin, 2007; Charlesworth, 1992). It has also been suggested that rare events of outcrossing in partially selfing plants could be enough to avoid the accumulation of deleterious mutations, and this could be promoted by the high recombination rates observed in selfers (Wright *et al.*, 2008). Moreover, selfing will increase K_a/K_s only if mutations have mild effects on fitness, which might not be the case: very little is known about the shape of the distribution of fitness effects of mutations (Glémin and Muyle, 2014). In agreement with this idea, selection on codon usage (involving small selective coefficients) is weaker in selfers than in outcrossers (Table 3).

Contrasting with results on K_a/K_s, most studies based on polymorphism (π_N/π_S) detected reduced selection in selfers: in *Arabidopsis*, *Capsella*, *Collinsia*, *Eichhornia* and in an angiosperm meta-analysis of about 30 species (Table 3). This suggests that selfing does result in selection being reduced but is probably of such recent origin that reduced selection can only be detected over short time scales (i.e., in polymorphism and not divergence data, Figure 2).

More efficient selection on recessive mutations in selfers than outcrossers could make positive selection and adaptation faster in selfers than in outcrossers (Glémin, 2007; Charlesworth, 1992). However, this is true for new beneficial mutations, and not for standing variation. When adaptation is underlain by standing variation, it is more efficient in outcrossers than in selfers (Glémin and Ronfort, 2013). In agreement with this idea, positive selection seems less efficient in selfers than in outcrossers (Table 3).

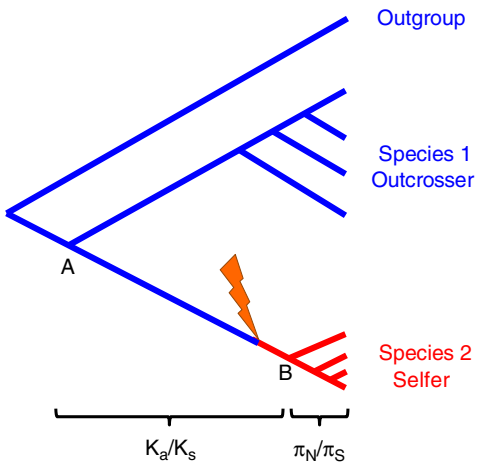

Figure 2 Timing of the transition from outcrossing to selfing and effects on divergence and polymorphism data. The transition is depicted by an orange lightning. If the transition is recent and a little older than the coalescent point of all mutations in the selfing population (node B), it will affect the polymorphism data (studied with π_N/π_S) strongly and the divergence data (studied with K_a/K_s) only weakly. This is because the divergence data will mainly reflect the evolution that took place from node A to B, whereas polymorphism will reflect the evolution that took place from node B to now. Adapted from Glémin, S., Marais, G., 2015. L'évolution du sexe: un carrefour pour la biologie évolutive. In: Thomas, F., Lefevre, T., Raymond, M. (Eds.), Biologie évolutive, Édition: Deuxième édition. Bruxelles: De Boeck Universite.

The Transition from Sexuality to Asexuality

Very few studies have so far compared sexual and asexual plant species (Table 3). Current data suggest adaptation is weak in asexuals. However, K_a/K_s analysis revealed no clear trend of reduced selection. No π_N/π_S analysis is available so that it is not possible to disentangle the possible effect of recent switches to asexuality from no accumulation of deleterious mutations.

The Transition from Hermaphroditism to Dioecy

The only study that tested the hypothesis of reduced selection in dioecious species using K_a/K_s is consistent with expectations when dioecy was several millions years old (Table 3). How the expectations are affected by the biology and ecology of the species remains to be explored: for example wind-pollinated dioecious species will not be affected by pollinator competition between sexes, and the reduction in N_e should not be as strong as in insect-pollinated species.

Breeding Systems and the Evolution of Genome Base Composition

Biased gene conversion (BGC) is a molecular process associated with recombination that drives genomic base composition toward a high GC content (Figure 3) (Lesecque *et al.*, 2013; Marais, 2003), which was shown to affect plant genomes (Glémin *et al.*, 2014; Serres-Giardi *et al.*, 2012; Muyle *et al.*, 2011). Because BGC will operate when recombination occurs in heterozygous loci, it will increase GC content in outcrossing and not in purely selfing species (Marais *et al.*, 2004). Because of the absence of recombination in asexuals, BGC should not affect GC content in these species. Also, as the effect of BGC is dependent on N_e (Lartillot, 2013), it should be reduced in selfers.

Observations fitted expectations for non-coding DNA (higher GC content in outcrossing Poaceae, Glémin *et al.*, 2006), unless a too small intron dataset was used (Brassicaceae, Qiu *et al.*, 2011). But studies using coding sequences gave conflicting results in Triticeae (Escobar *et al.*, 2010; Haudry *et al.*, 2008), or supported BGC but could not distinguish it from mutational biases in other groups (Hazzouri *et al.*, 2013; Wright *et al.*, 2007). More studies comparing selfing and outcrossing species are required, with appropriate methods that allow to distinguish BGC from mutational biases (equilibrium GC content, GC*, that a genome reaches if mutation and substitution rates remain constant, or derived allele frequency spectra) and large non-coding datasets in order to distinguish BGC from selection on codon usage. Similar studies also have to be carried out in asexual and sexual species.

Breeding Systems and the Evolution of Chromosomes and Genome Structure

Due to inefficient recombination, strong linkage disequilibrium is expected in selfers and was observed in the highly selfing *Arabidopsis thaliana* (Kim *et al.*, 2007; Nordborg *et al.*, 2005;

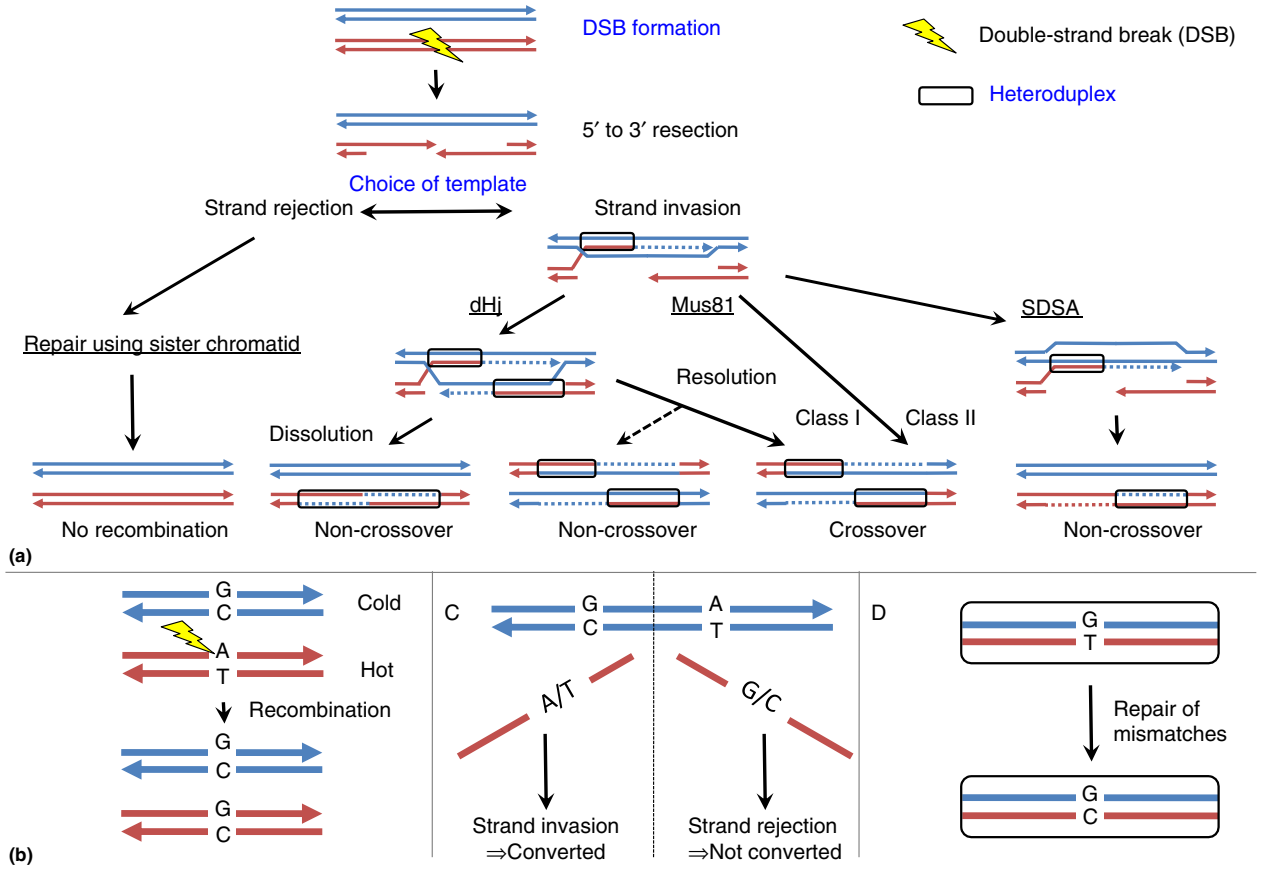

Figure 3 Possible mechanisms for the biased gene conversion (BGC). Different pathways for double-strand break (DSB) repair during meiotic recombination are shown in (a). Blue and red chromosomes indicate chromosomes from different parental origin. Recombination starts with the DSBs on one chromosome. Broken DNA is resected forming single-stranded DNA, which will be used to select a template for repairing the breaks. The mismatch repair (MMR) triggers the choice of template: if the degree of similarity between the single-stranded DNA and the potential template is sufficient, the template is invaded and a D-loop is formed. Otherwise, repair takes place by using the sister chromatin, no recombination happens. Recombination can take place by several pathways: the repair of the breaks by the MMR can lead to the formation of a double-Holliday junction (dHj) intermediate. Resolution of this intermediate may result in crossovers (COs) of class 1, i.e., showing CO interference (a mechanism by which 1 CO / arm / meiosis is ensured). Another pathway (Mus81) produces non-interfering COs. And the synthesis-dependent strand annealing (SDSA) pathway gives non-crosser (gene conversion) events only. Heteroduplexes (DNA with strands from different origins) can be found at various stages of the recombination pathway; they may include mismatches that are detected and repaired by different pathways (MMR and base-excision repair (BER)). BGC may result from different possible biases. (b) Initiation bias. If A/T alleles are recombinationally hotter than G/C alleles, G/C alleles will get copy-pasted onto the A/T alleles (recombination hot spots get converted by recombination cold spot, see the 'hot spot paradox,' Lesecque *et al.*, 2014), and this will increase GC content. (c) Choice of template bias. If the chance of the single-stranded DNA to invade the homologous chromosome is higher when it is GC-rich, this will also create BGC. (d) Repair bias. If repair of mismatches in heteroduplexes is biased in favor of G/C alleles, this may also generate BGC. A recent study in yeast suggests that BGC is found mostly associated with long gene conversion tracts and CO and may be due to MMR (Lesecque *et al.*, 2013). While evidence for BGC have been found in many organisms (Pessia *et al.*, 2012), in most cases the exact mechanism remains unknown and could be one of the three mechanisms outlined here. Adapted from Lesecque, Y., Mouchiroud, D., Duret, L., 2013. GC-biased gene conversion in yeast is specifically associated with crossovers: Molecular mechanisms and evolutionary significance. Molecular Biology and Evolution 30, 1409–1419. doi:10.1093/molbev/mst056.

Miyashita *et al.*, 1999; Wright *et al.*, 2008). Linkage disequilibrium decays with distance in *A. thaliana*, which fits with a transition to selfing >1 MY ago with some rare outcrossing events (Tang *et al.*, 2007). In the outcrossing *Collinsia linearis*, linkage disequilibrium decayed rapidly (over less than 1 kbp), whereas in the selfing *Collinsia rattanii* it could extend to several kbp (Hazzouri *et al.*, 2013).

Chromosomal rearrangements, when heterozygous, are deleterious as they cause chromosome disjunction and result in unbalanced gametes (Lande, 1985). However, selfing is associated with almost complete homozygosity and selection against chromosomal rearrangements will be weak. Fast karyotypic evolution is expected in selfers (Charlesworth, 1992). For example, *A. thaliana*'s karyotype includes 5 chromosome pairs and not 8, the standard karyotype in Brassicaceae (Hu *et al.*, 2011).

The mechanisms for sex determination is unknown in most of the ~15,000 dioecious species; sex chromosomes have been described in only 40 of them (Ming *et al.*, 2011). In male heterogametic systems males are XY and females XX, in female heterogametic systems females are ZW and males are ZZ, and in some species with a haplodiploid life cycle the

gametophytes have separated sexes and females are U and males are V (Bachtrog *et al.*, 2011). Y, W, U, and V chromosomes do not recombine which strongly impacts their molecular evolution. Self-incompatibility S-loci are also non-recombining and have similar properties (Castric and Vekemans, 2004).

Breeding Systems and the Evolution of Genome Size

Three major mechanisms influence genome size: ploidy levels, genomic parasite dynamics (transposable elements (TEs) and B chromosomes), and insertion/deletion events. We will review the effect of breeding systems on each mechanism impacting genome size and then consider the global effect of breeding systems on genome size.

Breeding Systems and Ploidy Levels

Virtually all apomictic plants are polyploid with some exceptions of recovered diploidy in apomictic hybrids (usually polyhaploid, see Asker and Jerling, 1992; Koltunow and Grossniklaus, 2003). This results in larger genomes in asexual compared to sexual plants as observed in *Hypericum* (Matzk *et al.*, 2003). Whether apomixis causes polyploidy, or whether polyploidy causes apomixis by disrupting developmental processes is, however, still unclear (Bhat *et al.*, 2005).

Gametophytic self-incompatibility systems can be directly broken down by polyploidy through the formation of diploid pollen grain (Robertson *et al.*, 2011). The inbreeding depression following self-incompatibility breakdown was proposed to trigger the evolution of dioecy in the Solanaceae family and 12 other genera (Miller, 2000). This proposed association was further tested in 22 genera (i.e., a small fraction of the dioecious genera) and hermaphroditism is indeed more common among diploid than polyploid species, whereas gender dimorphism (dioecy and gynodioecy) is more frequent among polyploid species (Ashman *et al.*, 2013). In most dioecious genera though, the transition to dioecy is probably not accompanied by polyploidization.

Liverworts are ancestrally dioecious and it has been suggested that polyploidy could happen more often in hermaphrodites. However, a test on 67 liverwort species, controlling for phylogenetic inertia, did not support this hypothesis (Bainard *et al.*, 2013).

Breeding Systems and Insertion/Deletion

A. thaliana has smaller introns than *Arabidopsis lyrata* because of different indel dynamics in both species (Wright *et al.*, 2002). A whole genome alignment of *A. thaliana* and *A. lyrata* showed that more than 50% of the *A. lyrata* genome is missing from the *A. thaliana* genome (Hu *et al.*, 2011), explaining the large difference in genome size between both species (*A. thaliana*: 125 Mb, *A. lyrata*: 207 Mb). Large deletions of TEs and intergenic regions, probably selected for, resulted in genome streamlining in *A. thaliana*.

Breeding Systems and TE Dynamics

TE amplification is predominantly deleterious when insertion occurs in or near functional regions or when functional regions are lost by ectopic recombination between TE copies (Charlesworth and Langley, 1989). The number of TEs in a population is determined by the balance between the capacity to invade the host genome through transposition and the efficiency of the host defense mechanisms against TEs. Old TEs end up being inactivated by the host defense mechanisms. A TE element will survive if it can jump from a host to another through horizontal gene transfer and contaminate new hosts, which do not have yet efficient defense mechanisms against that element (Schaack *et al.*, 2010).

In asexual species, TE do not have that chance and they will remain confined to a single host (Hickey, 1982). TE transposition is expected to decline on the long run (Bestor, 1999; Wright and Finnegan, 2001). However, reduced selection against TEs in asexuals might favor TE accumulation. The outcome of these two opposed effects is not easy to predict. It is not clear whether fewer or more TEs should be found in asexuals than in sexuals.

In *Hypericum*, the mean DNA content per chromosome for old asexual species is higher than that of closely related sexual species, suggesting an accumulation of TEs in asexuals (Matzk *et al.*, 2003). In a study comparing four pairs of closely related sexuals and asexuals for three TEs, no reduced selection against TEs was found in asexuals using K_a/K_s, suggesting their transposition capacity has not decreased. The recent origin of the asexuals could, however, explain the results, as shown by simulations (Docking *et al.*, 2006).

Similar expectations hold for selfing species: TE transposition should decline because of confinement to a lineage but this could be blurred by a globally reduced selection in selfers. But the dynamics of TEs in selfers are even more difficult to predict than in asexuals. Because of a high level of homozygosity, purifying selection is expected to purge more efficiently the deleterious recessive mutations from the population in selfers than in outcrossers. However, ectopic exchanges among TE copies will probably be rare in homozygotes (Montgomery *et al.*, 1991), and selection will not act against TEs this way in selfers. It is not clear which one of these many opposing effects should prevail (Charlesworth and Charlesworth, 1995; Wright and Schoen, 1999; Morgan, 2001; Boutin *et al.*, 2012).

Looking at some TE families have indeed returned contradictory results (Young *et al.*, 1994; Charlesworth and Charlesworth, 1995; Wright *et al.*, 2001; Lockton and Gaut, 2010; Tam *et al.*, 2007). However, more recently, a clearer view has started to emerge from whole genome comparisons between selfers and outcrossers. *A. thaliana* has less TEs than *A. lyrata* (24% and 30% of the genome respectively, Hu *et al.*, 2011). Interestingly, TEs are less frequent nearby genes in selfers than in outcrossers (Agren *et al.*, 2014; Slotte *et al.*, 2013). Also, deletions of TEs are selected for in *A. thaliana* (Hu *et al.*, 2011), clearly pointing to a more efficient selection against TEs in selfers than in outcrossers. In *A. thaliana*, most TEs are old, older than the *A. thaliana*–*A. lyrata* speciation. In *A. lyrata*, on the contrary, recent bursts of TEs have occurred (Hu *et al.*, 2011; Wright and Agren, 2011; Tsukahara *et al.*, 2012; de la Chaux *et al.*, 2012; Maumus and Quesneville, 2014). Similar

patterns were found in the selfers *Capsella rubella* and *Capsella orientalis* compared to the outcrosser *Capsella grandiflora* (Agren *et al.*, 2014; Slotte *et al.*, 2013). These observations suggest that reduced transposition and efficient selection against TE insertions are dominating TE dynamics in selfers.

Dioecy can be determined by sex chromosomes, which are known to accumulate TEs because of reduced selection triggered by the absence of recombination for the Y chromosome and a reduced N_e for the X chromosome. In papaya (trioecious), the male-specific and female-specific regions of the sex chromosomes have accumulated retrotransposons as predicted (Gschwend *et al.*, 2012; Wang *et al.*, 2012). In the dioecious plant *Silene latifolia*, the X and the Y chromosomes are much larger than other chromosomes, probably because of sex chromosome specific TE accumulation (Cegan *et al.*, 2012). Interestingly, *S. latifolia* has a much larger genome than *Silene vulgaris*, a non-dioecious close relative. However, whether dioecious species tend to accumulate TEs on the sex chromosomes only or on the whole genome as would predict the dead-end hypothesis is not clear.

Breeding Systems and B Chromosomes

B chromosomes are also considered parasitic DNA elements, which are mainly deleterious to their hosts. Their dynamics may be similar to TEs with outcrossing favoring their spread; they are therefore expected to be associated with outcrossing or to evolve into mutualists when associated with selfing (Burt and Trivers, 1998). A significant positive correlation was found between the presence of B chromosomes and outcrossing, when taking phylogenetic inertia into account (Burt and Trivers, 1998; Trivers *et al.*, 2004). However, B chromosomes do not seem to contribute significantly to the evolution of genome size (Trivers *et al.*, 2004).

Global Effect of Breeding Systems on Genome Size

An analysis of C-values of 14 pairs of closely related outcrossing and selfing species revealed that selfers tend to have smaller genome sizes than outcrossers, and this is not due to differences in ploidy levels (Wright *et al.*, 2008). A detailed analysis of genome size in the *Veronica* genus suggests that selfing, not annuality, is associated with genome size reduction after taking phylogenetic inertia into account (Albach and Greilhuber, 2004). The analysis of a dataset of 205 species showed a significant association between outcrossing and large genomes after taking phylogenetic inertia into account (Whitney *et al.*, 2010). However, these two studies used the phylogenic independent contrast method (Felsenstein, 1985) to correct for phylogenetic inertia. This simple approach (in which sister branches are compared along the tree and ancestral states of characters are inferred in internal nodes by averaging the values of the next lower nodes) has been criticized for the study of genome size as it neglects the possibility of parallel evolution (Lynch, 2011; Whitney *et al.*, 2011). The question of the global effect of selfing on genome size remains thus open. Studies on the effects of asexuality and dioecy on genome size are lacking.

Conclusion

Current data suggest that changes in breeding system can have a strong impact on genome evolution (**Table 4**). An important point is that the transitions to new breeding systems have to be recent for the data to fit with the theory. In the future, it will be important to date those transitions much more precisely than they are today (when such dating is available) to confirm this. Another important point is about the forces to explain the difference in genomic features between two species with different breeding systems. Breeding system is usually not the only difference, even among closely related species, demography for instance can be a confounding factor. This is a very difficult point; increasing the number of studied systems is probably the only way to address it. We are starting to be in this situation for the transition from outcrossing to selfing, which is the best studied. The transitions to asexuality and

Table 4 Summary of theoretical expectations and observations concerning breeding systems' effects on genome evolution in plants

		Selfing	*Asexuality*	*Dioecy*
Neutral diversity	Theory	Lower	Lower	Lower?
	Observation	Yes	Yes	–
Selection (K_a/K_s)	Theory	Reduced	Reduced	Reduced?
	Observation	No	No	Yes
Selection (π_N/π_S)	Theory	Reduced	Reduced	Reduced?
	Observation	Yes	–	–
Positive selection	Theory	Reduced	Reduced	Reduced?
	Observation	Yes	Yes	–
Codon usage bias	Theory	Weaker	Weaker	Lower?
	Observation	Yes	–	–
GC content	Theory	Lower	Opposing effects	Opposing effects
	Observation	Yes	–	–
Linkage disequilibrium	Theory	Stronger	Stronger	–
	Observation	Yes	–	–
Genome size	Theory	Opposing effects	Opposing effects	Larger
	Observation	Smaller	Larger	Yes

Selfing species are compared to outcrossing species, asexual to sexual, and dioecious to non-dioecious. Observation: Yes if observation fits theory; No, otherwise; –, if observation is unavailable.

dioecy have been less studied. Only a handful of studies are available on the effects of dioecy on genome evolution. However, dioecy is expected to drive substantial changes in evolutionary forces acting on genomes (**Figure 1(c)**). In particular, sexual selection should be much stronger than in non-dioecious species, probably driving the evolution of sex-biased expression for many genes in the genome. Future studies on dioecious species could provide data on sex-biased expression (for which we currently know very little in plants) and other aspects. It could help to understand why dioecy is surprisingly rare in angiosperms.

See also: Effective Population Size. Genetic Variation in Populations. Mating Systems in Flowering Plants. Recombination and Selection. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth

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Mating Tactics and Mating Strategies

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Glossary

Alternative mating tactics Discrete variation among individuals within a population in traits related to mating. These traits can be behavioral, physiological, and morphological.

Condition-dependent selection Selection that varies with individual condition such as age, size, energy reserves, or experience.

Genetic polymorphism Discrete genetic variation among individuals within a population.

Negative frequency-dependent selection Selection on a genotype or phenotype arising from a decrease in relative fitness as the frequency of that genotype or phenotype increases.

Phenotypic plasticity When the phenotype of an organism is not fixed but is instead plastic and depends on environmental conditions or individual circumstances.

Introduction

Evolution by natural selection tends to favor the genes or genotypes that allow some individuals to have more surviving offspring than others. One might, therefore, expect over evolutionary time that a single solution would arise to the problem of 'how best' to mate and reproduce in a given environment. What we actually observe is that many populations exhibit both continuous and discrete variation in reproductive behaviors and mating patterns (Oliveira *et al.*, 2008). Evolutionary biologists have puzzled for decades over how and why this variation is maintained (Austad, 1984; Dominey, 1984; Gross, 1984, 1996; Henson and Warner, 1997). A population is said to exhibit 'alternative mating tactics' (AMTs) or more generally 'alternative reproductive tactics' (ARTs) when there is discrete variation between individuals in how they mate or reproduce within the same population at one point in time. For example, some individuals might defend a reproductive territory to attract mates and rear young, while other reproductive individuals in the same population find mates without having a territory. Such alternative tactics can arise from a genetic polymorphism or because individuals respond plastically to an aspect of their environment or individual state. Traditionally (though not necessarily consistently), the term 'alternative tactics' has been used for alternative behaviors or phenotypes independent of their basis, while 'alternative strategies' is reserved for cases where the alternatives are known to be determined by a genetic polymorphism (Gross, 1984, 1996). This article focuses on AMTs, but it is worth noting that alternative tactics occur in many other aspects of an organism's phenotype.

What Form Do Alternative Mating Tactics Take in Nature?

In the bluegill sunfish (*Lepomis macrochirus*, a North American freshwater fish), three alternative male tactics coexist (Gross and Charnov, 1980; Gross, 1982). Some males build nests, attract females to these nests and take care of the developing eggs and young (called 'parental males'). Other smaller males called 'sneakers' do not build nests or care for young, but sneak in and release sperm when a parental male and female are spawning. Finally, 'female mimics' get close to another male's nest by looking and behaving like a female, but release sperm when a real female spawns in the parental male's nest. These three male types differ in their behavior, size, physiology, and morphology. And yet they are all one species. In meerkats (*Suricata suricatta*, African desert mammals from the mongoose family), individuals live in social groups with relatives and cooperate to defend and rear young (Clutton-Brock *et al.*, 2001; Russell *et al.*, 2007). Dominant males tend to sire young in their own social group, while subordinate males mainly sire young outside their social group, while helping to rear the young within their group instead of their own young (Young *et al.*, 2007). Subordinate and dominant male meerkats therefore exhibit AMTs with respect to their mating behavior and parental care. In the green-veined white butterfly (*Pieris napi*), females vary in their propensity to mate with one male (e.g., be monandrous) or multiple males (e.g., be polyandrous, Wedell *et al.*, 2002). The probability that a female will be monandrous or polyandrous depends not only on female genotype but also on the amount of energy and type of sperm they receive from their mate (Wedell, 2001, 2006; Wedell *et al.*, 2009). In goldeneye ducks (*Bucephala* sp.), and a variety of other water birds, some females build and lay eggs in their own nests, while others lay eggs in the nests of other females (a behavior called conspecific brood parasitism, Eadie and Fryxell, 1992; Eadie and Lyon, 1998; Lyon, 2008). Recent research on the common goldeneye duck (*Bucephala clangula*) suggests that females preferentially lay their eggs in the nests of related females and in nests more likely to be safe from predation (Poysa *et al.*, 2014). In guppies (*Poecilia reticulata*, a tropical freshwater fish), older females tend to choose mates independently while younger females often copy the mate choice of other females (Dugatkin and Godin, 1993). These examples illustrate that AMTs are found in both sexes, take a variety of forms, occur across taxa, affect patterns of mating, fertilization, and parental care,

and can involve discrete variation in behavior, physiology, and morphology.

Why Do Alternative Mating Tactics Interest Evolutionary Biologists?

First, the maintenance of genetic variation that underlies phenotypic variation is necessary for evolution by natural selection. Without such variation, evolution will not occur; understanding how and when variation is maintained remains an open question in evolutionary biology. Second, we learn more about how selection shapes reproductive behaviors and mating patterns by knowing how alternatives arise and affect individual fitness. For example, are some individuals territorial because they are larger and in better condition, while smaller or lower condition individuals cannot defend reproductive territories? Or, is there is a tradeoff between defending a territory and investing in some other aspect of reproduction, such as producing more gametes? Are these alternatives (e.g., being territorial with fewer gametes vs. not being territorial but making more gametes) two equally good solutions to the 'problem' of how best to reproduce? The answer to these questions informs our general understanding of selection on territorial behavior. Third, examining how the genome of a single species manages to generate strikingly different phenotypes helps us understand not only the mechanisms underlying the differences we see between species, but also what allows novel forms to arise over evolutionary time between species. Finally, in the case of alternative tactics arising from phenotypic plasticity, understanding more about how and why these alternatives exist can yield new insights into the evolution and expression of plasticity itself, a topic of general importance in biology. AMTs are therefore not only interesting in their own right, but also have the potential to yield insights into evolutionary innovations, the maintenance and production of diversity, and the mechanisms and evolutionary processes that allow and the limit the degree to which organisms respond plastically to their environment.

What Mechanisms Allow the Evolution of Alternative Mating Tactics?

As described above, AMTs can arise from a genetic polymorphism or phenotypic plasticity. While both can generate discrete AMTs, the evolutionary dynamics and predicted empirical patterns differ between these two mechanisms. This section reviews when and how these mechanisms are predicted to allow the persistence of AMTs and describes a few illustrative examples.

Discrete variation in mating patterns can exist if a genetic polymorphism (i.e., discrete genetic variation between individuals) affects which mating pattern an individual exhibits. For example, there could be a gene with two different alleles (or variants): one allele for being territorial and another for being non-territorial. Extensive theory has considered whether and when a genetic polymorphism of this kind will be maintained in a population at equilibrium (Rubenstein, 1980; Cooper and Kaplan, 1982; Maynard Smith, 1982; Gross, 1984; Shuster and Wade, 2003; Shuster, 2011). This theory predicts that the alternative tactics must have equal fitness on average for the genetic variation underlying the alternative tactics to persist. Otherwise, evolutionary theory predicts that one allele or genotype would become more common and eventually go to fixation in the population. How is a stable genetic polymorphism maintained? It is extremely unlikely that two alternatives would by chance have exactly equal fitness. Instead, negative frequency-dependent fitness is the most likely evolutionary explanation. Frequency-dependence arises when the fitness of a particular behavior or genotype is not fixed, but instead depends on the frequency of other behaviors or genotypes in the population. Negative frequency-dependence is when the relative fitness of an alternative decreases as it becomes more common (**Figure 1**). Consider the territorial/non-territorial example; it could be that when few individuals in the population are territorial that those individuals that defend a territory are able to attract more mates and raise more offspring because there is little competition for good territories. If everyone in the population is territorial, however, the cost of fighting for territories may be so high that non-territorial individuals attract more mates and have more surviving offspring. This is negative frequency-dependent fitness; the relative fitness of an alternative is higher when they are rare than when they are common (**Figure 1**). At equilibrium, however, the alternative tactics can coexist where they have equal fitness (i.e., where the two lines cross in **Figure 1**). Evidence for AMTs arising from a genetic polymorphism has been found in both sexes and in a variety of taxa from insects to birds (Neff and Svensson, 2013). While empirical examples certainly exist, it is still debated whether AMTs that arise from a

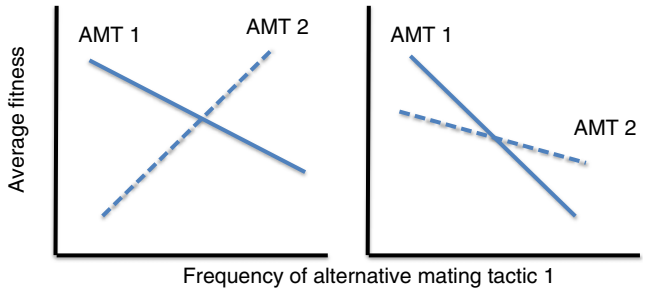

Figure 1 Negative frequency-dependent fitness: The fitness of each tactic declines with its frequency in the population. In both cases shown here, the two alternatives are predicted to occur on average at the frequency where the two fitness curves cross. Reproduced with permission from Henson, S.A., Warner, R.R., 1997. Male and female alternative reproductive behaviors in fishes: A new approach using intersexual dynamics. Annual Review of Ecology and Systematics 28, 571–592.

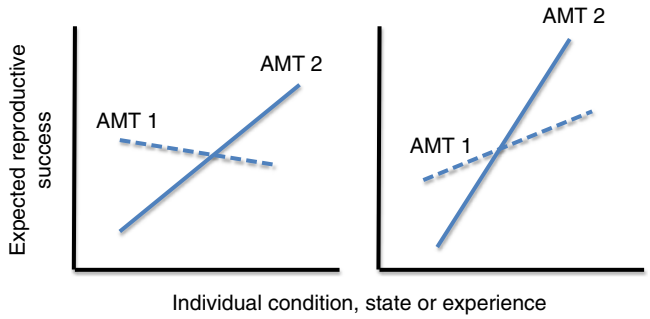

Figure 2 State-dependent fitness: The expected fitness of each tactics depends on individual condition or state (such as age, size, energy reserves, or experience). Individuals are predicted to adopt the tactics that leads to greater expected fitness for their state or condition. Reproduced with permission from Henson, S.A., Warner, R.R., 1997. Male and female alternative reproductive behaviors in fishes: A new approach using intersexual dynamics. Annual Review of Ecology and Systematics 28, 571–592.

genetic polymorphism are common in nature (Gross, 1996; Tomkins and Hazel, 2007; Oliveira *et al.*, 2008; Shuster, 2011; Neff and Svensson, 2013).

One fascinating and well-studied example of AMTs arising due to a genetic polymorphism is observed in the side-blotched lizard (*Uta stansburiana*). These lizards are found in western North America and reproduce in rocky grassland habitats (Sinervo and Lively, 1996). Males come in three discrete throat color morphs that differ not only in color but also in behavior and physiology (Sinervo and Lively, 1996; Calsbeek *et al.*, 2002). Orange males are more aggressive, defend large territories, and exhibit higher testosterone levels. Blue males defend smaller territories and are less aggressive while yellow males are non-territorial 'sneakers' (Calsbeek and Sinervo, 2002). The most likely explanation for their coexistence is negative frequency-dependent selection on a gene or gene region that codes for these differences (Sinervo and Lively, 1996; Svensson *et al.*, 2005; Bleay *et al.*, 2007). It is not only the males, however, that exhibit discrete variation in this species. Females exhibit two main throat color morphs (orange and yellow) that differ in their behavior as well as the size and number of eggs they produce (Svensson and Sinervo, 2000, 2004; Svensson *et al.*, 2001, 2002). These alternatives appear to be determined by the same gene region that affects male AMTs and selection on both male and female alternatives varies year to year. While the three male alternatives coexist in some populations, one or more AMTs have been lost in other populations (Corl *et al.*, 2010a,b).

In contrast to the relatively rare empirical evidence for AMTs arising from a genetic polymorphism, many examples exist where phenotypic plasticity appears to explain the existence of discrete ARTs within a population. One common pattern is that individuals adopt different reproductive behaviors depending on their age, condition, size, or social status (which can generally be called the individual's 'state'). For example, remember that male meerkats switch their mating and parental care behavior depending on whether they are currently subordinate or dominant in their social group (Clutton-Brock *et al.*, 2001; Russell *et al.*, 2007). And female guppies change from using mate choice copying when young to making independent mate choice decisions when older (Dugatkin and Godin, 1993). Why does this evolve? When the fitness of alternative tactics is differentially affected by the individual's state, the alternative that leads to greater reproductive success may change with individual state (**Figure 2**). If this kind of state-dependent fitness exists, it can select for phenotypic plasticity such that the alternative adopted by individuals depends on their state or experience (Gross, 1996; Alonzo and Warner, 2000c; Hazel *et al.*, 2004; Tomkins and Hazel, 2007). AMTs arising from phenotypic plasticity can take a variety of forms: individuals may switch frequently as their state changes (e.g., reversible alternatives), individuals may switch from one tactic to another over their lifetime as they grow or age (e.g., irreversible or sequential alternatives), and finally individuals may adopt only one alternative for life depending on their state at a key life history stage (e.g., fixed alternatives, Gross, 1984; Henson and Warner, 1997). These forms have the same underlying explanation but their expression and the mechanisms that control them will differ (Emlen, 2008).

In the ocellated wrasse (*Symphodus ocellatus*, a small fish found throughout the Mediterranean), three AMTs coexist: (1) Large colorful 'nesting' males build nests of algae, court females, care for eggs, and attempt to prevent mating by other males. (2) Smaller 'sneaker' males attempt to join a nesting male and female during mating, but do not court females or provide care. (3) 'Satellite' males court females, chase sneakers, and are tolerated by the nesting male, but sneak spawns at the nest and do not provide care. Females do not provide care and prefer to mate with nesting males (that provide care) but cannot avoid sneakers and satellites because fertilization is external (Alonzo *et al.*, 2000; Alonzo and Warner, 2000a,b; Alonzo, 2008). Sperm competition is intense and male types differ in the level of sperm competition they experience (sneakers the most, nesting males the least, Alonzo and Warner, 2000a). While males differ in many aspects of their behavior and physiology, we know that males can change between tactics over their lifetime. Male ARTs are determined by early differences in growth and fall along two life-history pathways (Alonzo *et al.*, 2000). Slow-growing males start reproducing as sneakers in their first year and then switch to being a satellite in their second and final year. Fast-growing males reproduce as a satellite male in their first year and switch to being a nesting male in their second and final year. While there is no evidence for a genetic polymorphism underlying these two life history pathways, it is possible that underlying

genetic variation interacts with environmental variation to generate differences in growth which then determine which of the two pathways males adopt.

Similar behavior differences exist in the Azorean Rock-pool blenny (*Parablennius parvicornis*) (Oliveira, 2001; Oliveira *et al.*, 2002; Ros *et al.*, 2006). When mating, males adopt either a nesting male, satellite or sneaker male phenotype. In this species, however, males seem to switch between AMTs over their lifetime as they become older and larger. In contrast, in many species of dung beetle (*Onthophagus* sp.) males develop either a horned (fighting) phenotype or a hornless phenotype during development (Emlen and Nijhout, 1999; Emlen, 2000, 2008; Moczek and Emlen, 2000). Although there is continuous variation in body condition during development, males with a body condition above a certain threshold develop horns with those below the threshold do not (Emlen, 1997, 2008) and the insect hormone 'juvenile hormone' seems to influence this developmental switchpoint (Nijhout and Emlen, 1998; Emlen and Nijhout, 2000). AMTs of this kind are a specific form of phenotypic plasticity, called threshold traits. There is an extensive general literature on phenotypic plasticity (extending well beyond discrete mating forms) and more specifically on the circumstances in which condition or state-dependent selection favors the kind of phenotypic plasticity that generates discrete alternative forms (Via and Lande, 1985; West-Eberhard, 1989; Scheiner, 1993; Via *et al.*, 1995; Sultan, 2000; Miner *et al.*, 2005; Ghalambor *et al.*, 2007; Roff, 2011). While this research does not necessarily focus on mating tactics, research on the evolution of phenotypic plasticity can help us understand the factors that lead to discrete alternative phenotypes in nature.

Some controversy exists regarding the best mathematical methods to use to model the evolution of phenotypic plasticity in the face of state or condition-dependent selection (Gross, 1996; Plaistow *et al.*, 2004, Tomkins and Hazel, 2007; Neff and Svensson, 2013, 2014; Buzatto *et al.*, 2014). Though the debate is methodological in focus, these differences have important implications for the kind of predictions one might test empirically (Tomkins and Hazel, 2007; Shuster, 2011). Debate also exists regarding whether plasticity or genetic polymorphisms are more likely to explain the many AMTs observed in nature (Shuster and Wade, 2003; Tomkins and Hazel, 2007; Shuster, 2011; Neff and Svensson, 2013, 2014; Buzatto *et al.*, 2014). In general, however, we know that alternatives arising from a genetic polymorphism should have equal fitness on average over time. In contrast, alternative tactics arising from phenotypic plasticity could but often will not have equal expected lifetime fitness. Given the challenges of tracking individuals over their lifetime let alone across generations in most organisms, unequivocal demonstration of either equal or unequal fitness of alternatives is most likely impossible. It is also worth noting that while we have discussed genetic polymorphisms and phenotype plasticity separately here, it is also possible that negative frequency dependent and condition-dependent selection operate in combination within single species. It is also known that phenotypic plasticity involves an underlying genetic component where the thresholds and developmental processes underlying plasticity respond to selection (Emlen, 2008) even when a genetic polymorphism does not underlie the alternatives. Therefore, AMTs, even when plastic, respond evolutionarily to selection on the underlying mechanisms that determine the degree of plasticity that exists and what form the AMTs take.

Conclusions and Future Directions

Clearly, controversy exists about some aspects of our understanding of AMTs. The existence of AMTs arising from both genetic polymorphisms and phenotypic plasticity, however, is undeniable. And these alternatives take a variety of fascinating forms in nature. We are only just beginning to understand how a single genome with either no or only small genetic differences can generate such diverse forms. While AMTs are just one specific example of how discrete variation in populations can be maintained, our understanding of them has implications for selection on mating behavior, the maintenance of diversity and the evolution and expression of novelty.

Extensive research, beyond the scope of this review, has also examined the neurological, hormonal, and physiological basis underlying AMTs. For a few species, we understand both the evolutionary processes and physiological or developmental mechanisms that generate discrete AMTs. For example, discrete throat color morphs in the size blotched lizard arise from a genetic polymorphism that initiates a cascade of hormonal, developmental, and immunological processes that shape their divergence in behavior and form. Few studies, however, integrate evolutionary and mechanistic perspectives simultaneously. Research that advances both evolutionary and mechanistic understanding of how AMTs arise within a single species has the potential to generate new insights that increase our understanding of the impressive variety of forms seen in nature as well as how novelty can arise from small change in the genome and the developmental and physiological systems it encodes. Finally, further knowledge of the evolution of the processes underlying alternative tactics will tell us what role these processes may play allowing and constraining organismal responses to a changing world.

See also: Developmental Plasticity and Phenotypic Evolution. Genetic Variation, Maintenance of. Life History Trade-offs. Mating Systems, A Brief History of. Natural Selection, Introduction to. Natural Selection, Measuring. Sperm Competition

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Maximum Likelihood Phylogenetic Inference

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Glossary

Dynamic programming A general method for solving complex problems by breaking them down into a collection of simpler subproblems that are similar in structure to the original problem.

Hessian matrix A square matrix of second order partial derivatives of a real-valued function. It is often needed within numerical optimization techniques.

Hill climbing A generic optimization strategy that relies on local searches when trying to find the global maximum of a given function. Hill-climbing procedures incrementally modify variables in an optimization problem and check to see if the modified variables have achieved a higher function value (i.e., 'climbed the hill').

Law of total probability A probability formula that states how to calculate the probability of an event given a partition of the sample space.

Metric space A set of objects (i.e., real numbers, phylogenies, etc.) equipped with a distance between any two elements.

Reversible substitution model A Markov substitution model that, if started at the equilibrium distribution, can be run backwards in time, with the resulting backward Markov model following the same probability law as the original forward model.

Simulated annealing A probabilistic method for approximating the global maximum of a given function. It is a useful technique that avoids getting stuck in local maxima.

Stationary distribution A marginal probability distribution over the states of a substitution model that can be interpreted as a long-run steady-state distribution as evolution occurs over time.

Substitution model A model that specifies probabilities of state changes for DNA (or amino acid) sequence data along the branches of a given phylogeny.

Introduction

Maximum likelihood estimation is an extremely popular statistical inference framework that is used to estimate the parameters in a probabilistic data generating model. This conceptually simple method provides parameter estimates that have good statistical properties. Before delving into maximum likelihood techniques for phylogenetic tree reconstruction, we present a simple example of maximum likelihood estimation to illustrate how this procedure works.

Suppose we have a coin that has some unknown probability p of landing on heads. We are interested in estimating this unknown probability based on the outcomes of observed coin flips. For example, one could imagine flipping this coin independently 10 times, resulting in an observed sequence of *HTTHTHTHTT*, denoted by D. The maximum likelihood principle suggests that the best guess for p is obtained by choosing p so that the probability of observing the sequence *HTTHTHTHTT* is the highest. The probability of observing the sequence *HTTHTHTHTT* from this coin is:

$$L(p) = \Pr(D;p) = p(1-p)(1-p)p(1-p)p(1-p)$$

$$p(1-p)(1-p) = p^4(1-p)^6 \qquad [1]$$

We are able to take a product of p and $1-p$ terms because of the independence of the coin flips. Equation [1] is referred to as the likelihood given parameter p. Because we are picking the value of p that makes $L(p)$ the largest, this likelihood function is treated as a function of p, not as a function of the data D. From basic calculus, we know that finding the p that maximizes a differentiable function w.r.t. p is equivalent to solving the equation $L'(p)=0$, which is:

$$2(-1+p)^5p^3(-2+5p) = 0 \qquad [2]$$

This results in three roots $p=0$, 0.4, 1. A quick inspection of the likelihood function, depicted in Figure 1, evaluated at all three roots shows that $p=0.4$ is the maximum likelihood estimate of p. This is an intuitive estimate because it is just the proportion of heads observed in the sequence *HTTHTHTHTT*. It turns out that this maximum likelihood estimator (MLE) of p tends to the true, unknown value of p as we observe more and more coin flips this is called consistency in statistics. Also, this estimator uses available data in the most optimal way, again as we observe more and more coin flips — this is called efficiency in statistics. Under fairly general and reasonable regularity conditions, consistency and efficiency hold for a large class of MLEs (van der Vaart, 1998). This easy-to-understand estimation principle along with the associated optimality properties for a wide class of likelihood models make maximum likelihood an attractive procedure for many parameter estimation problems, including the problem of estimating phylogenetic relationships from molecular sequence data.

Phylogenetic Likelihood

Likelihood Description

Throughout this presentation, we restrict our attention to DNA sequence data for simplicity (although the methods we describe are compatible with other discrete character datasets as

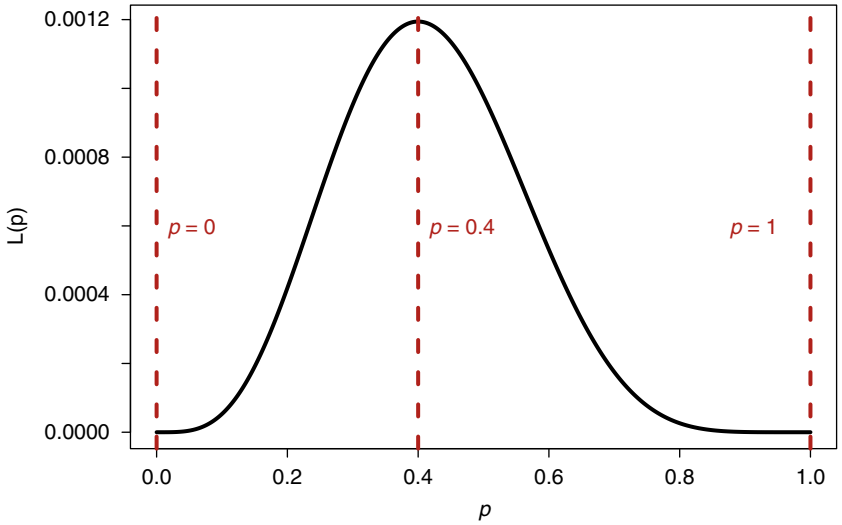


Figure 1 The likelihood of observing 4 heads in 10 coin tosses as a function of the unknown probability of heads p in a single toss. The likelihood function $p^4(1-p)^6$ is maximized at $p=0.4$, which corresponds to the observed proportion of heads. The red dashed lines represent critical points found by differentiating the likelihood function and setting it equal to 0.

well). Suppose we observe m aligned sequences of DNA (potentially corresponding to m distinct species), where each sequence has nucleotide observations recorded at n distinct sites. Gaps in the alignment are usually treated as missing data, but more accurate treatment of insertions and deletions is possible (Redelings and Suchard, 2005; Liu *et al.*, 2012). Just as we considered the likelihood of the observed coin flips as a function of the unknown parameter p, here we will examine the likelihood of the DNA sequence data as a function of the unknown tree topology and branch lengths. Given a tree topology with branch lengths, we can use a substitution model to calculate the probabilities of state changes along the branches of the tree; substitution models are described in great detail in the 'Models and Model Selection' article contained within this encyclopedia. Specifically if t denotes a branch length on a tree, substitution models allow us to calculate $p_{ij}(t)$, which denotes the probability of going from state i to state j on a branch of length t, where $i,j \in \{A,G,C,T\}$. Note that branch lengths are commonly measured in expected number of substitutions per site, not in clock time, because estimating substitution rates and branch lengths in units of clock time requires additional information about branching and/or sampling times in the phylogeny (Drummond *et al.*, 2006).

Two assumptions are made that are crucial to the rest of the analysis (Felsenstein, 2004):

1. Evolution at different sites (on a given tree) is independent.
2. Conditional on the internal node states, evolution proceeds independently on different branches of the phylogeny.

Let $L(\tau,\mathbf{t},\boldsymbol{\theta})$ be the likelihood corresponding to the $m \times n$ DNA sequence alignment matrix $\mathbf{y}$ for a given tree topology τ with branch length vector $\mathbf{t}$ and substitution model parameter vector $\boldsymbol{\theta}$. We can write the likelihood as:

$$L(\tau,\mathbf{t},\theta) = \Pr(\mathbf{y};\tau,\mathbf{t},\theta) = \prod_{i=1}^{n} P(y_i;\tau,\mathbf{t},\theta) = \prod_{i=1}^{n} L_i(\tau,\mathbf{t},\theta) \quad [3]$$

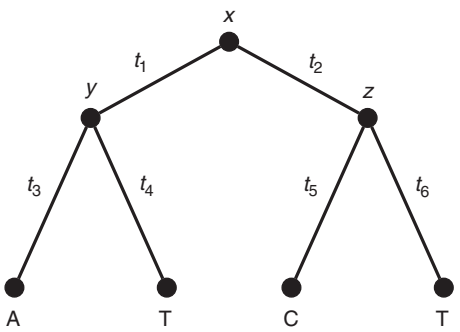


Figure 2 An example phylogenetic tree. Letters x,y,z represent the unobserved internal node states where x is associated with the root node, τ_{ex} specifies the tree topology, and $\mathbf{t}_{ex}=(t_1,t_2,\ldots,t_6)$ denotes the vector of branch lengths. Given the tree topology τ_{ex} and branch lengths $\mathbf{t}_{ex}$, we can calculate the likelihood of observing the nucleotide vector (A,T,C,T).

where γ_i is the $m \times 1$ vector of observed nucleotides at the i'th site and $L_i(\tau,\mathbf{t},\theta)$ is the site i likelihood. This factorization follows directly from the first independence assumption given above. Thus, we can find the likelihood of the whole sequence matrix by finding the likelihoods for each of the n sites. Suppose we observed the nucleotide vector (A,T,C,T) at a particular site (assuming there were only $m=4$ aligned sequences). We will use the example tree τ_{ex} given in Figure 2 to help illustrate how to calculate the likelihood at this site.

Using the tree τ_{ex}, we can deconstruct the likelihood of this nucleotide vector in the following manner:

$$\begin{aligned}\Pr(A,T,C,T;\tau_{ex},\mathbf{t}_{ex},\theta) &= \sum_x \sum_y \sum_z \Pr(A,T,C,T,x,y,z;\tau_{ex},\mathbf{t}_{ex},\theta) \\ &= \sum_x \sum_y \sum_z \pi_x p_{xy}(t_1) p_{xz}(t_2) p_{yA}(t_3) p_{yT}(t_4) p_{zC}(t_5) p_{zT}(t_6) \quad [4]\end{aligned}$$

where the summations are over the elements in $\{A,G,C,T\}$. We obtain eqn [4] by conditioning on the internal node states and

by invoking the assumption of independent evolution across branches. Note that, following common practice, we assumed that the initial distribution at the root of the phylogeny is $\boldsymbol{\pi} = (\pi_A, \pi_G, \pi_C, \pi_T)^T$, the stationary distribution of the substitution model (Page and Holmes, 2009). Looking at eqn [4], it is clear that we will need to take a sum over $4^3 = 64$ probabilities. For only $m = 4$ terminal nodes, this computation is reasonable; as m grows, the computation becomes problematic because the sum will involve 4^{m-1} terms over $m-1$ internal nodes. In the next section, we describe an algorithm that can efficiently calculate this site likelihood by eliminating redundant computations.

Likelihood Computation

First presented by Felsenstein (1973), the pruning algorithm is a standard technique used to efficiently compute phylogenetic likelihoods. This algorithm is a particular type of dynamic programming technique and takes advantage of the distributive law of algebra to achieve efficiency gains. For example, the expression $ab + ac + ad + ae$, where a, b, c, d, e are scalars, requires 7 computations (4 multiplications and 3 additions). By noting the scalar a appears in all 4 multiplications, we can use the distributive law to rewrite the above expression as $a(b + c + d + e)$, which requires 4 computations (3 additions and 1 multiplication). Thus by applying the distributive law to expressions that contain sums of product terms, like in eqn [4], we can reduce the number of computations required to evaluate them.

For the phylogenetic site likelihood given in eqn [4], we can utilize the distributive law by pushing the summations as far right as possible:

$$P(A,T,C,T|\tau_{ex},\mathbf{t}_{ex},\theta) = \sum_x \sum_y \sum_z \pi_x p_{xy}(t_1) p_{xz}(t_2) p_{yA}(t_3) p_{yT}(t_4) p_{zC}(t_5)$$

$$p_{zT}(t_6) = \sum_x \pi_x \left[\sum_y p_{xy}(t_1) p_{yA}(t_3) p_{yT}(t_4)\right]\left[\sum_z p_{xz}(t_2) p_{zC}(t_5) p_{zT}(t_6)\right] \quad [5]$$

This formulation suggests calculating $\sum_y p_{xy}(t_1) p_{yA}(t_3) p_{yT}(t_4)$ and $\sum_z p_{xz}(t_2) p_{zC}(t_5) p_{zT}(t_6)$ first, caching these intermediate results for all possible values x, and then computing the final sum over x. Note that we can visualize this procedure as traversing τ_{ex} bottom-up because the sums over y and z are evaluated before the sum over x. Instead of naively summing over $4^3 = 64$ terms, this reformulation requires a sum over $4 \times 3 = 12$ terms. For an arbitrary number of terminal nodes m, a similar reformulation will reduce the number of computations from being exponential in m to being linear in m.

The nested, bottom-up nature of the above computation leads naturally to a recursion for calculating site likelihoods. We define $L(k, i)$ to be the conditional likelihood of a subtree with root node k being in state i. Conceptually, it is the likelihood of the observed terminal nodes below node k, conditional on node k being in state i. For example, in τ_{ex}, $L(\gamma, i)$ represents the likelihood of observing (A, T) below node γ, conditional on node γ being in state i. For any internal node k (in state i) with children v,w and corresponding branch lengths t_v,t_w, Felsenstein (1973) defines the recursion to be:

$$L(k,i) = \left[\sum_{s_1 \in \{A,G,C,T\}} p_{is_1}(t_v) L(v,s_1)\right]\left[\sum_{s_2 \in \{A,G,C,T\}} p_{is_2}(t_w) L(w,s_2)\right] \quad [6]$$

for all states $i \in \{A,G,C,T\}$. This recursion uses the conditional likelihoods calculated for the children of node k to compute the conditional likelihoods for node k itself.

The derivation of the above recursion is based on the assumption of independent evolution across different lineages and the law of total probability. The former justifies decomposition of $L(k, i)$ into a product of the two terms, enclosed in square brackets, in eqn [6], where each term represents a conditional likelihood component from one of the two lineages below node k. The component from child v is computed by summing over all possible states ($s_1 \in \{A,G,C,T\}$) to which state i could have changed to and for each possible state computes the probability of changing to that state (i.e., $p_{is_1}(t_v)$) times the probability of everything that is observed below node v, given that node v's state is s_1. A similar reasoning can be used to describe the component corresponding the other child of k — node w.

To turn the recursion in eqn [6] into an algorithm, we need to initialize the recursion by defining the $L(k, i)$ values for all terminal nodes. For any given terminal node k, $L(k, i)$ is set to 1 when state i is the observed state and 0 otherwise. This initialization process can be adjusted to account for situations when data are either missing or partially observed at the terminal nodes (Felsenstein, 2004). Once all of the terminal node $L(k, i)$'s are initialized, we continue calculating conditional likelihoods for internal nodes up the tree, computing them only if the corresponding conditional likelihoods for their children have been computed. The procedure ends after calculating the conditional likelihoods at the root ($L(\text{root}, i)$, for all i); the resulting site likelihood is computed as $\sum_i \pi_i L(\text{root}, i)$, where $i \in \{A,G,C,T\}$.

Root Invariance and the Pulley Principle

Before discussing the specifics of maximum likelihood phylogeny estimation, it is essential to understand the types of phylogenetic trees that will be estimated. Even though it may seem like we are estimating rooted phylogenies, it turns out that under our assumption of substitution model reversibility and without further assumptions or external information maximum likelihood methods can only estimate unrooted trees. This result is a direct consequence of the Pulley Principle, first discussed by Felsenstein (1981). Under the assumptions of a reversible substitution model, unconstrained branch lengths, and a root nucleotide distribution at equilibrium, the Pulley Principle states that the root may be placed anywhere on the tree without affecting the likelihood. This implies that the root is unidentifiable using likelihood methods for phylogeny estimation because the likelihood is invariant to the placement of the root. In fact, we are not estimating a single rooted tree, but an equivalence class of rooted trees that corresponds to a unique unrooted tree (Felsenstein, 1981). In **Figure 3**, we present two rooted trees that lie in the same equivalence class; the unrooted tree that corresponds to this

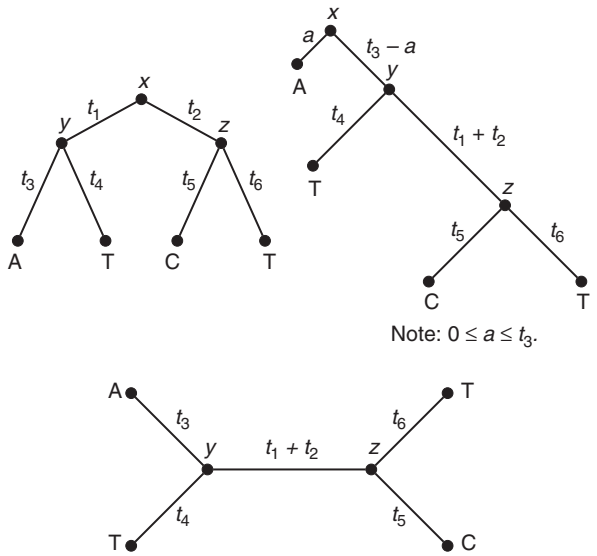


Figure 3 Three trees with equivalent likelihood values under the assumptions of the Pulley Principle. The Pulley Principle states that the root of a tree may be placed anywhere on the tree without affecting the likelihood value. The two rooted trees (top) are contained within a larger equivalence class of rooted trees that uniquely corresponds to the given unrooted tree (bottom).

equivalence class is shown below the two rooted trees. As we shift the root node associated with x, the tree topology and branch length parameters change, but the likelihood value remains the same.

Likelihood Maximization

Branch Length Optimization

Now that we have described how phylogenetic likelihoods can be computed, we will begin discussing how to maximize these functions with respect to the tree topology τ, branch lengths $\mathbf{t}$, and substitution model parameters $\boldsymbol{\theta}$. Phylogenetic maximum likelihood algorithms proceed by iterating between two major algorithmic steps: (1) for a given tree topology, find optimal branch lengths and substitution model parameters; (2) obtain a tree topology that maximizes the likelihood given branch lengths and substitution model parameters. We start with the continuous optimization problem.

The problem of optimizing the phylogenetic likelihood function, or equivalently the log-likelihood, over branch lengths and substitution model parameters falls into a class of nonlinear, non-convex optimization problems. This means that no existing optimization algorithm can guarantee to solve this problem. However, in practice, this problem can be solved by a myriad of hill-climbing optimization methods, including the Newton–Raphson method and the expectation–maximization (EM) algorithm. Although computational ingredients for the Newton–Raphson (Schadt *et al.*, 1998; Kenney and Gu, 2012) and the EM algorithm (Holmes and Rubin, 2002; Hobolth and Jensen, 2005) are available, often simpler methods are preferred. For example, many implementations of phylogenetic maximum likelihood estimation update branch lengths one at a time, rather than jointly (Guindon and Gascuel, 2003).

In addition to lack of optimization convergence guarantees, there is no theory that says that a phylogenetic likelihood will have a unique maximum, although multiple maxima are rarely seen in practice (Felsenstein, 2004). Steel (1994) provides an example that shows maximum likelihood branch lengths for a given tree are not necessarily unique. Chor *et al.* (2000) found sequence alignments that have multiple branch length optima on the same tree. In contrast, Rogers and Swofford (1999) performed numerous simulation studies and concludes that it's extremely unlikely that maximum likelihood estimation results in multiple local optima for a given phylogenetic tree. Given these intriguing results, it is not surprising that studying properties of the phylogenetic likelihood function remains an active research area.

Tree Topology Search

Because there exist finitely many tree topologies, we could, in principle, optimize branch lengths and substitution model parameters for every possible tree topology and choose the tree that had the highest likelihood value as the maximum likelihood tree. Unfortunately, the set of possible topologies is extremely large (Felsenstein, 1981) so naively searching over this tree space is computationally infeasible. Various heuristics are used to find the topology that has the highest likelihood. All of these methods use local modifications of the previously visited tree topologies to find a new tree with a higher likelihood. For example, early methods, such as the PHYLIP (Felsenstein, 1989) and PAUP* (Swofford, 2003) packages, traverse the tree topology space greedily by comparing the likelihood values between these modified trees and by choosing the topology that increases the likelihood the most; the procedure will end if there are not any trees that increase the likelihood. While this local tree search is faster than the exhaustive search over all possible trees, it is still inefficient because it has to optimize branch lengths and evaluate likelihoods for all of the rejected trees (Guindon and Gascuel, 2003).

Many other tree search heuristics have been introduced to improve upon the aforementioned hill-climbing methods. Salter and Pearl (2001) proposed a stochastic search algorithm that uses simulated annealing to move through tree space. This stochastic search was found to be faster and less likely to become trapped in local optima, when compared to PHYLIP and PAUP, for several simulated and real data examples. The improvement in speed is largely due to the fact that stochastic search algorithms gradually optimize branch lengths and other model parameters as the tree search goes on and avoid the full optimization steps used within hill-climbing methods for every candidate tree (Salter and Pearl, 2001). In addition, Guindon and Gascuel (2003) presented a simple hill-climbing algorithm that adjusts the tree topology and branch lengths simultaneously. By performing joint optimization, this procedure tends not to get stuck at local modes of the likelihood and produces extremely accurate estimates of the tree topology (Guindon and Gascuel, 2003). Although these optimization strategies work well in many practical situations, it is important to keep in mind that all local hill-climbing optimization

methods are prone to getting stuck in local maxima and may not return the true MLE phylogeny as a result.

Consistency of Maximum Likelihood Estimates

An important question of interest is whether the maximum likelihood phylogenetic estimation process is able to reconstruct the true phylogeny as we gather more and more data. More precisely, as we collect data at more and more sites for a fixed set of sequences, will the maximum likelihood phylogeny estimates tend to the true phylogenetic tree? As we briefly mentioned before, consistency holds for a wide class of MLEs under sufficiently broad regularity conditions (van der Vaart, 1998). Despite this, consistency for maximum likelihood phylogenies has been hard to establish due to the complex nature of the parameter space.

Felsenstein (1973) suggested that consistency could be proven for maximum likelihood phylogeny estimates by using a modified version of a general consistency proof found in Wald (1949), although the proof was never explicitly given. Chang (1996) presented one of the earliest proofs of consistency, but did not consider the branch length parameters in his setup. RoyChoudhury *et al.* (2015) provide a complete proof of consistency by verifying the Wald conditions in this setting; their proof is dependent on a previously constructed metric space for phylogenetic trees (Billera *et al.*, 2001). Thus, RoyChoudhury *et al.* (2015) show that consistency will hold for maximum likelihood phylogenies assuming the correct model of evolution is used. A discussion regarding phylogenetic MLE consistency under model misspecification can be found in Felsenstein (2004).

Bootstrap for Phylogenies

In the process of phylogenetic estimation, it is natural to desire an estimate of the uncertainty surrounding the reconstructed phylogeny. In this section, we explain how the nonparametric bootstrap, a general statistical technique for assessing sampling variability of an estimator (Efron, 1979), can be used to assign confidence to phylogenetic MLEs. Before getting into the details of the bootstrap procedure, it is instructive to imagine what would be a statistically ideal way to estimate phylogenetic uncertainty. Suppose we could repeatedly 'rerun' evolution along the same true phylogenetic history to obtain replicated molecular sequence alignments. Then, estimating phylogenies based on these alignments via maximum likelihood would tell us something about the sampling variability of our estimation procedure. For example, if the phylogenies obtained from the replicated reruns of evolution were nearly identical, we would conclude that our phylogenetic estimation procedure is very precise, enjoying low sampling variability. The idea behind the bootstrap is to get around rerunning evolution, which is clearly infeasible, by resampling the observed data.

Bootstrap Description

The bootstrap procedure starts by generating B replicate datasets. Each replicate dataset is obtained by sampling n alignment sites with replacement (i.e., sampling columns) from the observed alignment. Maximum likelihood estimation is then applied to each of these B bootstrapped sequence alignments, and uncertainty is assessed by summarizing the similarities between the bootstrapped phylogenies (Felsenstein, 1985). Figure 4 displays some bootstrap datasets drawn from an observed sequence alignment; note that each dataset consists of randomly sampled columns from this observed alignment. While bootstrap sampling is conceptually simple, summarizing bootstrapped trees is not an easy task so we devote the next subsection to this topic. For a more detailed account of bootstrap sampling, please see the other article contained within this encyclopedia.

Summarizing Bootstrapped Phylogenies

We are interested in understanding how to summarize the similarities among the B bootstrap trees because a high degree of similarity corresponds to a low degree of variability. For instance, if a particular tree split – a bipartition of the species set – appears in most of the bootstrap trees, then we will be more confident about that tree split being in the true phylogeny. One way to keep track of which tree splits appear most often in the bootstrap trees is to construct a majority-rule consensus tree – a tree that is constructed from tree splits that appear in a majority of the bootstrap trees. This is done by first enumerating all of the tree splits that occur on the bootstrap trees and then retaining only those splits that appear in more than 50% of the trees. We can then construct the consensus tree using this remaining collection of splits. In building the

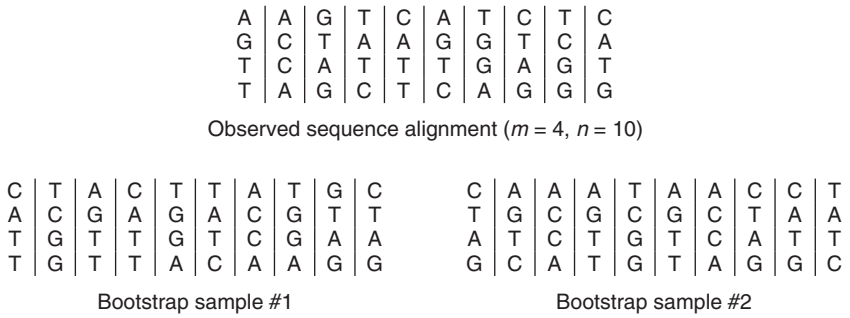
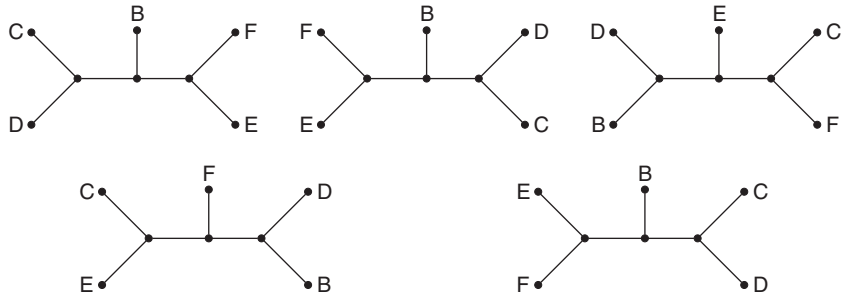


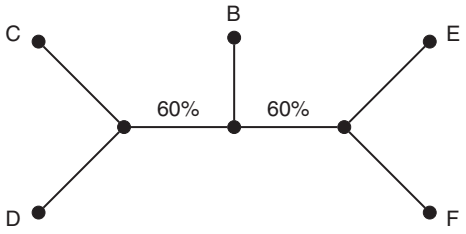
Figure 4 An example of bootstrap sampling for sequence alignment data. We obtain bootstrap datasets by randomly sampling $n=10$ sites (i.e., columns) with replacement from the observed sequence alignment (top). We provide two examples of possible bootstrap datasets (bottom).



Tree collection

Tree splits	Counts
$\{C, D\}\|\{B, E, F\}$	3
$\{B, C, D\}\|\{E, F\}$	3
$\{B, D\}\|\{C, E, F\}$	2
$\{B, D, E\}\|\{C, F\}$	1
$\{C, E\}\|\{B, D, F\}$	1

Number of appearances of each tree split



Majority-rule consensus tree

Figure 5 Consensus tree example with five species $\{B,C,D,E,F\}$. At the top of the figure, we display a sample collection of trees from which we'll build a consensus tree. A table enumerating all possible tree splits from this collection and their respective counts is given in the middle of the figure. At the bottom of the figure, we display the only consensus tree that can be constructed from the majority tree splits, which are $\{C,D\}|\{B,E,F\}$ and $\{B,C,D\}|\{E,F\}$. Note that we label each tree split with the corresponding percentage of the tree collection it appears in.

consensus tree, the procedure always avoids putting two splits that might conflict on the same tree because there will always be at least one bootstrap tree where the two splits coexist (Felsenstein, 1985). Thus, it is guaranteed that a consensus tree can be built from these tree splits. Once the tree is constructed, it is common to label each split with the percentage of bootstrap trees it appears in. This helps us understand which parts of the consensus tree we should have strong or weak confidence in. Figure 5 illustrates how this tree building process works by constructing a majority-rule consensus tree for a simple collection of trees. For a more comprehensive treatment of consensus trees, please see the other article contained within this encyclopedia.

Maximum Likelihood and More Complex Models of Evolution

So far, we have limited our discussion of phylogenetic maximum likelihood estimation to the very basic models of molecular evolution. However, assuming more complex models does not significantly change the maximum likelihood machinery. For example, it is widely recognized that when modeling evolution of molecular sequences it is important to account for a possibility of different substitution rates across sites (Yang, 1994). Therefore, most implementations of phylogenetic maximum likelihood estimation include models that deal with such rate heterogeneity. Model extensions that relax the assumption of site independence (Hobolth, 2008) and that impose constraints on substitution rates across branches of the phylogeny (Rambaut and Bromham, 1998) are also possible. Increasing model complexity may eventually lead to a situation where maximum likelihood estimation ceases to produce a unique solution, so studying identifiability of models becomes an important avenue of research (Rhodes and Sullivant, 2012).

Example: Primate Phylogeny Estimation

In this section, we present an example of maximum likelihood phylogeny estimation applied to an alignment of mitochondrial DNA sequences from seven different primate species: human, chimpanzee, bonobo, gorilla, Bornean orangutan, Sumatran orangutan, and gibbon (Yang *et al.*, 1998). The length of the alignment is 9993 sites. We are interested in understanding the ancestral relationships among these species. We use a general time-reversible substitution model, with a gamma distribution used to model rate variation across sites. After finding the maximum likelihood phylogeny estimate using the PhyML package (Guindon *et al.*, 2010), we perform

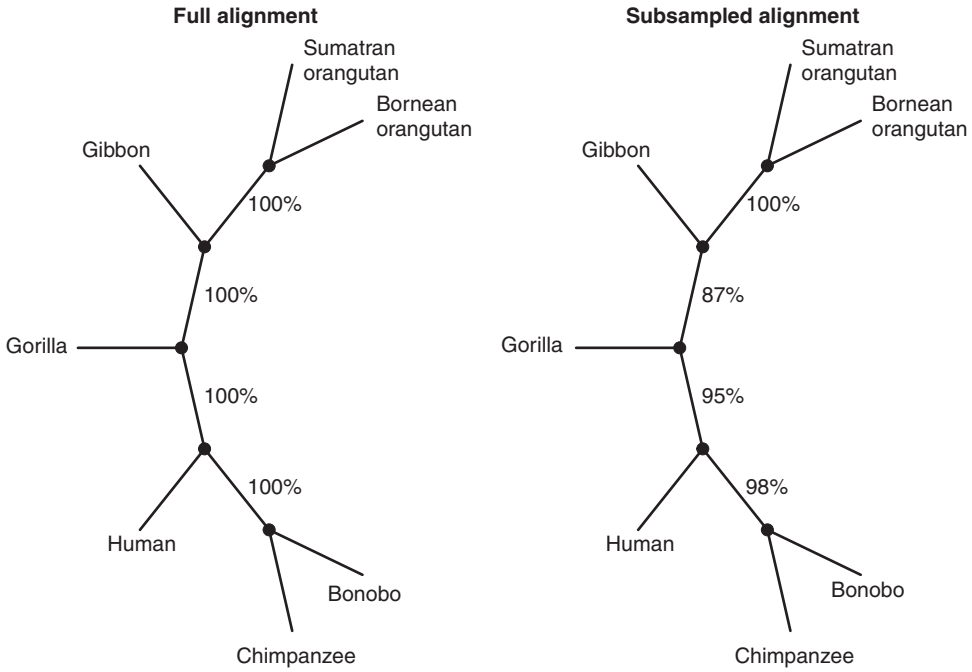


Figure 6 Maximum likelihood phylogenies with corresponding bootstrap percentages for the primate example using both the full sequence alignment and a randomly subsampled alignment containing 500 sites.

the bootstrap and obtain 1000 bootstrapped trees. We carry out this procedure on the full sequence alignment and on an alignment that we constructed by randomly subsampling 500 sites from the original alignment.

Figure 6 displays the maximum likelihood trees with corresponding labeled bootstrap percentages; note that the majority-rule consensus trees have been omitted from Figure 6 as they coincide with the maximum likelihood trees in this example. However, maximum likelihood trees and bootstrap consensus trees could be different, which usually happens when maximum likelihood phylogenetic inference is not very precise. In this example, maximum likelihood trees of both the full and subsampled alignment share the same topology and have similarly sized bootstrap percentages. However, notice that artificially reducing the size of the data yields more uncertainty reflected in lower bootstrap support of internal branches of the reconstructed phylogeny on the righthand side of Figure 6. Our analysis suggests an accepted relationship among the primates, with bonobos and chimpanzees being most closely related to humans (Mailund *et al.*, 2014).

See also: Consensus Methods, Phylogenetic. Distance-Based Phylogenetic Inference. Molecular Evolution, Models of. Phylogenetic Invariants. Support Measures, Phylogenetic Tree

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EDITOR IN CHIEF

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Richard M. Kliman, PhD, is Professor of Biological Sciences at Cedar Crest College in Allentown, Pennsylvania. He received his BA from Colby College in biology and music. His graduate work at Wesleyan University focused on quantitative genetics of circadian rhythms and photoperiodism in the Djungarian hamster, *Phodopus sungorus*. As a postdoctoral fellow at Rutgers University and Harvard University, he studied molecular evolution and population genetics. Prior to Cedar Crest College, he taught at Radford University in Virginia and Kean University in New Jersey. He has also served as a program director in the Division of Environmental Biology at the US National Science Foundation (NSF).

Kliman's research interests center on questions in molecular evolution, including the evolution of codon usage bias in a variety of organisms; speciation and natural history; and ecology and conservation. Much of this work has relied on population genetics/genomics and bioinformatics approaches. He has also collaborated with Cedar Crest colleague John Cigliano on an Earthwatch-supported "before-after-control-impact" study on the effects of a new marine reserve in Belize on queen conch populations. His research in evolutionary and ecological genetics has been supported by the US National Institutes of Health and by Conservation International.

Kliman has served on the editorial boards of *Genetica* and *The Journal of Molecular Evolution*. He has been deeply involved in evolution education, helping to coordinate "Undergraduate Diversity at SSE/SSB," an NSF-supported program to bring a diverse group of undergraduates to the annual Evolution research conference. He was a lead editor of population/quantitative genetics and evolutionary genetics for *Nature Education/Scitable* at its inception. He is a member of the Education and Outreach Committee of the Society for the Study of Evolution, and editor of the society's peer-reviewed educational resource, the *EvoEd Digital Library*.

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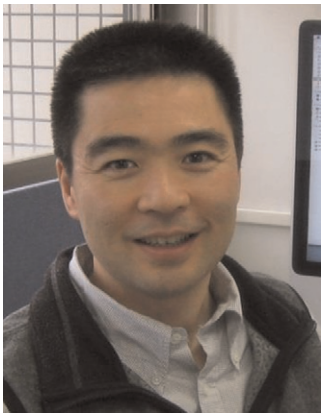


Hiroshi Akashi is a Professor of Evolutionary Genetics at the National Institute of Genetics, Japan. He worked with Marty Kreitman for his PhD in Ecology & Evolutionary Biology from the University of Chicago (1996) and with John Gillespie as a postdoctoral fellow at UC Davis. He has been a faculty member at the University of Kansas (1998–2000), Penn State University (2000–2008), and NIG (2009–present). Akashi's research focuses on inferring causes of genome evolution, especially weak selection, from within and between species sequence variation. His studies of codon usage employed population genetic methods to detect natural selection acting at its limit of efficacy and identified a phenotypic basis of natural selection (translational accuracy) from sequence comparisons in *Drosophila*. Extensions of this work revealed constraints related to biosynthesis that act globally on compositional properties of microbial proteins. The interplay of weak evolutionary forces appears to shift frequently among closely-related species and current interests include tests of adaptive changes in protein/DNA composition.

Tim Coulson's primary interest is in creating better links between the fields of ecology and evolution. He does this by developing theory, parameterising models for field and laboratory systems, making predictions from these models, and, where possible, testing these predictions with experiments. He works on a range of systems, from bulb mites within the laboratory, to guppies living in streams in Trinidad, to wolves in Yellowstone. His motivation to do this comes from observations that ecological and evolutionary change can be observed occurring on similar time scales, yet ecological theory typically ignores evolutionary processes and vice versa.

Tim was awarded his PhD in plant ecology from Imperial College, London, in 1994. He moved on to research genotype-by-environment interactions as Natural Environment Research Council (NERC)-funded post-doc at the Institute of Zoology in London. He remained at the Institute on a fellowship where he developed models to investigate the economic and life history consequences of a range of population management strategies. In 2000 he moved to the University of Cambridge, where he briefly lecturered in the Zoology department. In 2004 he moved back to Imperial College London as a senior lecturer where he started developing models that allow the simultaneous investigation of the dynamics of life history, populations, and quantitative characters. In 2007 he became Professor of Population Biology at Imperial College London. He left Imperial in 2013 to take up his current position as Professor of Zoology at the University of Oxford. He is also a Professorial fellow of Jesus College, Oxford.

Andrew Forbes

Rosemary Gillespie is a Professor at the University of California, Berkeley, where she also holds the Schlinger Chair in Systematics. She is Past President of the *International Biogeography Society* and Trustee and Fellow of the *California Academy of Sciences*, and serves as Associate Editor for *Molecular Ecology*. Gillespie was born and educated in Scotland, receiving her BSc in Zoology from Edinburgh University in 1980. She came to the US to conduct graduate work on the behavioral ecology of spiders at the University of Tennessee. After her PhD she spent several months at the University of South in Tennessee, and then started work at the University of Hawaii in 1987, initially as a postdoc, and then in 1992 as Assistant Professor in Zoology and Researcher in the Hawaiian Evolutionary Biology Program. It was during her first year in Hawaii that she discovered an adaptive radiation of *Tetragnatha* spiders. She left Hawaii in 1999 to join the faculty at the University of California in Berkeley, where she continues her research focus on the islands of the Pacific, Hawaii in particular, using islands of known age and isolation to assess the combined temporal and spatial dimension of biogeography and determine patterns of diversification, adaptive radiation, and associated community assembly.

David Guttman received his PhD from Stony Brook University in 1994 working with Daniel Dykhuizen on questions related to the role and importance of recombination in structuring genetic diversity in bacterial populations. He followed this with a postdoc in molecular evolution with Brian and Deborah Charlesworth at the University of Chicago, and a second postdoc at the University of Chicago with Jean Greenberg to gain experience in the fields of molecular plant pathology and plant-microbe interactions. He started his faculty position at the University of Toronto in 2000, and is currently a Professor in the Department of Cell & Systems Biology (CSB). He is also the Associate Chair for Research in CSB, founder and Director of the University of Toronto Centre for the Analysis of Genome Evolution & Function, and Canada Research Chair in Comparative Genomics. He has served as the Chair of the American Society for Microbiology, Division R (Evolutionary and Genomic Microbiology), and was the *PLoS Pathogens* Section Editor for Bacterial Evolution & Genomics.

Dr. Guttman runs a highly diverse research program generally focused on bacterial evolutionary genomics, with three major foci: (1) the evolution of host specificity and virulence in plant pathogenic bacteria; (2) microbial comparative genomics; and (3) studies of the human and plant-associated microbiome. He is best known for elucidating and linking evolutionary and mechanistic processes that determine the course and fate of bacterial infections, and characterizing the impact of genetic variation on the balance between disease and immunity.

Norman A. Johnson, the section editor for Applied Evolution, is an evolutionary geneticist and author. He received his PhD from the University of Rochester in 1992 and did postdoctoral research at the University of Chicago. His research interests have generally focused on aspects of speciation, specifically those related to the genetics and evolution of hybrid incompatibility: sterility, inviability, or other reduction of fitness in hybrids between species. Dr. Johnson, an adjunct professor in the Biology Department at the University of Massachusetts at Amherst, has taught classes there, as well as at Hampshire College, the University of Texas at Arlington, and the University of Chicago.

Dr. Johnson also has a long-standing commitment toward improving the communication of science in general and evolutionary biology in particular to other scientists, educators, and the public at large. He is the author of *Darwinian Detectives: Revealing the Natural History of Genes and Genomes* (Oxford University Press: 2007), a book geared to general audiences that shows how biologists use DNA sequence data to make inferences about evolutionary processes. He also was the lead organizer for a working group on communicating human evolution at the National Evolutionary Synthesis Center (NESCent).

Laura Kubatko received a PhD in Biostatistics from The Ohio State University (OSU) in 1999. After seven years on the faculty at the University of New Mexico, she returned to OSU in the Fall of 2006, and is now Professor of Statistics and of Evolution, Ecology, and Organismal Biology at OSU. Laura served as an Associate Director of the Mathematical Biosciences Institute at OSU from 2013–2015. At OSU, she is a Faculty Affiliate of the Initiative in Population Research, and a Faculty Affiliate in Translational Data Analytics (TDA@OSU). She holds appointments as an Affiliate Faculty Member at the Battelle Center for Mathematical Medicine at Nationwide Children's Hospital in Columbus and as an Adjunct Research Scientist at Lovelace Respiratory Research Institute in Albuquerque, NM. Laura's research interests are in statistical genetics, with a focus on the development of statistical methods for inferring phylogenies from molecular data. Her recent work in this area concentrates on bridging the gap between traditional phylogenetic techniques and methodology used in population genetics analyses, primarily through the application of coalescent theory to species-level phylogenetic inference. She develops and distributes several software packages for phylogenetic inference, and has been an active member of the *Society of Systematic Biologists*. She has served as an Associate Editor for the journal *Systematic Biology* since 2007.

Amy Litt has been studying plant evolution and diversity since her PhD on floral structure and evolution in the neotropical plant family Vochysiaceae, known for its beautiful but unusual flowers many of which have only one petal and one stamen. While completing her PhD in plant systematics and morphology in the joint City University of New York/New York Botanical Garden Plant Sciences program under Scott Mori and Dennis Stevenson, she became interested in the molecular basis of plant diversity. She did her post-doc in the developmental genetics lab of Vivian Irish at Yale University on the evolution of a family of transcription factors involved in flower development, and she continues to study the functional evolution of this gene family currently. After one year on the faculty of University of Alabama, she moved back to The New York Botanical Garden as Director of Plant Genomics, where she developed her research program studying the evolution of plant form along two paths: studying evolutionary changes in genes to see how those changes affected flower and fruit form; and identifying the genes that underlie differences in form among closely related species. Dr. Litt also served as a program director in Plant, Fungal, and Microbial Development and Evolutionary Development at the National Science Foundation. She recently moved to the University of California at Riverside, where she continues to study the genetic basis of plant diversity.

Maria E. Orive is a professor of evolutionary genetics in the Department of Ecology and Evolutionary Biology at the University of Kansas. Her research in theoretical population genetics aims to develop mathematical models that provide a conceptual framework for exploring important questions in evolutionary biology and analytical tools for demographic and genetic data. Her work has considered levels of selection and mutation in organisms that reproduce both sexually and asexually, the relationship of population structure and life-history attributes to gene flow and genetic diversity, and models of within- and between-host pathogen and symbiont population dynamics. Orive received her BS from Stanford University and her PhD from the University of California at Berkeley. After spending two years as a postdoctoral researcher in genetics at the University of Georgia, she was an NSF-NATO Postdoctoral Fellow at the University of Edinburgh. Her research has been funded by multiple grants from NSF and NIH. In 2007–2008, she was the Carl and Lily Pforzheimer Foundation Fellow at the Radcliffe Institute for Advanced Study (Harvard University), and has served as the University Faculty Ombudsman for the University of Kansas since 2007.

Daniel Ortiz-Barrientos is an Associate Professor in evolutionary genetics in the School of Biological Sciences at The University of Queensland, Brisbane, Australia. During his scientific career he has investigated the ecological and genetic basis of speciation both in plants and animals. His current research program explores the early stages of speciation, the molecular basis of parallel speciation, and the interplay between recombination and natural selection during the origin of new species. His research funds come from The Australian Research Council. He is married to Antonia Posada, and is the father of three energetic and beautiful kids.

Claudia Russo was born in Leeds, England, but has lived in Rio de Janeiro, Brazil since she was two years old.

Claudia has an academic major in Ecology from Universidade Federal do Rio de Janeiro completed in 1989, and finished her Master's thesis in 1991 on population genetics of two actiniid species of sea anemones with different reproductive strategies, under the supervision of Associate Professor Antonio Mateo Sole-Cava. Her PhD dissertation was on the diversification of drosophilids and on the use of a known phylogenetic tree to estimate the reliability of tree building methods. The dissertation was completed in 1995 under the supervision of the Evan Pugh Professor Masatoshi Nei who recently received the prestigious Thomas Hunt Morgan Medal. Her graduate degrees were obtained as a student at the Genetics Program from the Universidade Federal do Rio de Janeiro and as a visiting scholar at the Pennsylvania State University (1992–1995).

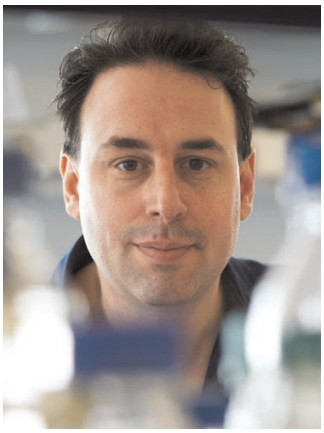
Claudia is currently the Head of the Genetics Department at the Federal University of Rio de Janeiro, having been a member since 1997. Claudia has supervised 13 Master's dissertations, eight PhD theses and seven post-docs, of which eight are now Assistant Professors at universities in Brazil and abroad. She has published 42 academic papers that have been cited over 1,200 times. Her *h-index* is 14. Since 2012, Claudia has been a member of the editorial board, and an associate editor of the *Molecular Biology and Evolution* journal. Since 2012 she has been a council member for the Pan American Association of Computational Interdisciplinary Sciences and since 2009 for the Brazilian Association for the Advancement of Science.

Claudia's general academic interests are on key aspects of animal phylogenetics, including their diversification patterns in time and space. She has worked with various metazoans groups but more prominently on marine sponges, sea anemones, arthropods, passerine birds, and mammals. Claudia has also published on the use of known phylogenetic trees to estimate the efficiency of phylogenetic methods in recovering and rooting those trees. More recently, she has developed some interesting *hands-on* educational tools for evolutionary biology practices in the classroom.

Karen E. Sears is an evolutionary developmental biologist whose primary research goal is to determine how developmental variation within a species produces congenital malformations in humans, and among species generates new evolutionary forms in mammals. Dr. Sears earned her PhD from the University of Chicago, did postdoctoral research at in the Howard Hughes Medical Institute (HHMI) lab of Dr. Lee Niswander, and joined the faculty of the University of Illinois at Urbana-Champaign. At Illinois she holds positions as an Associate Professor in the Department of Animal Biology, a Faculty Member in the Institute of Genomic Biology, and an Affiliate of the Program in Ecology, Evolution and Conservation Biology and the Department of Cell and Developmental Biology. She is also the President of the Pan American Society for Evolutionary Developmental Biology. She has authored or co-authored over 35 publications including first-authored publications in *Nature*, *Proceedings of the National Academy of Sciences*, and *Evolution*. She has served as a principal investigator on multiple, nationally-funded research projects, and presented invited seminars at more than 30 institutions and symposia. She is routinely ranked among the top 10% of Illinois professors for her teaching, and was a featured scientist in the PBS/HHMI documentary "*Your Inner Fish*."

Vassiliki "Betty" Smocovitis is Professor of the History of Science in the Department of Biology and in the Department of History at the University of Florida. Her areas of expertise include the history of evolutionary biology, genetics and systematics and the history of botany. She is best known for her contributions to understanding the historical event known as the "evolutionary synthesis" and in gaining greater understanding of the origins of the discipline of evolutionary biology. She has published extensively on both the intellectual and social aspects of the history of evolutionary biology including a history of the Society for the Study of Evolution, a history of the Darwin Centennial of 1959, and the integration of botany, genetics, and anthropology into the evolutionary synthesis. She was the contributor to the *Oxford Bibliographies* entry on Charles Darwin at over 25,000 words and the entry on the modern synthesis. She is the author of *Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology* (Princeton: Princeton University Press, 1996).

Nina Wedell is a professor of evolutionary biology with research interests focused on the evolutionary ecology of sex. She has worked extensively on various aspects of sexual selection and sexual conflict, in particular on the role of selfish genetic elements in reproductive biology. Nina is the Academic lead for the Behaviour research group at the University of Exeter.



Jason Wolf is Professor of Evolutionary Genetics in the Department of Biology & Biochemistry and The Milner Centre for Evolution at the University of Bath. His research is unified with a special focus given to understanding the influence that frequently ignored or under-appreciated sources of genetic variation have on the genotype-phenotype relationship and how this, in turn, influences evolutionary processes. He integrates theoretical, computational and empirical quantitative and population genetic techniques to achieve this goal. He is particularly interested in understanding the evolutionary consequences of various types of interactions, including gene interactions (epistasis), parent-offspring interactions and social interactions. He received a PhD from the University of Kentucky, after which he was a postdoctoral researcher at Indiana University and a US National Science Foundation Postdoctoral Fellow at Washington University School of Medicine. Prior to moving to the University of Bath he held positions at the University of Tennessee and the University of Manchester. He won the Dobzhansky Prize from the Society for the Study of Evolution, a Young Investigator's Prize from the American Society of Naturalists and the Scientific Medal from the Zoological Society of London.

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Alphabetical Contents List

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

All articles within the encyclopedia have an extensive list of cross-references which appear at the end of each article, for example:

Adaptive Mutation Controversy

> *See also*: Directional Selection and Adaptation. Microbial Experimental Evolution. Mutation, Population Genetic Models of. Natural Selection, Introduction to. Recombination in Bacterial Populations

3. Subject Classification List

A subject classification list has been developed by the editors to group articles under broad topics. This index is useful for making broad connections between articles and locating the relevant article for a topic that is covered in more than one article.

4. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

5. Contributors

A full list of contributors appears in the front mater of each volume.

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Animal Diversification

Amniotes, Diversification of
Amniotes, the Origin of
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Evolutionary Medicine IV. Evolution and Emergence of Novel Pathogens
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History of Evolutionary Biology

Life History Evolution

Microbial Evolution

Molecular and Genome Evolution

Phylogenetic Methods

Plant/Fungus Diversification

Population Genetics

Quantitative Genetics

Sex, Recombination, and Mating Systems

Speciation and Hybridization

PREFACE

The *Encyclopedia of Evolutionary Biology* was developed to provide an authoritative overview of the current state of evolutionary biology. It was an ambitious goal, especially given that the field did not pause for the two and a half years needed to complete the project. The encyclopedia's 15 section editors collaborated to ensure that content gaps were kept to a minimum, and their efforts show. When the project was completed, we had compiled 256 entries, covering a broad range of topics selected by the editors to ensure a comprehensive resource. It was a privilege to read every one of these entries, and I was truly humbled by the collective efforts of hundreds of authors to communicate the excitement and sophistication of a field of study that touches on every conceivable topic in biology today.

There are many ways to envision an encyclopedia of evolution, and we had to choose an approach that would lead to a cohesive resource. Readers will note that, in the more organismal-focused entries (edited by David Guttman, Amy Litt, and Claudia Russo), there is an emphasis on *diversification* of life. We did not set out to provide an overview of the diversity of life, as such a goal would be untenable; rather, we focused on the evolutionary processes and key events responsible for diversity. Numerous entries deal with speciation, life history evolution, evolutionary biogeography, and coevolution. These entries (edited by Daniel Ortiz-Barrientos, Tim Coulson, Rosemary Gillespie, and Andrew Forbes) bring to light how the evolution and diversification of life is intimately entwined with ecology. Of course, there is extensive coverage of population genetics, quantitative genetics, evolutionary developmental biology, the evolution of sex and mating systems, molecular/genome evolution, and phylogenetic analysis (edited by Maria Orive, Jason Wolf, Karen Sears, Nina Wedell, Hiroshi Akashi, and Laura Kubatko), all fundamental to our understanding of evolutionary processes. And as thematic bookends, several entries (edited by Betty Smocovitis and Norman Johnson) cover the history of evolutionary biology and applications of evolutionary biology.

Readers of the encyclopedia will find that entries are generally pitched at a somewhat advanced level, although with great effort by authors to make entries as accessible as possible to a broad audience. Encyclopedias, like living organisms, are compromises. If all entries could be readily understood in their entirety by first-year university students, this encyclopedia would be of limited value to experts. At the other extreme, if entries were extremely technical – and our authors were undoubtedly capable of producing such entries – the encyclopedia might be inaccessible to students. While there is, by necessity, variation among entries in this regard, we settled on a general target: the majority of an entry should be accessible to a motivated, advanced undergraduate. Readers are, of course, directed to additional resources, with authors providing bibliographies and lists of further reading.

As with any undertaking of this scale, there are many individuals who should be recognized for their roles in the development of this encyclopedia. Special thanks go to Norman Johnson for early discussions that helped us develop the general structure of the encyclopedia. The dedicated and distinguished team of section editors deserves the credit for drafting the table of contents, recruiting authors, and working extensively with authors to ensure the highest quality product. It should go without saying that the high quality of this encyclopedia ultimately reflects the efforts of the editors and authors. Finally, the project management and development teams at Academic Press were always ready to assist, and while it is not possible to name everyone who contributed to the effort, I am particularly indebted to Simon Holt, Will Bowden-Green, Paula Davies, and Justin Taylor.

Richard Kliman
Editor in Chief

FOREWORD

What is life, how did it originate, and what accounts for its great diversity? These are fundamental scientific questions that have and will always be the source of endless fascination and wonderment. Charles Darwin and Alfred Russel Wallace provided an answer to the latter question through the grand idea of evolution and the process of natural selection. Darwin also speculated on the where question of the origin of life by hypothesizing it originated long ago in a warm lagoon. Most importantly, however, Darwin shattered the notion that the natural world is static and replaced it with a biology that is dynamic and continually changing. Species are not fixed, typological entities. Rather, they are related by common descent in a great tree of life. Analogous to tracing one's ancestors back in time in a pedigree, one can climb down the tree along its branches and boughs that connect species in a hierarchy of phylogenic relationships until reaching the base of its trunk and the common ancestor of us all. One can also climb up the tree and quickly realize that evolution produces a seemingly endless array of new forms (and sometimes extinction). Thus, as the tree of life grows, populations are continuously evolving and diverging from one another, creating novel varieties and races (showing slight differences) that eventually evolve into new species (separated by distinct gaps). And natural selection – the differential survival of individuals in populations possessing heritable traits favorable for their survival and reproduction – is the primary materialistic process causing evolutionary change and the origin of new species.

The *Encyclopedia of Evolutionary Biology* chronicles our current state of understanding of the dynamics of evolution and its product, Darwin's great tree of life. A diversity of seminal topics are covered including overviews of the history of the field, the origin of life, the history of life (including the phylogentic methods used to reconstruct life's history), the myriad ways and means (including mechanisms other than natural selection) that evolution is affected, and the important roles that conflict versus cooperation, and mergers and acquisitions, occurring within across varying levels of biological organization, play in the narrative of life. In so doing, the *Encyclopedia* highlights the grandeur in Darwin's view of life. We are not separate, but rather a twig along a branch of life, a twig that has evolved the ability to comprehend the existence of and our connectedness to the tree, and climb around its branches to see what has been and think about what may come. It is a wonder of life that it can look at and understand the meaning of itself.

But Darwin's grand view has even larger ramifications, going beyond providing a materialist basis for organismal change and putting us in our place. The reality of evolution also answers the question of what life is. If pressed to define life, most of us would reply with a list of the things that living organisms do. For example, living organisms metabolize, grow, develop, move, behave, mutate (are variable), and reproduce with inheritance. One can investigate these different characteristics of life separately and discern the mechanistic basis for the different processes that constitute life – the "how" of life. And such studies represent the basis for many fields of the life sciences. However, these are only the components of life and, in isolation, produce a static view of the natural world. Rather, the seminal insight is that populations of living beings possessing these characteristics have the emergent property that they evolve. Darwin's "*On the Origin of Species*" therefore not only describes how populations evolve, and as a logical extension how new species form, but also conveys the essence of what life itself is – evolution. Thus, as Theodosius Dobzhansky famously stated "nothing makes sense except in the light of evolution." The Encyclopedia wonderfully brings this view of life to light, providing the reader with the breadth of knowledge and overview of the current state of the field of evolution needed to appreciate and participate in the next major ongoing synthesis in our understanding of life, the so-called "Omics Revolution."

The study of evolution is in an accelerated phase of discovery brought about by major technical advances in our ability to DNA sequence whole genomes (genomics), and to generate profiles of mRNA transcription (transcriptomics), protein levels and enzymatic activity (proteomics), and metabolic products (metabolomics) at varying stages in the life cycle and development of organisms. This "Omics Revolution" may not change foundational evolutionary principals, per se. Our understanding of evolution has been heightened by a series of such advances in the past, including the "Modern Synthesis" when Mendelian genetics was wedded to Darwinian thinking and the "Molecular Revolution" in which genetic technology increasingly allowed allele frequencies in natural populations to be analyzed. The Omics Revolution is an extension of these pervious advances, but one in which the workings of whole organismal systems and the composition of entire communities can be gleaned at once.

Perhaps, the most important discoveries in Omics will come from linking an understanding of the process occurring at the cellular and microevolutionary level with large scale patterns and trends at the macroevolutionary scale. Previously, processes occurring within and interactions occurring among cells could be studied in at least some detail. Omics is providing an opportunity to fully understand how all of these processes interact simultaneously to result in the development and functioning of integrated, multicellular systems of life. At the other end of the spectrum, fossils attest to the evolution of new life forms through time and the creation of great and observable morphological diversity. Genomic sequencing is providing a powerful means to help accurately place these fossils within the framework of a fully resolved molecular phylogenic tree to better understand the history of life, including major trends, themes, and variation in the tempo and mode of evolutionary change. But it is the middle of the micro and macro at the branching points in the tree of life that Omics may prove most insightful. Now it is possible to not only DNA sequence large numbers of individuals within populations, but to equate these genetic differences within and between populations to morphological, physiological, and behavioral phenotypes, and discern the developmental

and physiological mechanisms by which these genetic changes produce organismal variation, diversity, and reproductive isolation – the stuff of evolution and speciation itself. Thus, we will be able to not only understand the everyday processes responsible for how the tree of life grows, but be able to translate this into a mechanistic appreciation of how these processes result in new branches on the tree forming and others dying out, giving shape to the history of being on our planet.

The field of evolution is currently inundated with a mass of Omics data and the bottleneck is the development of bioinformatic analytical tools to edit, analyze, and interpret the results. However, it is clear that many new insights are on the horizon, and even if they do not affect the root principles of evolution, soon a deep connection of the how and why of life will emerge to help forge a truly integrative evolutionary biology: The *Encyclopedia of Evolutionary Biology* is an excellent guide to prepare readers to assimilate these new findings, keeping bioinformatics grounded in the bio and providing a valuable source for seeing the tree through the forest to understanding the grand synthesis of life that is flowering.

Jeffrey L. Feder
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†Deceased.

M

Metazoans, Origins of

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Origin of Metazoans

Metazoans are also known as animals. These are heterotrophic organisms with a multicellular organization that develop via blastula. Their cells are eukaryotic and they lack chloroplasts or walls and are organized into tissues. All metazoans probably form a monophyletic group (**Figure 1**; e.g., Wainright *et al.*, 1993; Cavalier-Smith, 1996; Borchiellini *et al.*, 1998; Philippe, 2006, 2009, 2011) with a single hypothetical last common ancestor called the Urmetazoan. It has also been suggested that the Urmetazoan was a marine organism that first appeared around 600 million years ago (Mya) (Erwin *et al.*, 2011). Nevertheless, the shape and the origin of this ancient form are yet to be ascertained, and many different theories are still being debated. One aspect concerning the origin of the metazoans is not under heavy debate; the ancestor of all animals was a protozoan (King, 2004). But, what did the ancestor look like?

A Protozoan Ancestor

When the first animals arose from protozoan ancestors, this crucial evolutionary step was a result of genomic innovation, environmental forces, natural selection, and chance (Carroll, 2001; King, 2004). In order to become multicellular, protozoans had to develop integration systems. Such systems started with the formation of molecules involved in signal transduction, adhesion, and differentiation to facilitate the communication between the cells of a multicellular organism (King, 2004).

In this sense, the advent of multicellularity would permit cell specialization and the formation of tissues that would enable the organism to perform multiple and complex tasks (Maldonado, 2014). The easiest way to enlarge your body is to increase the number of cells, a single cell has a size limit beyond which the performance of its functions would be impaired. In this sense, even the first metazoans would have been more protected against predation favoring their survival.

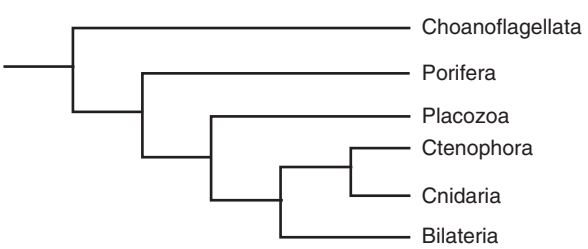

Figure 1 Tree of life showing the monophyletism of Metazoa.

The origin of the gene families involved in signaling and adhesion between cells is likely to have occurred in the first metazoans, but more recent molecular studies indicate that these gene families were already present in the protozoan ancestors. As molecular analyses strongly suggest that the choanoflagellates are the sister group of the Metazoa (Lang *et al.*, 2002; Ruiz-Trillo *et al.*, 2007, 2008; Shalchian-Tabrizi *et al.*, 2008), some of these studies also reveal the expression of genes involved in signaling in these protozoans. This finding indicates that these genes probably played a crucial role in the transition to multicellularity. But, in what context did the protozoan ancestors evolve to a multicellular life?

Another possibility is that the first metazoans appeared merging the life cycle of protozoan lineages, such as flagellates and ciliates. In this sense, flagellated and ciliated cells are not capable of division and motility at the same time. This condition might have favored mixed colonies alternating functions through the life cycle, such as the amoeboid for reproduction and the flagellated for dispersion. After some time, both cell types could have coexisted in the same colony that would be favored by natural selection and evolved to a metazoan life (Buss, 1983).

In light of this scenario, in which cell differentiation preceded the formation of colonies (Mikhailov *et al.*, 2009), the 'Synzoospore theory' (Zakhvatkin, 1949) was proposed. According to this theory, temporal cell differentiation would have preceded spatial cell differentiation in the origin of animals (Mikhailov *et al.*, 2009) and, hence, the metazoan ancestor would have been a protozoan with a complex life cycle. In time, the colony would have sedentary (or less mobile) cells capable of changing their phenotype from flagellated to amoeboid for example. The flagellated phenotype would remain attached to each other, forming the blastula (synzoospore), a primary flagellated larva (Mikhailov *et al.*, 2009; **Figure 2**). Even though it is a possible scenario, this is not the only theory for the origin of Metazoa and not even the most accepted. Traditionally, the most accepted theories are the Syncytial and the Colonial Theories.

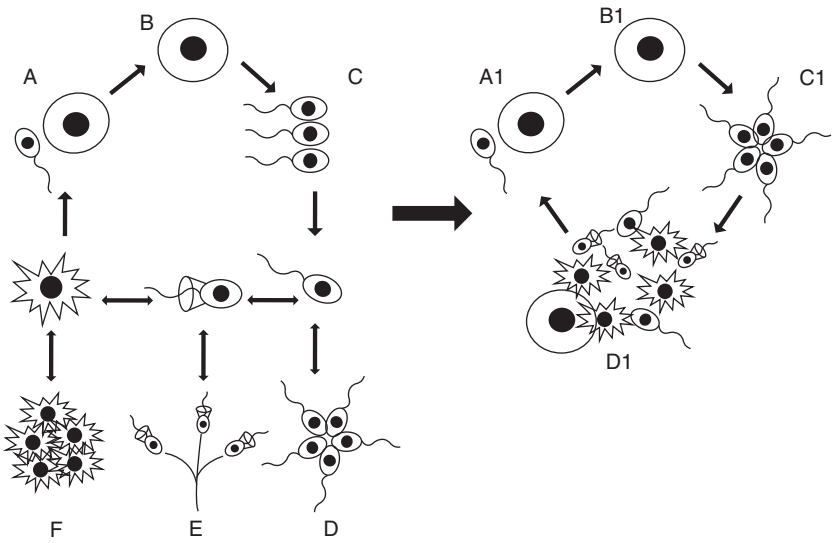

Figure 2 Synzoospore theory. A – gametes; B – zygote; C – non-feeding zoospores generated by palintomic cleavage of the zygote; D – free-living form of flagellates colony; E – sessile form of trophic flagelattes colony; F – reproductive form of amoeboid colony. A1 – gametes; B1 – zygote; C1 – synzoospore generated by palintomic cleavage of the zygote; D1 – sedentary colony with differentiated cells (trophic and reproductive cells). Redrawn from Mikhailov, K.V., Konstantinova, A.V., Nikitin, M.A., *et al.*, 2009. The origin of Metazoa: A transition from temporal to spatial cell differentiation. BioEssays 31, 758–768.

The second most accepted theory is the Syncytial Theory (Hadzi, 1953; Hanson, 1977), but it has met no strong support in current molecular analyses. The theory suggests that a multinucleate, bilaterally symmetrical, ciliate became benthic and went through cellularization, forming a cellular epithelium (**Figure 3**). This organism, that would be very similar to a flatworm (acoelomate triploblastic bilateria), would have been the ancestor of all metazoans. This would imply that simpler organisms such as sponges, ctenophores, and cnidarians would have derived from a more complex animal.

The Colonial Theory, originally proposed by Haeckel (1874), is the currently most accepted to explain the origin of metazoans. According to this theory, unicellular flagellates aggregated forming a spherical hollow colony of identical cells (the blastaea). In time, the colony went through an invagination event that produced a double layer of cells and, only then, cell differentiation started. The second stage was named gastraea by Haeckel (Mikhailov *et al.*, 2009; **Figure 4**).

Several modifications were proposed for this theory. For instance, the blastaea might have suffered ingression rather than invagination and might have been solid rather than hollow (Metchnikoff, 1883; **Figure 5**). On the other hand, Bütschli (1883) proposed the placula, a hypothetical animal that had two layers of cells and bilateral symmetry. The separation of these two layers had permitted the invagination of the ventral cells, forming the gastraea (**Figure 6**). This modification on Haeckel's original theory regarding the placula gained more strength when the *Trichoplax adhaerens* (Phylum Placozoa) was first discovered (**Figure 7**).

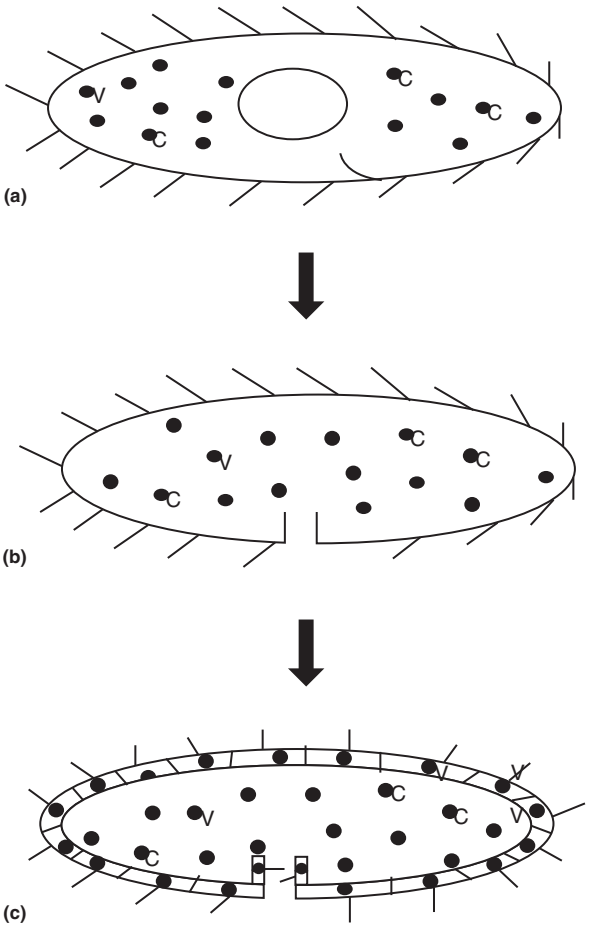

Figure 3 Syncytial theory. (a) – A multinucleate, ciliated protozoan. (b) – This protozoan became benthic and developed a ventral mouth. (c) – Cellularization resulted in the formation of an epithelium.

The Oldest Extant Metazoan

One critical step to comprehend the early diversification of metazoans is to discover which lineage branched off first in the

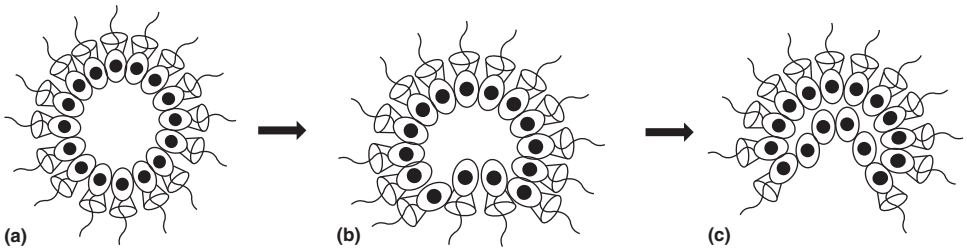

Figure 4 Gastraea theory according to Haeckel. (a) – Flagellates colony (Blastaea). (b) – Invagination of the colony. (c) – Gastrula-like animal (Gastraea).

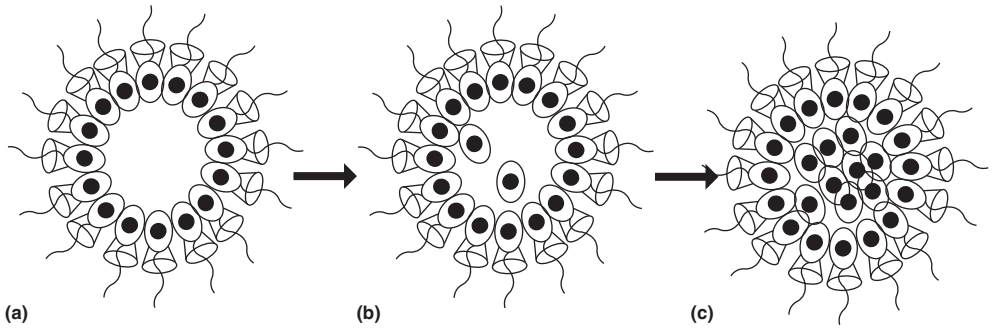

Figure 5 Gastraea theory according to Metschnikoff. (a) – Flagellates colony. (b) – Cell differentiation and with some flagellate cells become amoeboid. (c) – Ingression resulted in a hollow Gastrula-like animal (Gastraea).

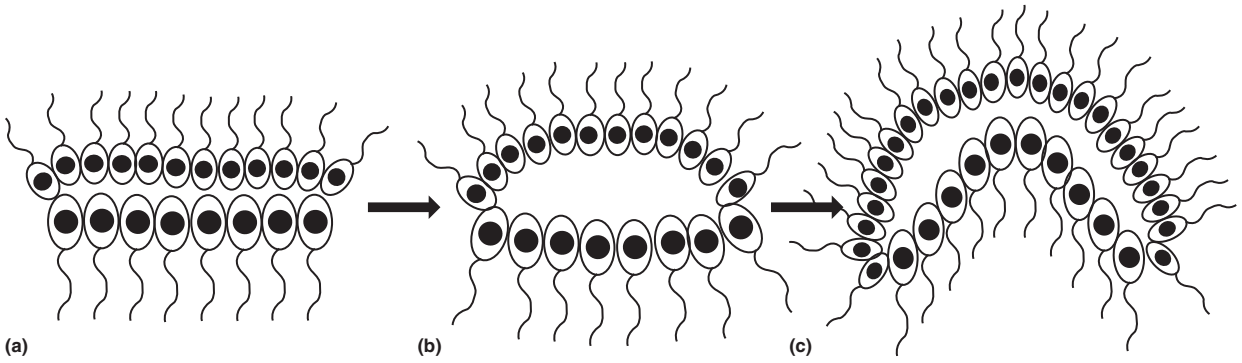

Figure 6 Placula theory. (a) – The placula, an hypothetical animal with bilateral symmetry. (b) – Separation of the two cell layers. (c) – Invagination of ventral cells (digestive chamber) resulting in the formation of the Gastraea.

Metazoan tree of life. Although most researchers agree that sponges are the sister group of other animal lineages (Philippe *et al.*, 2009; Pick *et al.*, 2010; Worheide *et al.*, 2012), other authors suggest that Placozoa (Schierwater and DeSalle, 2007; Schierwater *et al.*, 2009) or Cnidaria and Ctenophora (Dunn *et al.*, 2008; Hejnol *et al.*, 2009) is the first branched off in the metazoan radiation.

The phylogenetic position of Ctenophora (**Figure 8**) as sister group of all Metazoa was originally proposed based on molecular analyses (Dunn *et al.*, 2008). This hypothesis was first received with caution as their taxon sampling was rather low (Pick *et al.*, 2010). Improving the taxon sampling, Pick *et al.* (2010) and, later, Philippe *et al.* (2011) found Porifera and not Ctenophora branching off first. Nevertheless, a very recent analysis indicated that this conclusion may be revised soon as other datasets indicate Ctenophora as the sister to the metazoans (see Halanych, 2015).

The hypothesis of Placozoa being the sister group of all the other Metazoa was proposed based on the morphological simplicity (i.e., the lowest number of cell types – 4–5 – lack of symmetry, organs, nerve or muscle cells, basal lamina, and extracellular matrix) of these organisms (Schierwater and DeSalle, 2007), and also by a combined molecular and morphological dataset (Schierwater *et al.*, 2009). Some criticism was made to this study, leading to a reanalysis of the same dataset a new topology was obtained with sponges branching off first (Philippe *et al.*, 2011; see also Miller and Ball, 2008; Srivastava *et al.*, 2008).

Therefore, although this discussion continues, sponges (**Figure 9**) are now considered by most researchers to be the

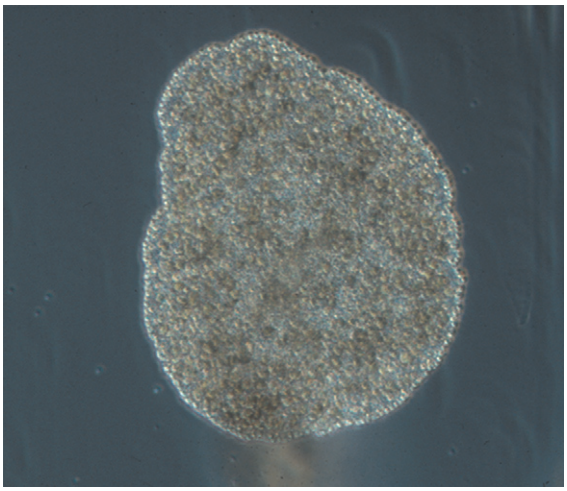

Figure 7 The Placozoa *Trichoplax adhaerens* (http://en.wikipedia.org/wiki/File:Trichoplax_adhaerens_photograph.png). Reproduced from Eitel, M., Osigus, H.-J., DeSalle, R., Schierwater, B., 2013. Global diversity of the placozoa. PLoS ONE 8 (4), e57131. doi:10.1371/journal.pone.0057131.

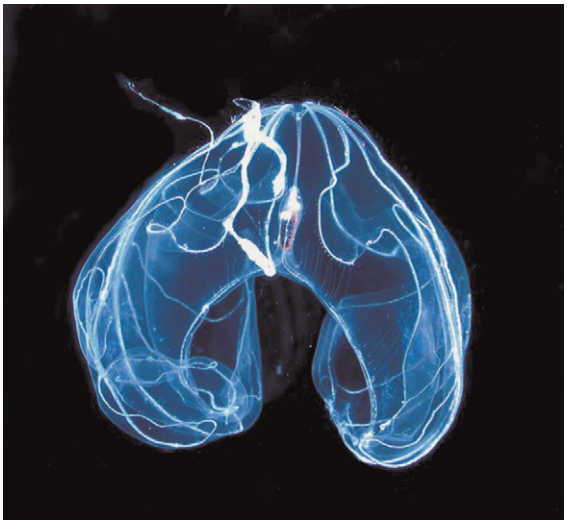

Figure 8 The Ctenophora *Bathocyroe fosteri* (http://en.wikipedia.org/wiki/File:Bathocyroe_fosteri.jpg).

oldest extant animals as most molecular analyses show Porifera as the sister group of all the other Metazoa (e.g., Philippe *et al.*, 2009; Pick *et al.*, 2010; Worheide *et al.*, 2012). Besides, they are morphologically simple and have a special cell type (the choanocyte) that is very similar to choanoflagellates. The hypothesis of choanoflagellate-like organisms being ancestors of the metazoans is related to the similarities between those protozoans and sponge choanocytes (King, 2004). Although the homology between sponge choanocytes and choanoflagellates has not yet been properly tested, DNA sequences do show that these lineages share several genes and that they are sister lineages (e.g., Wainright *et al.*, 1993; Cavalier-Smith, 1996; Philippe *et al.*, 2004). Moreover, if Ctenophora or Placozoa were the sister group of Metazoa,

Figure 9 Sponge (*Clathrina* sp.). Photo by A. Bispo.

these lineages and the ancestors of Cnidaria and Bilateria would have lost their choanocyte-like cells, which are not the most parsimonious scenario (Worheide *et al.*, 2012).

Origin of the Sponges

Sponges were most possibly originated from a benthic colony of choanoflagellate-like organisms that might have had cell types with distinct phases to feed (protochoanocytes) and to reproduce (protoarchaeocytes) (Valentine, 2004). However, to become a metazoan, sponges had to develop multicellular bodies with differentiated cell types and an extracellular matrix.

Cleavage was probably a vital step to a rapid development and to organize the differentiation and arrangement of cell lines (Valentine, 2004). Adding support to this early origin of eggs and cleaving embryos, fossils near 600 Mya have been found (Xiao and Knoll, 2000). Indeed, fossils that were originally identified as the protist group acanthomorphic (i.e., spinose) acritarchs were in fact hulls of diapause animal eggs (Yin *et al.*, 2004, 2007). This finding might suggest that sponges, and perhaps other animals, could have appeared before 580 Mya (before the Ediacaran fauna!) (Telford and Littlewood, 2009). Recently, a fossil sponge in a geological stratum of 600 Mya was reported (Yin *et al.*, 2015).

Although sponges are supposed to be morphologically simple, they have many features that are shared with metazoans. Apart from multicellularity, homebox genes, and task division, they exhibit collagen, septate junctions, integrins, fibronectins, and all apparatus to link the extracellular matrix to the cytoskeleton (Valentine, 2004). Sponges also developed cell specialization, although not rarely their cell differentiation is not final. The continuous totipotency/pluripotency of sponge cells provides these animals with a high level of plasticity that probably enabled them to survive along in the past 600 million years.

Sponges do not show nervous system or even organs. Basal lamina, a distinctive metazoan characteristic, is reported in a single out of the four extant Porifera classes. Currently, there are five recognized classes of sponges: Archaeocyatha, Calcarea, Demospongiae, Hexactinellida, and Homoscleromorpha. Archaeocyatha is a class of marine sponges with calcium

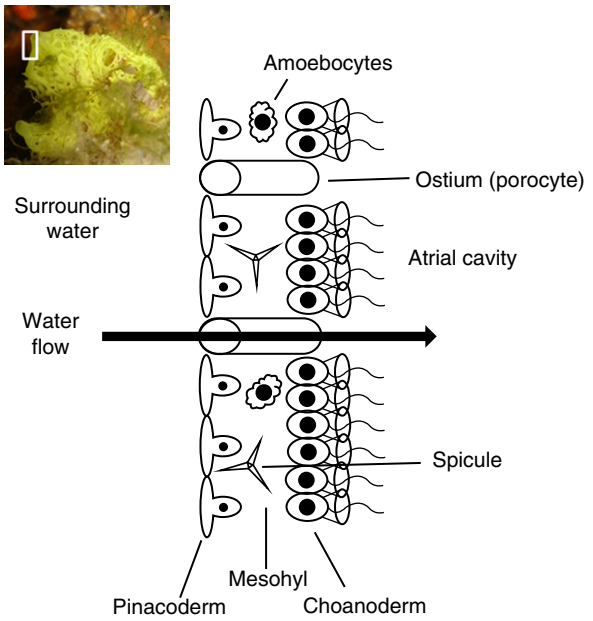

Figure 10 Schematic section of an asconoid sponge. Photo by E. Hajdu.

carbonate skeleton and that were reef builders in the Cambrian, when they became extinct. Calcarea is the only extant class that produce calcium carbonate spicules whereas the other three have silicium spicules. Demospongiae is the most speciose class, representing almost 85% of the phylum. These are the only sponges that have invaded freshwater and that developed carnivory. Hexactinellida are mainly deep-sea sponges and are the only ones that present syncytial tissues. Until recently, Homoscleromorpha was considered a subclass of Demospongiae, but it was recently elevated to a class status.

In order to grow in size, sponges developed a system of inhalant and exhalant canals and chambers with flagellate cells (choanocytes) (**Figure 10**) that continuously pump water through the body of the sponge. As the water goes through, the sponge is able to acquire oxygen and food (bacteria and macromolecules) and also to eliminate residues. These connected channels and chambers consist of the aquiferous system, the main synapomorphy of Porifera, but some carnivorous sponges lack this feature (Vacelet and Boury, 1995).

Sponges do not possess true symmetry, but they come in different forms such as spherical, cylindrical, radial, and even some present bilateral symmetry (i.e., hexactinellids) and they are morphologically very simple, as they do not have organs, or sensorial cells not even nervous system. As sponges lack nervous cells, all communication between cells is mediated possibly by chemical messengers. Additionally, sponge tissues are not considered to be true tissues, as most of the species do not have a basal lamina. The basal lamina is a membrane of proteins (laminins and collagens) working as a barrier to avoid that cells from one tissue infiltrates other tissues and, among sponges, it is encountered in Homoscleromorpha. As most sponges do not have this barrier, neither strong cell junctions to isolate their epithelia, their cells can move freely throughout the sponge body. Associated with cell totipotency, this feature makes sponges very plastic animals a key step that surely contributed for their adaptation and survival.

Although most sponges lack basal lamina, they share with other metazoans several characteristics, including multicellularity, type IV collagen, septate junctions, and work division among cells. If in one hand the monophyletic origin of Porifera has been already questioned (e.g., Lafay *et al.*, 1992; Borchiellini *et al.*, 2001; Sperling *et al.*, 2009), on the other hand studies with very large matrices of DNA sequences are showing that sponges are monophyletic (Philippe *et al.*, 2009, 2011; Pick *et al.*, 2010), but phylogenetic relationships among the classes are less clear. Many recent molecular studies have shown that Calcarea is more related to Homoscleromorpha and Demospongiae is closely related to Hexactinellida (Philippe *et al.*, 2009; Pick *et al.*, 2010), but more comprehensive studies are still required to settle this matter.

See also: Animal: What Is an Animal?. Cambrian Explosion: A Molecular Paleobiological Overview. Complexity, the Role of Oxygen in Evolution of

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Microbial Experimental Evolution

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Glossary

Alleles Forms of a gene or the products of a gene carrying different mutations.

Allozyme Allozyme is an allele of an enzyme.

Chemostat It is a device to maintain a constant growth rate of bacteria at a rate between the maximum and very slow growth.

Electrophoretic alleles They refer to alleles that run at different rates in an electrical field. All electrophoretic alleles have mutations that change an amino acid, but not all mutations that change an amino acid are electrophoretic alleles.

Enzyme kinetics V_{max} and K_m are measures of an enzyme's activity. V_{max} is the maximal velocity of a reaction given excess substrate and K_m is the concentration of substrate given half the maximal rate of reaction.

Epistasis It describes the observation that the fitness of two mutations cannot be predicted by measuring the fitness of each mutation. Sign epistasis is when the fitness of the combined mutations is less than the fitness of either mutation alone.

Growth-limiting resource It is the resource that is in short supply such that it limits growth and the organisms compete for it.

Isogenic strains Strains that are identical genetically except at specified differences.

Mutation It is a change in the order of nucleotide bases along a strand of DNA.

Population A group of individuals of a species that live in close enough proximity that they interact.

Turbidostat It is a device that maintains the growth rate of a population of bacteria at its maximum rate by measuring the turbidity of the culture.

Introduction

Experimental Evolution

Experimental evolution is the use of the experimental method to study evolution. This simple definition hides considerable complexity and ambiguity. What are the minimal conditions to call something an experiment? What would be an ideal experiment? What do we want to know in evolution that can be tested experimentally? In this short essay, these questions will be answered so the reader can understand the strengths and limitations of experimental evolution. Also this essay will focus on microbial experimental evolution. For a discussion of the current state of experimental evolution generally, see 'Experimental Evolution: concepts, methods, applications of selection experiments' (Garland and Rose, 2010). They consider anything that is experimental and dealing with any question of evolution as experimental evolution. This paper narrows the definition of experimental evolution to exclude natural experiments, directed evolution, artificial selection and domestication from my definition of experimental evolution.

Natural selection as studied in experimental evolution is different from artificial selection. In artificial selection, the scientist selects the individuals who best exemplify the desired traits and breeds from those individuals. In experimental evolution, the scientist creates the environment where natural selection happens. However, the line between artificial selection and natural selection can be fuzzy when the investigator sets an environmental condition that is simply there to select a certain defined phenotype (e.g., selection for multicellularity, see below).

Experiments

An experiment is the controlled testing of a hypothesis, using an orderly and well-described procedure. An experiment should be done in such a way that any other scientist anywhere in the world can repeat the experiment and, by using the same logical analysis of the results, obtain the same conclusion. Usually experiments are designed to provide insight into cause and effect, but also can be used to describe an array of possible outcomes from an initial condition. When possible, experimenters try to design controls such that unknown or unexpected causes, such as the phases of the moon, the micro-contaminates of the water, etc., can be ruled out as a cause of the results. Repeatability is the hallmark of the experimental method.

Comparative Method

We can talk about natural experiments, meaning similar, independent and repeated outcomes that seem to have the same precursors. The controls are assumed to be the different outcomes that are assumed to have different precursors. For example, animals that swim through water, for example, fish, whales, penguins, etc., have similar shapes and different from the shapes of animals that swim through air, for example, bats, birds, and pterosaurs. However, this is not an example of the experimental method, but of the comparative method. The comparative method has been used with great success in evolutionary biology and should continue to be used regularly. The experimental method has been used very little in evolutionary biology. This is due to a number of reasons, some important and some trivial.

Experimental Method

The experimental method requires that the investigator create the conditions under which the experiment is carried out in such a way that the experiment can be repeated. This is usually done in a laboratory where conditions are not natural – constant temperature, regular light intensity, defined media without the mix of enzyme inhibitors, toxins, tannins, and other non-metabolizable compounds found in nature. Also, generally there are no predators or parasites in the laboratory along with the organisms studied. In such unnatural conditions, what can you learn about the evolution in the world out there? The traditional answer is: 'Not much.' In one respect this answer is correct but in another respect, incorrect. It is correct in that experimental evolution will tell us little about the evolution of a particular phenotype of a species in nature. But it is incorrect in that experimental evolution can be used to understand fundamental patterns in nature. Further, we will never really understand ecology and evolution without doing experiments.

It has been argued that experiments in physiology, biochemistry, and genetics can be carried out in the laboratory because the physiological, biochemical, or genetic system is not changed when pulled out of its natural environment. The DNA is the same whether it is in an organism in nature or in the laboratory. But, one cannot bring a piece of the environment into the laboratory and have it remain the same. The environment is too complex and too much of a network of cause and effect to do this. If you take a sample of a pond in a jar, the sample in that jar very quickly changes such that the dynamics in the jar become different than the dynamics in the lake. The conclusion is that the environment cannot be moved into the laboratory the way an organism can.

While true, this is an incorrect way to look at the problem. The environment in the laboratory does not need to be natural. When we measure the properties of enzymes, we measure the V_{max} and k_m in dilute salt solutions with excess substrate, an enzyme environment very different from its environment in a cell. The environment of an enzyme in a cell is almost a gel of other compounds, not a dilute salt solution, and contains little substrate, rather than an excess. But yet these parameters of V_{max} and k_m can predict the fitness ranking of allozymes in their natural environment of the cell (Dykhuizen and Dean, 1990). Thus, we believe that characterizing enzymes in dilute salt solutions with excess substrate gives us real information about enzyme function in its natural state. Likewise, we can study organisms in very unnatural conditions and learn about the process of evolution.

Controls in Short-Term Experiments

A second reason that experiments have been little used in evolutionary biology is that it is really easy to do dumb experiments. Let us pick some arbitrary environments and random genetic differences and study the differences in fitness. There will be results, but what do they mean? Very little! Instead, one has to start with a theory that can be experimentally tested: for example, different electrophoretic alleles of 6-phosphogluconate dehydrogenase (6PGD) from *E. coli* are selectively equivalent. Then the experiments have to be very carefully designed with all the proper controls. Controls! Controls! Do the proper controls so that the results can be correctly interpreted. As Michael Travisano (2010) has written:

> Unfortunately experimental evolution studies are prone to faulty analysis, largely because investigators assume they have greater control of the experimental conditions than they actually achieve. Responses to selection are determined by the actual selective conditions, not the selective conditions supposed by the experimenter. (p. 127)

The importance of controls, is illustrated by the experiment done to show that the electrophoretic variants of 6PGD are selectively neutral or nearly neutral (Dykhuizen and Hartl, 1980). First, strains have to be constructed such that different alleles are in isogenic backgrounds along with a wild-type allele and a mutation that renders 6PGD inactive (a null mutation). Then the environments are constructed. One environment is minimal media (media without any toxins, inhibitors etc.) with growth-limiting amounts of gluconate (the selective media). Another environment (the control environment) is minimal media with growth-limiting amounts of another sugar (in this case ribose + succinate to mimic the products of gluconate metabolism). It is assumed ribose and succinate can be metabolized without using the enzyme 6PGD. This assumption needs to be tested. The bacteria are not grown just in the media suspended in space, but the media has to be in something, a chemostat, a 96-well plate, an agar plate, etc. These containers create part of the environment in which the *E. coli* grows. In this case, the bacteria are grown in chemostats because, in the chemostat, the cells are always competing for the growth-limiting resource. This should promote and magnify any selective differences between isogenic strains that contain different alleles of 6PGD. With this experimental framework established, controls are done to show there is selection in the gluconate-limiting environment but none in the control environment. These are the system controls. When a wild-type strain is competed against a strain with null mutation, the selection in the selective medium will be maximal. This selection should be large, showing that 6PGD is an important enzyme in the metabolism of gluconate, but the selection need not be 100%. In the control media, the two strains should be selectively equivalent, since the control media has been designed such that 6PGD is not required for growth. If not, first remake the strains because, by chance, one might have picked up a mutation in the background during the strain construction. If the selective difference remains, pick a different control media, until one is found that the null and wild type are selectively equivalent. Now that the selective and control environments are determined, each different allele is competed against an isogenic standard strain in both control and selective media. In the control media, the strains should be selectively equivalent; otherwise there are background differences. These background effects will prevent assigning any fitness differences in the selective media to the allelic variant of 6pgd. Lastly, each allele is competed against the wild type in selective media to see if they are selectively equivalent or not. When this experiment was done on four different alleles, three were equivalent and one was selected against, with a limit of detectability of selection of 0.2%. The enzyme kinetics gave the

same result – the k_m of the allele selected against was significantly higher than the k_m of the other alleles. This allele was also the least frequent (0.011) in a sample of *E. coli* from nature (Dykhuizen and Hartl, 1980). Thus, we can conclude that most electophoretically distinguishable alleles are selectively equivalent, but that some are not. For a summary of the results of many different experiments using a number of different genes, and a discussion of the interpretation of these experiments, see Hartl and Dykhuizen (1985). This result was important in establishing the idea that many electrophoretic variants are selectively neutral, but some are nearly neutral, supporting the theories of Kimura and Ohta (see Kimura, 1983).

Long-Term Experimental Evolution

While the experiment described above had definite predictions with the required repetition and controls, there is another approach that is often used in experimental evolution. This approach starts with a number of identical populations in the same environment and follow the fitness trajectory. Richard Lenski and his collaborators have done the classic experiment of this type (Wiser *et al.*, 2013). They evolved 12 populations of *E. coli* for over 50 000 generations in glucose minimal media in flasks, diluting daily 1/100 into fresh media. The listing of the environmental conditions is important because we do not yet know the generality of the results for different conditions. This experiment has told us that identical cultures evolve generally the same, but with significant differences and that the rate of fitness increase slows over time.

The observed results of this experiment are about what was expected. The true value of this experiment is as a resource to test various hypotheses. Samples from this experiment were frozen over time giving a record of the evolution dynamics. Previous to the start of this long-term experiment, Lin Chao had shown that a mutator gene is selected in chemostats by raising the mutation rate of advantageous mutations. Then by hitchhiking, these mutators increase in frequency as the advantageous mutations are selected and increase in frequency. However, the mutator strain will decrease in frequency if the mutator strain is at such low numbers that it is unlikely that a single advantageous mutation will happen within it (Chao and Cox, 1983). What happens to new mutations that increase the mutation rate in these long-term experiments? Are they lost because they are so rare or do they get fixed in the population because of increased frequency of advantageous mutations? Using 10 000-generation strains, Sniegowski *et al.* (1997) showed that 3 of the 12 populations are fixed for mutators. By going back into the frozen cultures, the three mutators were fixed at about 3500, 8600, and 2500 generations. Another two cultures had picked up mutators by 40 000 generations at about 18 000 generations and at 26 500 generations (Kussell, 2013). As the fitness increase slows in these populations, the fixation of mutators also slows. This result shows that while mutations to strains with elevated mutation rates are common, most strains with mutators are lost because of rarity, but sometimes, by chance, they become common enough that the selection for higher mutation rates fix these mutator strains.

The short-term experiments described above that showed the selective equivalence of the 6GPD alleles and the density-dependent selection for the mutator gene are what have been described as bottom-up experiments while the long-term experiments are top-down. In the bottom-up experiments, various alleles are competed against each other to see which one is selected and by how much. Bottom-up experiments have the advantage that they can directly test hypotheses, but it is not clear that the alleles chosen would be selected if one ran a top-down, long-term experiment under the same conditions. The top-down experiments allow evolution of the whole system, but it is hard to test well-defined hypotheses. Dykhuizen and Dean (2010) describe a bottom-up analysis of the lactose system in *E. coli*, and Travisano (2010) discusses long-term experiments: the need for them and their successes and challenges.

Evolution

Microevolution

Experimental evolution has been most useful when studying the evolutionary change in populations, using either top-down (long-term) or bottom-up (short-term) experiments. Evolution can be studied either as a change in a trait (phenotypic change) or as a change in gene frequencies (genotypic change). I shall first discuss an example of experiments selecting for a trait change and then discuss the examples associated with gene frequency change.

Phenotype Change

When selecting for a novel trait in long-term experiments, the environment and experimental procedure have to be constructed in such a way that the trait should be selected. If a particular experiment is unsuccessful and the trait is not selected, the failure may be because the environment was wrong, the genetic potentiality was not there, or the experiment did not run long enough. It is very hard to run controls, so only successful experiments are usually reported. One of the successful experiments is the selection for multicellularity. Ratcliff *et al.* (2012) selected multicellular yeast colonies from unicellular yeast by selecting for cells that settled most quickly. These colonies evolved specified apoptosis such that colonies split more or less equally. Using *Chlamydomonas reinhardtii*, Ratcliff *et al.* (2013) repeated the experiment. Rather than getting many lineages that evolved multicellularity as with the yeast, only one out of twenty cultures evolved multicellularity. This single lineage had a complex life cycle, with single-cell dispersal when the environment was flush (right after transfer to fresh media) and then growth to large clusters at the end of the growth phase when the selection for rapid settling was applied. The complexity of the life cycle suggests that this life cycle had evolved in some distant ancestral species and then suppressed in *C. reinhardtii*, rather than have evolved during the experiment. If multicellularity had been strongly selected against in a recent ancestor of *C. reinhardtii*, a strong block on the development of multicellularity would have evolved and many genetic steps would be required to break the block.

The evolution of aerobic utilization of citrate in one of the Lenski long-term lines has been described as an example of the

evolution of novelty. However, the genetic analysis of this evolutionary dynamic shows that a number of genetic changes were required before this cit+ strain could dominate the culture (Blount *et al.*, 2012), suggesting that the ancestor had metabolized citrate aerobically, but this ability had been suppressed very effectively. Thus, one has to be careful not to think that the evolution of a new function as the evolution of novelty. It could be an example of reverse evolution!

Gene Frequency Change

Mutation, genetic drift, migration, and natural selection are the forces that change gene frequency. In microbial experimental evolution, population size is usually large enough that genetic drift is unimportant. However, in very small populations with a high mutation rate, detrimental mutations can become fixed, decreasing the fitness of the population. If this continues to happen, generation after generation, it is theoretically possible that multiple detrimental mutations are fixed until the population becomes unviable. This idea is called Muller's ratchet (Muller, 1964). When an RNA virus is passaged through a single plaque, the fitness decreases (Chao, 1990), showing that Muller's ratchet can effect populations. But as fitness decreases, compensatory mutations that increase fitness increase (Poon and Chao, 2005), reversing the effects of Muller's ratchet. Thus, it is extremely unlikely that Muller's ratchet will continue until the population becomes unviable.

Migration

Normally migration is called contamination in experimental evolution and is actively blocked through all the protocols of sterile technique. However, to study the evolutionary dynamics of metapopulations, migration should be included in the experimental protocol. It is required when extinction is likely within any single population. For example, a metapopulation was created by distributing the pathogen, phage T7, and the host, *E. coli*, across a number of wells in a 96-well plate (Kerr *et al.*, 2006). In some wells, both the phage and the host will be present. In these wells, the pathogen numbers will increase as the host becomes extinct. In some wells with only host, the host population will prosper. In wells with only pathogen, there will be no growth of the pathogen. Regularly, an inoculum from each well of the plate is diluted into a well in the same position of a fresh plate. If there is no migration, the phage will be diluted to extinction and only the wells with bacteria alone will have a continuous population. With migration, sometimes phage will be added to wells containing a thriving bacterial population, causing extinction of the bacteria, but an increase in the numbers of phage. Also, sometimes bacteria will be added to empty wells establishing new populations of the host. Depending on migration distance and rate, various stable arrays of populations can be established (Kerr *et al.*, 2006).

Mutation

Mutations continuously happen. They cannot be avoided, but fortunately happen at a low frequency. All the long-term experiments rely on advantageous mutations to evolve traits or increase fitness. The short-term experiments that measure fitness are limited to about 100 generations because of the accumulation of advantageous mutations rising to high frequency.

The properties of mutation accumulation are non-intuitive in large microbial populations. The numbers used in this calculation are from experiments using *E. coli* growing in chemostats. There are 3×10^9 bacteria per culture and the per nucleotide mutation rate for *E. coli* is about 10^{-10}. This means that about one-third of the nucleotides are mutated in at least one cell in the population every generation. Thus, in a few generations all single nucleotide mutational changes will be present in the population. There will also be insertion–deletion mutations happening at about the same rate. Even if advantageous mutations are rare, they will be present in the culture from the start of the experiment.

Evolutionary biologists have assumed that evolution is mutation limited. However, in microbial populations, advantageous mutations are common. This is a very different evolutionary model than one derived from animals and plants where population size is much smaller.

Natural Selection

The usual definition of natural selection is the differential survival and reproduction of different phenotypes, when at least some of the difference in phenotypes is generated by differences in genotype. This definition is incomplete because phenotypes are generated by genotypes in an environment (the epigenetic environment) and natural selection is generated by differences in phenotypes in an environment (the selective environment). As seen in **Figure 1**, the interaction of genetic variation, epigenetic environment, phenotypic variation, and the selective environment generate natural selection. These are the 'causes' of natural selection. The 'effects' of natural selection produce changes in allele frequencies giving rise to adaptive evolution. I think that the most important function of experimental evolution will be to figure out the causal rules or laws of natural selection. I have previously made the analogy of natural selection evolution with force in physics (Dykhuizen, 1995). Newton described the effects of force; the understanding of the causes of force were done over the next 300 years leading to an understanding of electromagnetism, thermodynamics, atomic energy, etc. This understanding has led to most of the practical applications from physics. Hopefully, the same can be done for natural selection as has been done for force.

The study of causes of natural selection will be an area of evolution where the experimental method is essential. An example of an experimental study of natural selection is discussed in the section above, 'Controls in Short-Term Experiments.'

The Environment

The environment for natural selection is not just out there in nature, but also in the laboratory. We are just beginning to realize that there are major differences between the commonly

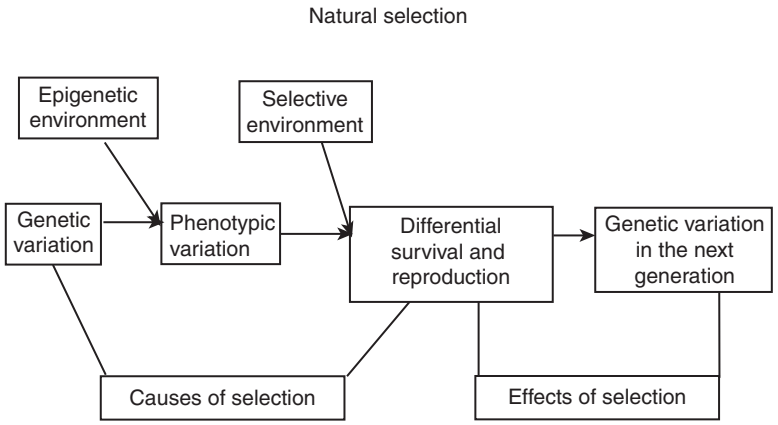

Figure 1 The current model of natural selection indicating the complexity of its causes and distinguishing the causes from the effects. Population genetics studies the effects of natural selection.

used environments in experimental evolution. The major environments are agar plates, tubes or flasks containing media, and chemostats. The agar plates can be a defined minimal media of salts and a sugar plus other defined nutrients, as an amino acid or vitamin or the agar plates can be a complex media made of yeast extract and tryptone or other semi-defined components. These different plates will undoubtedly give different epigenetic and selective environments. However, all plate environments have fixed neighbors. The interactions will primarily be with a few neighboring clones and this is very different than the selection in tubes or chemostats, which are well mixed. For example, a strain that produces an expensive-to-make toxin can invade a population of sensitive competitors on agar plates, but not in liquid, because the toxin concentration around a single colony on an agar plate will be high enough to kill the neighboring cells, but in liquid media the toxin will be diluted and not effective (Chao and Levin, 1981).

The tubes or flasks, and now 96-well plates, when used as growth chambers, can be shaken or not. If they are shaken, the environment will be homogeneous. If they are not shaken, the environment can be partitioned into different populations under different selective pressures (Rainy and Travisano, 1998). The media can be a simple defined media or a broth culture. While this has not been investigated, it is expected that the broth culture would provide many more niches, leading to more specialization and thus maintaining more genetic diversity. Normally, a simple defined media is used for experiments. The Lenski long-term lines (Wiser *et al.*, 2013) use a defined media, pH controlled by a phosphate buffer, with limited glucose (energy-carbon source). The cultures are diluted every day, meaning they go through a cycle of a lag after dilution, exponential growth until the glucose runs out, and then stationary phase until the next dilution. While most of the selection is to increase the maximal growth rate, some of the selection is to decrease the lag and some for the competition for limiting glucose as they go into stationary phase. Thus, the selection in this type of experiment does not have a single cause. One can isolate these components to select purely for maximal growth rate using the turbidostat or competitive ability using a chemostat.

A turbidostat is a continuous culture device that measures the turbidity in a culture by passing light through the culture and recording the turbidity. If the turbidity is too high, the dilution rate of the culture is increased, washing out cells faster than they can grow; if it is too low, the dilution rate is decreased, washing out cells slower than they can grow. In this way a turbidostat maintains a constant culture density while the cells are growing at their maximal rate in whatever environment used (media, temperature, etc.). Since the cells are growing at their maximal rate, any mutation that increases the maximal rate of growth would be selected. Even though in theory turbidostats sound like an ideal machines to carry out evolution experiments, trying to run one is hellish. Turbidostats are highly unstable because of the positive feedback in the selection for wall growth. You can go home in the evening thinking everything is OK and come back in the morning and the culture has been washed out because the growth on the wall has become thick enough to register as high turbidity, causing the pump to speed up in the futile effort to reduce the turbidity. A wall-less turbidostat or an Evolugator solves this problem (de Crécy *et al.*, 2007). They (de Crécy *et al.*, 2007) increased the maximal growth rate of a strain lacking elongation factor *P* threefold over 200 generations.

A chemostat is another continuous culture device where fresh media is added at a dilution rate less than would be required for the maximal growth rate and exhausted media and cells removed at the same rate. The dilution rate is set by the investigator, giving growth rates near the maximal rate to a rate just above stationary. For *E. coli*, which has a maximal growth rate in minimal media of about 1 h, chemostats are usually run at growth rates 2–6 h. One essential nutrient is added to the media at a concentration much lower than required, such that this nutrient is the nutrient first exhausted during growth. This is the limiting nutrient. If glucose is the limited nutrient, the chemostat is said to be 'glucose-limited.' The theory and use of chemostats in studying natural selection and adaptive evolution is given in Dykhuizen (1993). The selection in chemostats is for the efficient uptake and use of the limiting nutrient and for the deactivation of the global regulator for the transition into stationary phase (Gresham and Hong, 2015).

The environment can be made more complicated by using two limiting nutrients. These can be mixed or cycled. They can be substitutable or not. Sublethal concentrations of antibiotics could be added. All kinds of complicated environments could be used in experimental evolution. However, these environmental complications should be used only when the hypotheses to be tested require them. Complications for the sake of complication should not be done. But, still, environmental complications in experimental evolution will be needed to understand the complicated environment in nature.

Future of Experimental Evolution

Clearly, the major strength of experimental evolution is use of the experimental method. However, to utilize the experimental method at full strength, a complete array of controls is required. Even if a full array of controls are used, the conclusions could apply only to the particular organisms and environments used in the experiment. Is the strain used typical of the class of organisms considered (i.e., is strain K12 typical of *E. coli*? Or of bacteria? Or of life?) or is it some kind of outlier? Likewise, is the environment typical or not? Since organisms are a product of the historical process of evolution, there are no universal constants and the generality of any conclusion has to be determined by further experiments.

Experiments, where controls are not present, particularly long-term experiments, can be set up such that one can compare present results with past results. For example, if the experiment is to be run with succinate as the carbon source, a control with glucose should be run to check that all the other variables that might be different in this experiment from experiments run elsewhere by others with different strains of bacteria are not creating the difference between your experiments with succinate and the previous experiments with glucose. Only then can you say that succinate is a different resource than glucose. And only then will experimental evolution have a future.

The perceived greatest weakness of experimental evolution may be its greatest strength. The reason that it is so very hard to know how to argue that a constructed environment is meaningful is that the structure of the environment is very poorly understood. Our theory and, thus, our intuition may be wrong. For example, existing ecological theory says that two strains cannot coexist on two fluctuating resources, even when one is fitter on one resource and the other on the other, given that the selection is not frequency dependent. The strain with the highest average fitness over both environments will win. This is an example of the competitive exclusion principle and has many scientists wondering why there are so many coexisting species. As Yi and Dean (2013) have shown, using experimental evolution, the above theory is true only if the population growth is density independent, but if population growth has periods of density dependence, the two populations can coexist.

Experimental evolution gives us a way of seeing the environment as the organism sees the environment by following their evolution. Environments where the organisms select for different arrays of mutations are different. Environments that select for the same or very similar arrays of mutations are the same. The array of mutations selected in any one environment will tell us the changes in physiology arising in this environment.

At present, I do not see the limitations of experimental evolution. It will change ecology and evolution in the same way that applying the experimental method starting with Galileo changed physics.

See also: Directional Selection and Adaptation. Mutation, Population Genetic Models of. Natural Selection, Introduction to

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Microbiome

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Glossary

Alpha diversity The diversity within a single biological specimen.
Amplicon A specific region of DNA amplified by the polymerase chain reaction (PCR).
Ancestral state reconstruction The process of inferring the ancestral states of extinct genomes or organisms using a phylogenetic tree.
Archaea The 'third domain' of life, lacking nuclei like the bacteria but more closely related to eukaryotes than to bacteria.
Bacteria A monophyletic group characterized by cells that lack nuclei, for example, *Escherichia coli*.
Beta diversity The dissimilarity in communities between a pair of samples, or the change in communities over a gradient.
C4 plants Plants such as corn and other grasses that assimilate carbon dioxide via a 4-carbon intermediate metabolite rather than a 3-carbon intermediate, as is more characteristic.
Chitinase An enzyme that digests chitin, a structural long-chain polymer found in insects and fungi.
Distance metric A method of measuring dissimilarity that meets several mathematical criteria (always nonnegative), distance from A to B same as B to A, and satisfying the 'triangle inequality' such that there can be no point C where the distance from A to B is longer than the distance from A to C to B.
Endemism Specificity to a particular environment.
Endosymbionts Microbes that live inside a host cell and contribute to their mutual benefit.
Eukaryotes A monophyletic group characterized by cells with nuclei, for example, human cells.
Foregut fermentation A physiological process where the front part of the gut is expanded to digest plants.
Hindgut fermentation A physiological process where the back part of the gut is expanded to digest plants.
Horizontal gene transfer The process by which genes can move from one strain to another (contrast with vertical transmission).
Microbiome The collection of genes within a microbiota.
Microbiota The collection of microbial cells that inhabit a specific organism, or a specific body site within that organism.
Myrmecophages Animals that eat ants and termites.
Shotgun metagenomics The practice of fragmenting all DNA in a biological specimen, sequencing it, and piecing it back together to draw conclusions about the organisms that live in the community.
UniFrac A method of measuring the distance between two communities in terms of the difference in coverage over a phylogenetic tree.
Vertical transmission Transmission of genetic material from parent to offspring.

Introduction

Host-associated microbial communities have received considerable attention recently, primarily because advances in DNA sequencing have made them far easier to access than was previously possible. Technically, the microbiota consist of all the microbes in a given body habitat; their genes comprise the microbiome. Because DNA sequences suitable for amplicon sequences are approximately 1 million times cheaper to acquire than they were 10–15 years ago, the capacity to ask evolutionary questions about microbiota and microbiomes has expanded considerably (Gonzalez and Knight, 2012). However, most taxa have still not been investigated systematically, and the process of studying coevolution of the whole microbiome with its host has only just begun. Although the 'microbiota' and 'microbiome' are often used to refer just to the bacterial members of a microbial community, which are easier to study due to the better state of the relevant reference databases and computational tools, and the distribution and evolution of marker genes, these terms should properly refer to all microbes, including archaea, microbial eukaryotes (including fungi), and, under some definitions, viruses.

How Do We Find Out About Microbiomes?

Three main strategies have been used to find out about host-associated microbes. The first strategy is to study individual microbial genomes. This has been especially effective in arthropod-associated microbial communities, as many arthropods have endosymbionts or intracellular pathogens such as *Wolbachia* (Werren *et al.*, 2008) or very simple gut microbial communities that can consist of as few as one species (Moran *et al.*, 2008), vertically transmitted. Building phylogenetic trees of host and microbial genomes (or marker genes taken from these genomes) can be illuminating for testing the degree of vertical transmission; the ability to sequence the complete microbial genome can then be useful for determining the ecological relationship between microbe and host, for example, the microbe may derive sugars and other nutrients from the host diet, and synthesize otherwise unattainable amino acids and enzyme cofactors (Moran *et al.*, 2008). The second strategy is to use a phylogenetic marker, such as the 16S rRNA (Pace, 1997), to obtain a readout of the microbial community in terms of which taxa are present. Because microbial communities are often very dissimilar from

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one another, even the coarse-grained phylogenies obtainable using a small fragment of the 16S rRNA gene are often usable for this purpose, especially if the 16S rRNA fragment chosen is carefully considered (Liu *et al.*, 2007; Wang *et al.*, 2007; Soergel *et al.*, 2012). The third strategy is to perform shotgun metagenomics, in which all the DNA in a given sample is fragmented into small pieces and sequenced (Rondon *et al.*, 2000). The sequences so obtained can either be analyzed individually, by matching them to a reference database, or can be assembled into longer fragments. The advantage to shotgun metagenomics is that this technique can in principle recover the gene functions, and hence the repertoire of possible functionality, from a microbial community; this is especially important when key functional genes are horizontally transferred, or gained or lost, between closely related microbial strains. Although horizontal gene transfer can confuse the relationship between gene trees and species trees, in general horizontal gene transfers occur among arbitrary pairs of taxa and therefore do not obscure the overall phylogenetic signal. In other words, although genes are sometimes horizontally transferred (sometimes extensively: of the genes in the first three genomes of *Escherichia coli* to be sequenced, only 40% were in all three genomes (Welch *et al.*, 2002)), because the pattern is not consistent in terms of which genes or which taxa are transferred, overall phylogeny is a good guide to gene content (Konstantinidis and Tiedje, 2005). In fact, this property can be exploited to predict the metagenome from the taxon content, sometimes with high accuracy. The general strategy is to collect a list of genes in each known genome, use ancestral state reconstruction to predict what the genes would have been in unknown genomes, and then match the taxa detected in a sample (e.g., by 16S rRNA amplicon sequencing) back to these genomes. For well-characterized samples, such as those from the human gut microbiome where an intensive effort has taken place to obtain new reference strains, this technique can predict the metagenome with over 80% accuracy from far cheaper amplicon data (Langille *et al.*, 2013).

Several data analysis techniques have been especially effective in understanding complex microbial community data, and scale to the larger numbers of samples that it is possible to process today. Taxonomic inventories, essentially stacked bar charts or heat maps showing the relative abundance of each taxon (at some level, often phylum or genus level) provide a rapid way of comparing samples in terms of their overall composition (an equivalent technique for gene functions is also useful for shotgun metagenomic data). Alpha diversity measures, which essentially ask how many kinds of organisms (at some taxonomic level) are within a sample, sometimes taking the evenness of representation of the different taxa into account, are useful for getting a sense of whether samples differ in overall diversity. Beta diversity measures, which essentially ask how dissimilar the communities are in two different communities, are useful for getting a sense of which factors structure changes in microbial communities overall. UniFrac, a phylogenetic measure of beta diversity that takes evolutionary history into account in comparing two samples, has been especially effective in revealing biological patterns among communities (Lozupone and Knight, 2005), although many different beta diversity measures with varying strengths and weaknesses, and different theoretical motivations, are available (Kuczynski *et al.*, 2010).

What Differentiates Host-Associated Microbiomes from Environmental Samples?

Host-associated microbiomes, and especially the vertebrate gut, are very different from microbial communities in free-living environments such as water, soil, sediment, etc. In general communities in the vertebrate gut tend to consist primarily of microbes in the phyla Firmicutes and Bacteroidetes, with contributions from many other minor phyla including Proteobacteria, Verrucomicrobia, Fusobacteria, Actinobacteria, etc. In general, free-living microbial communities tend to have a much greater diversity at the phylum level but less at finer phylogenetic levels: this has been compared to a 'palm tree' structure for the vertebrate gut, versus an 'oak tree' structure for free-living environments (Dethlefsen *et al.*, 2007). Communities such as the skin and the mouth tend to bridge the gut to free-living environments, with sharing of Proteobacteria, Actinobacteria, and/or Spirochetes with free-living samples and Firmicutes and Bacteroidetes with the gut (Ley *et al.*, 2008). The level of microbial endemism is still a matter of controversy as it is still difficult and expensive to recover complete genomes from environmental samples, although this is rapidly improving with deeper shotgun metagenomic sequencing and improved algorithms to recover longer genomic fragments from environmental samples, including human samples (Sharon *et al.*, 2013). For example, the same strain of *E. coli* might be recovered from spinach, the human gut, and water; in contrast, even closely related species of insects may have distinct microbial inhabitants. Projects such as the Earth Microbiome Project (Gilbert *et al.*, 2010), by processing a large number of samples with common techniques, are seeking to address the issue of the general distribution of microbes across environmental samples. Interestingly, at the whole-community level, host-associated communities and especially the vertebrate gut are highly distinct from free-living communities.

What Factors Shape Microbiomes Across Different Animal Species?

Most metazoa are relatively poorly characterized in terms of their microbiota (exceptions include ants and termites, which have been studied extensively to study coevolution and for biofuels, respectively). However, some general principles are emerging. Microbes associated with invertebrates tend to resemble environmental samples, for example, microbes associated with the earthworm gut tend to resemble soils, and those associated with sponges and corals tend to resemble seawater, at least in terms of their overall composition (Ley *et al.*, 2008). Differences in the gut microbial communities in different mammalian species are dominated by diet; diet and lineage (which is often confounded with diet) have far stronger effects on the overall composition and structure of the gut microbiome than other factors such as geographical region, wild, or zoo specimen, etc. Interestingly, foregut and hindgut fermentation, each of which have independently evolved many times in different mammalian lineages as ancestrally carnivorous lineages switched to herbivory with the rise of the C4 grasslands about 20 million years ago, have

distinct and reproducible gut microbiota signatures. However, most microbes are unique to a given species, with relatively few taxa shared even among different members of the same species (Ley *et al.*, 2008). Intriguingly, this observation extends to skin microbes: even amphibians from the same pond pick up different, species-characteristic microbes from the same milieu, suggesting that host factors are critical for colonization (McKenzie *et al.*, 2012). One exception to the general pattern of diet effects is the bears, where differentiation from an ancestral omnivorous bear lineage into species that are strict folivores (pandas), strict carnivores (polar bears), and a range of diets in between still result in clustering by lineage rather than diet (Ley *et al.*, 2008). Current thinking in the field is that 5 million years may be insufficient time to differentiate the microbiota in the characteristic fashion seen on longer timescales, although detailed follow-ups in other taxa where lifestyle switches of this type appear are really required in order to answer this type of question regarding evolutionary timescale. Of note, however, humans eating diverse calorie-restricted diets tended to resemble the standard human microbiome rather than the microbomes of different nonhuman mammals. Interestingly, on the scale of different mammalian orders, there is a statistically significant but small influence of co-phylogeny on the overall microbial community (Ley *et al.*, 2008), but on smaller timescales, such as within the Great Apes, such co-phylogeny can be established (Ochman *et al.*, 2010; Moeller *et al.*, 2014), suggesting that co-phylogenetic signal may be lost as time passes. Moving from taxon inventories to gene inventories using shotgun metagenomics (Muegge *et al.*, 2011), we see a striking concordance between the taxonomic community structure and the collections of gene functions within each species, and a substantial core of metabolic functions shared among most microbiomes. However, in some cases, the directionality of key metabolic pathways (such as the connection between glutamine biosynthesis and the TCA cycle) run in opposite directions in carnivores and herbivores, underscoring the importance of checking the directionality as well as the abundance of genes in each key metabolic pathway. Interestingly, taxa with unusual diets, such as myrmecophages (ant- and termite-eaters), seem to converge in their gut microbial community metabolism, for example, by increasing the abundance of chitinases that can break down insect exoskeletons (Delsuc *et al.*, 2014). Confirming the general extent of convergent evolution in the microbiome during adaptation to different diets and lifestyles remains an exciting direction for investigation.

What Factors Shape the Microbiome Within a Species?

By far the best-studied microbiome is that of our own species, *Homo sapiens*, in part due to a $173-million investment by NIH in the Human Microbiome Project (Gevers *et al.*, 2012). The Human Microbiome Project primarily focused on characterization of a 250-member healthy cohort, sampled at up to 3 timepoints and at 18 locations on the body, using both 16S rRNA amplicon profiling (to reveal the members of the microbial communities at each site) and shotgun metagenomics (to identify gene functions) (Human Microbiome Project, 2012a, 2012b). Together with earlier efforts (Costello *et al.*, 2009; Grice *et al.*, 2009), this work provided the first overall picture of microbial distributions across the human body. One surprise was the high level of differentiation between human body sites, even compared to different free-living microbial communities. For example, even though they are topologically connected, the human mouth and gut share almost none of their microbes, and the differences in overall community structure between different human body sites are comparable to the differences between communities in different habitats, for example, soil and seawater (Ley *et al.*, 2008). A second surprise was the variation in microbial communities among different individuals. Remarkably, even within a body site, diversity between different subjects was very high: at the phylum level, some people had as much as 90% Bacteroidetes, whereas others had fewer than 10%, with many individual genera showing similarly broad ranges. Consequently, although human microbiomes resemble one another (for a given body site) more than they resemble the microbiomes of other species, they are still remarkably heterogeneous. In fact, even estimates of the 'core microbiome' typically suggest that the most dominant species in some humans are still as rare as 1 cell in 10 000 in others (Qin *et al.*, 2010). The relative importance of different sources of variation remain sources of considerable debate, as most attention has focused on proving that some factor is statistically significantly associated with differences in the microbiome, rather than using consistent methods to reveal the relative importance of different factors. Some of the variables that have been linked to the human gut microbiome with large effect sizes include age (especially the first three years of life, where the differences within the gut of a single individual over time are comparable to the differences among different species, or even different body sites) (Palmer *et al.*, 2007; Koenig *et al.*, 2011; Yatsunenko *et al.*, 2012), population (although typically location is confounded with many other factors, including diet and host genetics) (Yatsunenko *et al.*, 2012), use of antibiotics (Dethlefsen *et al.*, 2008; Ubeda *et al.*, 2010; Dethlefsen and Relman, 2011), and long-term diet (Ley *et al.*, 2006; Wu *et al.*, 2011). In humans, short-term diet typically has relatively little effect (Wu *et al.*, 2011), although extreme short-term diets such as all meat, eggs, and cheese can induce large shifts in just one day (David *et al.*, 2014). In contrast, in laboratory mice, many different manipulations can modify the microbiome drastically in a single day (Crawford *et al.*, 2009; Turnbaugh *et al.*, 2009). Similarly, in mice, many single-gene knockouts have been shown to have large effects on the microbiome, yet in humans genetic effects on the microbiome have been surprisingly elusive: only studies with hundreds of twin pairs and focusing on subsets of the microbiome have been able to find genetic effects (Goodrich *et al.*, 2014), whereas smaller studies have found no significant effect on the microbiome overall (Turnbaugh *et al.*, 2009) or only weak effects (Yatsunenko *et al.*, 2012). Interestingly, the strongest heritable effect in humans is in Christensenella, which causes increases in adiposity when experimentally transmitted to mice (Goodrich *et al.*, 2014). Relatively few studies have directly compared genetic and diet effects in mice, but the evidence to date suggests that host genetic effects on the microbiome are relatively small compared to dietary effects (Parks *et al.*, 2013). Understanding

these principles over evolutionary time is likely to be especially interesting, although preliminary indications from studies with wild species such as sticklebacks suggest that interactions such as diet × sex may be important for structuring the microbiome and its physiological correlates (Bolnick *et al.*, 2014).

Outlook for Evolutionary Biologists Interested in the Microbiome

The improved capacity for understanding the microbiome through better DNA sequencing methods and computational tools opens up many exciting possibilities for evolutionary biologists. The microbiomes associated with most body habitats in most taxa have not been characterized at all, and understanding the complex phylogenetic relationships of host and microbe holds considerable promise for understanding the microbiome, which can contain the majority of genes and the majority of cells in what we think of as a physiological organism. Understanding patterns of horizontal gene transfer, especially among microbial species exposed to a wide range of hosts and physiological environment, holds considerable theoretical importance for understanding its contribution to adaptation, as well as practical importance in the case of antibiotic resistance and the evolution of pathogenicity islands. Techniques such as genome-wide mutagenesis provide the capacity to understand which genes are critical for a given strain to establish in an ecological niche, including a complex ecosystem in which these genes must interact with genes housed in other microbes. Understanding how the microbiome ties into evolutionary ecology, from pathogen resistance to maintenance of sexually selected signals such as coat or feather condition to responses to stress, is very much an emerging direction. Modeling the dynamics of complex microbiomes, and extending models designed for a few species to hundreds or, thousands, remains both a practical and theoretical challenge, as does defining the repertoire of possible microbiome states and the trajectories that link them. Consequently, although our knowledge of microbiomes has expanded dramatically over the past decade, there is still much to discover.

See also: Bacterial Diversity, Introduction to. Evolutionary Medicine I. An Overview and Applications to Cancer. Mutualism, the Evolutionary Ecology of

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Mitochondrial and Nuclear Genome Coevolution

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Glossary

Co-regulation Correlation between the regulation of biological factors in either level of action, such as transcription, translation, or posttranscriptional/translational regulatory processes.

Cybrids These cytoplasmic hybrids (cybrids) are cultured cells, in which the original mitochondrial DNA was removed, and were re-populated using mitochondria (harboring their own mitochondrial DNA) from a different donor cell line. This can be performed when the donor and recipient cells belong to the same species, and to a certain degree between different species. Cybrid cells enable testing the functional effects of various mitochondrial genetic backgrounds in the same nuclear genetic background.

Dobzhansky–Muller model of speciation A hypothesis raised independently by two renowned geneticists, Theodosius Dobzhansky and Hermann Joseph Muller, during the first half of the twentieth century. This hypothesis suggests that interfering with interactions (genetic or physical) between two functionally important factors could create genetic incompatibilities, hybrid breakdown, and lead to speciation events.

Epistasis Genetic interactions between biological factors.

Haplogroup Group of phylogenetically related haplotypes.

Haplotype A combination of alleles from linked polymorphic sites (on the same chromosomal region).

Isoforms Proteins that share a function but are encoded by different genes (frequently ancient duplicates) and often differ in sequence.

Mitophagy The selective removal of mitochondria via a multi-component mechanism. This mechanism is thought to sense reduced mitochondrial membrane potential, remove dysfunctional mitochondria from the cellular mitochondrial network via mitochondrial fission, and in turn recycle their components.

mtDNA The mitochondrial genome.

Negative selection An evolutionary force acting to preserve the structure and function of biological factors (purifying selection).

Positive selection An evolutionary force acting to generate changes in the structure and function of biological factors as part of adaptive processes.

Introduction

The mitochondrion is the only organelle in animal cells with its own genome (the mtDNA) and is thought to have originated from an ancient alpha-proteobacterium (Lane and Martin, 2010; Gray, 2012). However, the mtDNA of higher eukaryotes harbors only a minority of the genes required for the operation of the mitochondria. As an example, the mtDNA in vertebrate animals has just 37 genes (see section Primer on Mitochondrial Genetics and Genomics), while the remaining genes required for mitochondrial function ($N \sim 1500$) are encoded by the nucleus. The latter are translated by the cytoplasmic ribosomes and, in turn, delivered into the mitochondrion via mitochondrial import machineries.

From where did these nuclear genes originate? One possibility is that during the course of evolution many nuclear genes acquired their mitochondrial functions subsequent to the origin of the mitochondrion. Indeed, many enzymes (such as uracil-DNA glycosylase, PEPCK, aconitase, and malate dehydrogenase) have cytosolic and mitochondrial isoforms (Ballard and Hanson, 1967; Domena and Mosbaugh, 1985; Yogev and Pines, 2011; Carrie and Small, 2013), or several variants resulting from alternative splicing (Kravchenko *et al.*, 2005; Kim and Gladyshev, 2006), suggesting ancient gene duplications and subsequent acquisition of a mitochondrial function. A non-mutually exclusive alternative is that genes with mitochondrial functions were once encoded by the genome of the mitochondrial bacterial predecessor, and were gradually transferred to the eukaryotic host nucleus during the course of evolution. The more than two billion years since the occurrence of the symbiotic event that gave rise to all Eukaryotes (Gray, 2012) is more than enough time for both processes to have occurred in tandem.

Two mitochondrial machineries are comprised of both mtDNA and nuclear DNA-encoded factors. These include the oxidative phosphorylation ATP production system (OXPHOS) and the mitochondrial-specific protein translational machinery (**Figure 1**). Additionally, both mtDNA replication and transcription of mtDNA-encoded genes are mediated by nuclear DNA-encoded proteins that bind mtDNA recognition sites (**Figure 2**; Bestwick and Shadel, 2013; She *et al.*, 2011). This bi-genomic cooperation introduces three problems: (1) whereas mtDNA-encoded factors are already within the mitochondria, nuclear DNA-encoded factors have to be imported into the organelle; (2) each cell has multiple individual mitochondria (~ 1000 per human somatic cell), each requiring the correct quantity of nuclear DNA-encoded factors; (3) in animals, the mtDNA evolves an order of magnitude faster than the nuclear genome. The solution to this third problem is mediated by natural selection: coevolution between factors encoded by the mtDNA and the nuclear DNA is necessary to preserve mitochondrial function. This process, and its consequences for cells, is the focus of this article.

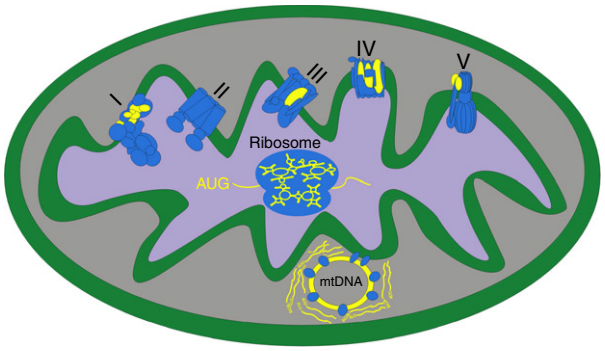

Figure 1 Bi-genomic systems within human mitochondria – an illustration of a single mitochondrion. Shown are the five oxidative phosphorylation complexes, the mitochondrial ribosome – representing the mitochondrial translation system and the mtDNA with its adjacent proteins and RNA products – representing the replication and transcription systems. Blue objects – nuclear DNA-encoded proteins that are imported into the mitochondria. Yellow objects – mitochondrial 'native' elements including the mtDNA, mitochondrial RNAs, and the mtDNA-encoded oxidative phosphorylation complexes' subunits I–V: OXPHOS protein complexes. The physical location of the mtDNA and 'cloud' of synthesized mRNA is based on our interpretation of recent high-resolution microscopic investigation of the mitochondrial nucleoid, i.e., the mtDNA-protein structure that undergoes replication and transcription (Brown *et al.*, 2011).

Primer on Mitochondrial Genetics and Genomics

The mitochondrion is the major source of cellular energy (Wallace, 2007). The vast majority of eukaryotic cells cannot survive without mitochondria and the mitochondrion cannot survive independently of its host cell. Most of the genes ($N \sim 1500$) required for the various activities of the mitochondria (such as apoptosis, nucleotide biosynthesis, fatty acids metabolism, the metabolism of iron and copper etc.) are encoded by the nuclear genome, whereas the mitochondrial energy producing system – oxidative phosphorylation (OXPHOS), and the mitochondrial translation machinery are encoded by both the nuclear and mitochondrial genomes (reviewed in Ryan and Hoogenraad, 2007). The small, circular mtDNA encodes for 37 genes in vertebrates (**Figure 2**): (1) 13 protein-coding genes, comprising members of four out of the five multi-subunit OXPHOS protein complexes. These include 7 protein subunits (ND1-6, ND4L) of NADH ubiquinone oxidoreductase (OXPHOS complex I), one subunit (cytochrome *b*) of cytochrome bc1 (OXPHOS complex III), three subunits (COI-III) of cytochrome *c* oxidase (OXPHOS complex IV) and two subunits (ATP6,8) of F1-F0 ATP synthase (OXPHOS complex V); (2) Two ribosomal RNA genes (*12SrRNA* and *16SrRNA*), which are components of the mitochondrial ribosome; and (3) 22 *tRNA* genes. These 37

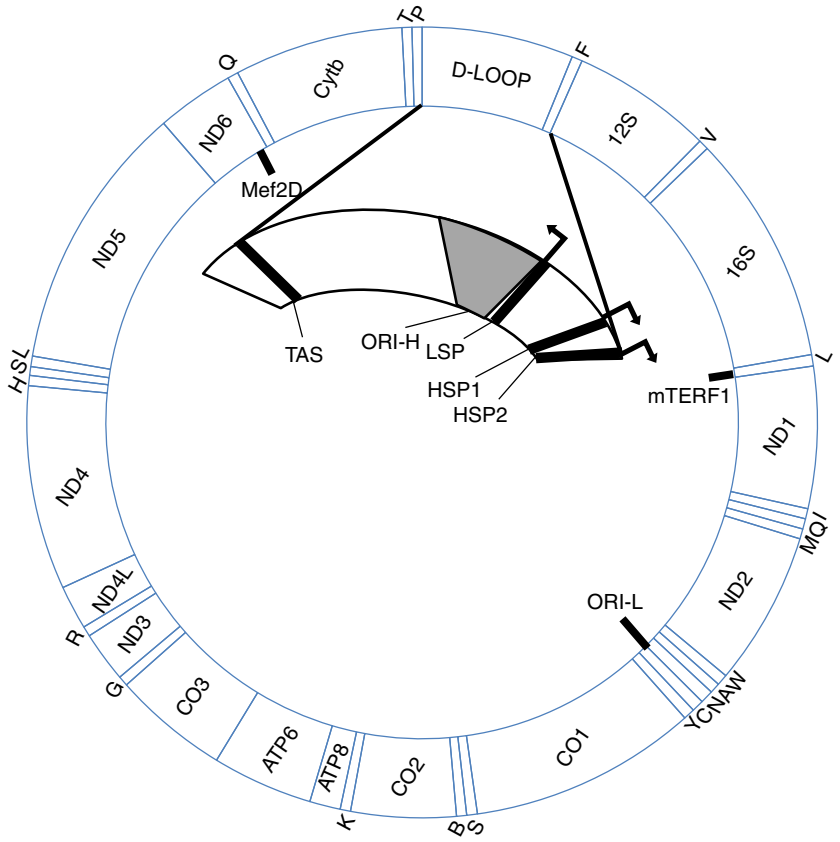

Figure 2 The human mitochondrial genome. Locations of all coding regions, transcription factors binding sites (such as MEF2D and MTERF1) and known regulatory elements are indicated. Letters on the outside of the circle refer to tRNA gene symbols. The D-loop and its known regulatory elements are expanded for clarity. LSP, light strand promoter; HSP1, heavy strand major promoter; HSP2, heavy strand minor promoter; ORI-H, heavy strand origin of replication; ORI-L, light strand origin of replication; TAS, replication termination associated sequence.

factors encompass more than 90% of the mitochondrial genome in vertebrates, although differences in gene order have been reported among species (Wallace, 2007). The remaining ~10% of the mtDNA comprises noncoding sequences harboring regulatory elements: (1) the D-Loop (~1000 base pairs in length), which includes the promoters for each of the two complementary DNA strands (e.g., the heavy and light mtDNA strands) as well as the origin of replication for the heavy strand (Ori-H), and (b) a shorter noncoding region (several tens of base pairs in length) encompassing the light strand origin of replication (Ori-L) – a stem and loop structure ~5000 base pairs apart from the D-loop. Although the heavy and light strand promoters (LSPs) are distinct entities in most vertebrates, the mitochondrial genomes of the African clawed frog (*Xenopus laevis*) and most fowl in super-order Galloanserae have a bidirectional promoter that governs the transcription of both the heavy and light strands. This alternative arrangement raises the attractive possibility that mutations in such sequences may affect transcription of both strands (L'Abbe *et al.*, 1991; Bogenhagen and Romanelli, 1988; Randi and Lucchini, 1998).

Mitochondrial–Nuclear DNA Mutation Rate Differences, Resultant Incompatibilities, and Coevolution

The mitochondrial and nuclear genomes of plants have similar mutation rates. In contrast, the calculated mutation rate of the mtDNA in animals in general, and in vertebrates in particular, is approximately an order of magnitude faster than that of the nuclear genome (Bar-Yaacov *et al.*, 2012b; Castellana *et al.*, 2011). This rate difference poses a particular problem for mitochondrial processes that require the interactions of factors, of which some are encoded by the mtDNA, and some by the nuclear genome. These include: protein–protein interactions within OXPHOS protein complexes I, III–V (but not within OXPHOS complex II which comprises only nuclear DNA encoded proteins), interactions between nuclear DNA-encoded proteins and mtDNA-encoded rRNA within the mitochondrial ribosome, and interactions between nuclear DNA-encoded transcription and replication factors and their mtDNA recognition sites. In order to cope with these rate differences, tight coevolution has occurred between interacting factors encoded by the two genomes (Gershoni *et al.*, 2010, 2014; Grossman *et al.*, 2004; Schmidt *et al.*, 2005; Yadava *et al.*, 2002; Rand *et al.*, 2004; Meiklejohn *et al.*, 2007; Rand, 2008). High-resolution 3D structures of OXPHOS complex IV have enabled investigation of coevolution between nuclear and mitochondrial DNA-encoded subunits (Schmidt *et al.*, 2001). Correlated mutations among mtDNA- and nuclear DNA-encoded factors allow for predictions about and experimental verification of protein–protein interactions within human OXPHOS complex I (Gershoni *et al.*, 2010, 2014; Mishmar *et al.*, 2006).

When coevolution is interrupted, mitochondrial dysfunction can occur (Gershoni *et al.*, 2014). This is the case for human cells in which the mitochondria were replaced either by chimpanzee or gorilla mitochondria (xenomitochondrial cybrid cells) (Barrientos *et al.*, 1998). These cybrids experienced a 40% reduction in the activity of OXPHOS complex I. Similar reduction in complex I activity was described in human children with inherited mutations in complex I subunits, which led to generalized hypotonia, developmental arrest, and death before their second year of life (Rubio-Gozalbo *et al.*, 2000). These findings suggest that functional incompatibilities between the mitochondrial and nuclear genomes of humans and our close phylogenetic relatives affect mitochondrial function in a manner analogous to known mitochondrial diseases. Likewise, reduced activity of OXPHOS complexes I and IV have been found in interspecific rodent cybrids (Dey *et al.*, 2000; Yamaoka *et al.*, 2000; McKenzie and Trounce, 2000). Moreover, backcross experiments have demonstrated that incompatibilities between the mitochondrial and nuclear genomes can affect mitochondrial function (Sackton *et al.*, 2003), sex-specific fitness in *Drosophila* flies (Rand *et al.*, 2001) and yeast (Lee *et al.*, 2008), and mortality rate in the parasitoid wasps *Nasonia girault*i and *Nasonia vitripennis* (Niehuis *et al.*, 2008; Ellison *et al.*, 2008).

Mitochondrial–nuclear coevolution can also impact interactions between populations of the same species. A series of inter-population breeding experiments in the copepod *Tigriopus californicus* revealed reduction in hybrid fitness and activity of OXPHOS complexes (mainly complex IV) (Burton *et al.*, 2006; Ellison and Burton, 2010). Similarly, reduced fitness in interpopulation breeding in *Drosophila* was attributed to mito–nuclear interactions (Dowling *et al.*, 2007; Rand *et al.*, 2001). A cybrid clone series carrying a single common human nuclear genetic background matched with a range of mtDNAs from diverse human or mouse lineages exhibited reductions in mitochondrial function (Kazuno *et al.*, 2006; Moreno-Loshuertos *et al.*, 2006; Kenney *et al.*, 2014) and variation in nuclear gene expression. This likely occurred via signals carried by small molecules from the mitochondria to the nucleus, termed retrograde signaling (Kenney *et al.*, 2014). Finally, mitochondrial–nuclear incompatibility may have played a role in the divergence of natural sparrow populations (Trier *et al.*, 2014) and possibly in other vertebrates (Bar-Yaacov *et al.*, 2012a). Such observations have led to the suggestion that mito–nuclear incompatibility could play a role in the generation of reproductive barriers, an essential step toward the emergence of new species (Gershoni *et al.*, 2009).

Coevolution and Physical Interaction

Interaction between factors is not unique to the mitochondria. Most biological activities require the interactions of several factors. Interacting residues within biological proteins are frequently conserved to retain physical contact and corresponding biological activity. However, it has been shown that in many cases interacting biological factors undergo correlated changes during the course of evolution (De Juan *et al.*, 2013). Such changes reflect adaptive processes which act to retain biological activities in response to changes in environmental conditions. In order to maintain critical biological functions and prevent incompatibilities, a mutation within a gene that codes for a particular protein can trigger selection for compensatory mutations either elsewhere in that same protein or in other physically or genetically interacting factors

(Pazos and Valencia, 2008). This phenomenon has been utilized to predict protein–protein interactions, in combination with parameters mainly derived from 3D structural information (Lewis *et al.*, 2010). Other modes of physical interactions between molecules, such as those between RNA and proteins in either the cytosolic or mitochondrial ribosomes, or between DNA binding sites and their associated transcription factors, frequently occur in biological systems, and are profoundly important in sustaining biological activities.

Indeed, co-adaptation occurs among physically interacting factors in the context of many biological functions. However, unlike most cellular activities which are coded by the nuclear genome, the relatively fast mutation rate of the mitochondrial genetic system requires especially tight coevolution among its interacting factors (Saccone *et al.*, 2006).

Mitochondrial–Nuclear Coevolution in the Mitochondrial Protein Translation System

The OXPHOS and the mitochondrial translation machinery are the only two mitochondrial complexes consisting of factors encoded both by the mitochondrial and the nuclear genomes. Similar to the OXPHOS system (which is discussed in section Primer on Mitochondrial Genetics and Genomics), mtDNA-encoded rRNAs and their interacting nuclear DNA-encoded ribosomal proteins (Desmond *et al.*, 2011; Smits *et al.*, 2007) have likely coevolved to maintain structure and function of the ribosome (Barreto and Burton, 2013). Additionally, it has been reported that the RNA component of the mitochondrial ribosome is reduced in size compared to its bacterial homologue, with a compensatory increase in protein content to maintain the 3D structure (Mears *et al.*, 2006; Greber *et al.*, 2014), further supporting mito–nuclear coevolution. Coevolution between the mtDNA-encoded rRNAs and their interacting proteins has yet to be studied, though it is known that the nuclear DNA-encoded mitochondrial ribosomal proteins evolve faster than cytosolic ribosomal proteins (Barreto and Burton, 2013).

In order to better investigate patterns of coevolution of interacting factors within the mitochondrial ribosome, high-resolution structural information is desirable. However, unlike the OXPHOS mammalian complexes for which high-resolution structural information is available (excluding complex I, and part of complex V) the structure of the mammalian mitochondrial ribosome was, until recently resolved only in relatively low resolution (Greber *et al.*, 2014; Kaushal *et al.*, 2014). Recent high resolution structures will enable elucidating the direct physical interactions between nuclear DNA-encoded proteins and the mtDNA-encoded rRNAs (Amunts *et al.*, 2015; Greber *et al.*, 2015). Additionally since the closest structural relative of the mitochondrial ribosome is the bacterial one, and since the ribosome structure is highly conserved from bacteria to human (Ben-Shem *et al.*, 2011), patterns of coevolution within the bacterial ribosome are likely to assist in understanding the nature of evolutionary dynamics and interactions within the mitochondrial ribosome. Indeed, patterns of correlated changes have been observed between the 23S rRNA (the orthologue of mtDNA-encoded 16S rRNA) and a directly interacting protein–alpha helix 3 of ribosomal protein L11 in bacteria (Guha Thakurta and Draper, 1999). Recent advances in identification of mitochondrial RNA-binding proteins may assist in isolating the binding proteins of mtDNA-encoded rRNAs and investigating their coevolution (Wolf and Mootha, 2014).

Another aspect of mito–nuclear RNA-protein coevolution is reflected in the need for compatibility between the mtDNA-encoded tRNA Tyr and the nuclear DNA-encoded tRNA Tyr-synthase to maintain normal development and mitochondrial function among *Drosophila* taxa (Hoekstra *et al.*, 2013; Meiklejohn *et al.*, 2013). As such, mito–nuclear coevolution is not restricted to protein–protein interactions.

Mitochondrial–Nuclear Coevolution of Regulatory Elements

The genome of the mitochondrial progenitor is believed to have harbored all the genes required for its activities, including all of the OXPHOS genes which are currently divided between the mitochondrial and nuclear genomes. Similar to its free living relatives, contemporary mtDNA genes are transcribed in a polycistronic manner, thus retaining their ancestral prokaryotic mode of regulation. Accordingly, it is reasonable to assume that the genome of the mitochondrial free-living bacterial ancestor harbored genes that were co-regulated and co-transcribed as a polycistornic RNA molecule. However, the genes currently encoding the protein subunits of the OXPHOS protein complexes are dispersed throughout the human genome and are mapped to different chromosomes; hence the problem of their co-regulation is a major issue as they have to collaborate within the multi-subunit protein complexes in many different tissues. Indeed, co-expression has been identified among genes that encode protein subunits that participate in the same OXPHOS complexes (Duborjal *et al.*, 2002; Garbian *et al.*, 2010; Van Waveren and Moraes, 2008). Accordingly, the expression pattern (mRNA) of genes belonging to the OXPHOS pathway was jointly altered in type 2 diabetes patients (Mootha *et al.*, 2003). Furthermore, changes in the expression pattern of nuclear DNA-encoded proteins have been described in cells originating from patients with mtDNA-encoded tRNA disease-causing mutations, suggesting signals delivered from the mitochondria to the nucleus and coordinated regulation (Rabilloud *et al.*, 2002; Chae *et al.*, 2013). These pieces of evidence point to the possible existence of a mechanism (or mechanisms) that govern co-regulation of mtDNA and nuclear DNA-encoded factors of the OXPHOS system.

If such mechanism indeed exists, there should be factors that are involved in the joint regulation of mitochondrial genes and nuclear genes. Indeed, some transcription factors, including NRF1, NRF2, PGC1a, and YY1, have been suggested as regulators of multiple proteins related to the OXPHOS system (Van Waveren and Moraes, 2008; Leigh-Brown *et al.*, 2010). NRF1 and NRF2 also regulate the transcription of factors that directly operate mtDNA transcription such as mitochondrial transcription factor A (TFAM) (Scarpulla, 2008). Furthermore, changes in the methylation status in promoters of nuclear DNA-encoded genes with mitochondrial function have been observed in a tissue-specific manner, suggesting differential regulation of

mitochondrial transcription across tissues (Takasugi *et al.*, 2010). Finally, other transcription factors, such as the thyroid hormone receptor, MEF2D, (**Figure 2**) and the glucocorticoid receptor, are known to regulate the transcription of nuclear genes but are also imported into the mitochondria, where they bind the mtDNA and regulate its transcription (Enriquez *et al.*, 1999; Leigh-Brown *et al.*, 2010; Psarra and Sekeris, 2011; She *et al.*, 2011; Szczepanek *et al.*, 2012). Similar to the components of mitochondrial transcription, some mtDNA replication components, such as hDNA2, APE1, Pif1, and DNA ligase III, co-localize and perform their functions both in the mitochondria and in the nucleus (Duxin *et al.*, 2009; Chattopadhyay *et al.*, 2006; Futami *et al.*, 2007; Lakshmipathy and Campbell, 1999). These findings indicate that nuclear and mtDNA transcription and replication could be co-regulated by a set of shared factors.

In addition to the mitochondrial localization of regulatory factors with known nuclear function, non-mitochondrial cellular localization has also been reported for some mitochondrial proteins (Yogev and Pines, 2011). Among these are the mtDNA transcription factor A (TFAM) (Pastukh *et al.*, 2007), and the mtDNA RNA Polymerase (POLRMT) (Kravchenko *et al.*, 2005), which are localized and involved in transcription both in the mitochondria and in the nucleus, although recent evidence question these interpretations (Kühl *et al.*, 2014). Hence, co-regulation of mtDNA and nuclear DNA-encoded proteins with mitochondrial activity is plausible (Bar-Yaacov *et al.*, 2012b).

The above findings raise the possibility of coevolution between factors that directly regulate mtDNA transcription and/or replication along with their binding sites in the mtDNA. This possibility is supported by the finding that human mitochondrial RNA polymerase, POLRMT, cannot bind and initiate transcription at the mouse light strand mtDNA promoter and vice versa (Gaspari *et al.*, 2004). Additionally, human mtDNA genetic variants can alter *in vitro* transcription and affect the binding capacity of TFAM (Suissa *et al.*, 2009). Certain genetic variants in TFAM have been associated with altered tendency to develop Parkinson's disease in Polish patients, in close correlation to the mtDNA genetic background haplogroup HV, suggesting that interfering with mitochondrial–nuclear interaction at the transcription level is involved in the etiology of the disease (Gaweda-Walerych *et al.*, 2010). Although such an association was discovered in the Polish population, no association was identified in the Spanish population (Alvarez *et al.*, 2008), thus implying the involvement of other factors that modulate the phenotypic effect of TFAM. Thorough investigation of the coevolution between nuclear DNA-encoded transcription factors and their mtDNA binding sites both within and between species should be performed in the near future. As selective constraints are different in noncoding versus gene-coding sequences one should take into account the rapid change rate of noncoding regulatory elements within the mtDNA (Montooth *et al.*, 2009). With this in mind, it is also plausible that there are regulatory elements that reside within the mtDNA-coding sequences, as was recently shown in the nuclear genome (Birnbaum *et al.*, 2012; Blumberg *et al.*, 2014). This suggests dual roles for such putative sites – they may code for genes but also promote binding of regulatory factors (Stergachis *et al.*, 2013). The discovery of such sites could pave the way toward new models of coevolution governed both by constraints acting on both gene sequences and transcription factor binding sites.

Heteroplasmy and Its Effect on Mitochondrial–Nuclear Coevolution

Unlike the nuclear genome, the mtDNA resides in multiple cellular copies that may differ in sequence, thus creating a mixed population of mtDNA molecules within a cell, termed heteroplasmy (Larsson, 2010). This phenomenon adds another dimension to mitochondrial–nuclear interactions: intracellular diversity within a single individual. Heteroplasmic mutations may be inherited as existing mtDNA diversity within the ovum, but may also appear *de novo* during the lifetime of the individual (Avital *et al.*, 2012). Pathogenic mtDNA mutations and deletions are thought to cause disease only when they constitute more than 80% (point mutations) or 60% (deletions) of the mtDNA population within the diseased tissues (Schapira, 2012; Zeviani and Di Donato, 2004). However, patterns of heteroplasmy in monozygotic twins demonstrate that even low-abundance heteroplasmic mutations are subjected to strong negative selection (Avital *et al.*, 2012). This suggests that even low-grade heteroplasmy can have functional implications and that a mechanism for the selective removal of dysfunctional mitochondria, such as mitophagy (Twig and Shirihai, 2011), can act at the subcellular level, maybe even on the activity of the single mitochondrion.

One could hypothesize that disease-causing heteroplasmic mutations affect mitochondrial–nuclear interactions, thus partially explaining the molecular basis underlying the phenotypic impact of these mutations (Carelli *et al.*, 2003). Support for this hypothesis comes from the fact that mtDNA pathological mutations have partial penetrance which is modulated by nuclear DNA modifiers (Hudson *et al.*, 2005; Shankar *et al.*, 2008; Luo *et al.*, 2013; Carelli *et al.*, 2003). Finally, it has been shown that while artificially creating a heteroplasmic cell with mixed mtDNA haplotypes, during cell divisions there has been a skew toward overrepresentation of the mtDNA molecules from the same strain of the nucleus (Lee *et al.*, 2008). All these pieces of evidence set the basis for the attractive possibility that mitochondrial–nuclear coevolution is subjected to strong selective constraints acting to protect mitochondrial–nuclear factor interactions from mutations at the population level, but even from heteroplasmic mutations.

Interference with Mitochondrial–Nuclear Interactions Cause Diseases

Since coevolution between interacting factors is important to maintain function, it is reasonable that interfering with such coevolution may have phenotypic consequences. This thought led two renowned geneticists, Theodosius Dobzhansky and Hermann Joseph Muller to independently suggest during the first half of the twentieth century that changes in one factor without compensatory response from its genetically interacting partner play an important step in the erection of reproductive barriers and, eventually, speciation events (Dobzhansky, 1936;

Muller, 1942; Gavrilets, 2003). Since interactions among mtDNA and nuclear DNA-encoded factors are important for cellular function and since some modes of co-regulation exist, at least at the transcriptional level (Leigh-Brown *et al.*, 2010), it is logical that disruption of the mito–nuclear association may cause diseases. Indeed, the penetrance of mtDNA mutations that cause Leber's hereditary optic neuropathy (LHON) was shown to be modulated by X-linked nuclear DNA encoded elements (Shankar *et al.*, 2008; Hudson *et al.*, 2005). Nuclear DNA-encoded modifiers of mtDNA mutations causing hearing loss were also suggested (Luo *et al.*, 2013; Johnson *et al.*, 2001; Guan, 2011; Kokotas *et al.*, 2011). A recent study in mitochondrial-nuclear exchange mice (which were generated by repeated backcross experiments) showed that mitochondrial–nuclear genetic interactions affect the tendency to develop nonalcoholic fatty liver disease (NAFLD) (Betancourt *et al.*, 2014). Work in conplastic strains of rats, i.e., strains with identical nuclear genomes but divergent mitochondrial genomes, which encode amino acid differences in OXPHOS proteins, exhibit differences in major metabolic risk factors for type 2 diabetes (Pravenec *et al.*, 2007). Accordingly, the association of a certain mtDNA genetic background (haplogroup J1) with a tendency to develop type 2 diabetes mellitus in Jews is modulated by nuclear DNA-encoded factors (Feder *et al.*, 2009; Gershoni *et al.*, 2014). Moreover, the susceptibility to develop type 2 diabetes mellitus in Indian patients depends on specific association of mtDNA and nuclear DNA common variants (Rai *et al.*, 2007). The disease-causing phenotype of a mutation in *NDUFA1*, a nuclear DNA-encoded subunit of OXPHOS complex I, is dependent on mtDNA-encoded factors (Potluri *et al.*, 2009). Disruption of mito–nuclear interaction due to disease-causing and evolutionary convergent mutations in mitochondrial tRNAs has also been reported (Kern and Kondrashov, 2004). Taken together, all these findings suggest that disruption of the connection between certain mtDNA-nuclear DNA-encoded factors can lead to diseases (De Magalhaes, 2005).

See also: Adaptive Molecular Evolution: Detection Methods. Coevolution, Introduction to. Endosymbiotic Theory. Evolutionary Genetics, History of. Gene Interactions in Evolution. Genetic Variation in Populations. Molecular Evolution, History of. Mutation and Genome Evolution. Natural Selection, Introduction to. Speciation Genes. Speciation Genomics. Symbiogenesis, History of

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Model Systems: The Key Roles of Traditional and New Models in Evolutionary Developmental Biology

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Glossary

Convergence It is the evolutionary concept of the existence of similar structures or genes that have evolved independently as a result of adaptation to similar environments.

Fate map It is used to trace a particular cell or region from an early embryo into a differentiated body plan.

Homology It is the evolutionary concept of the existence of similar structures or genes due to shared ancestry.

***In situ* hybridization (ISH)** It is a technique for localizing specific nucleic acid targets (DNA or RNA) in prepared tissues, cells, or even whole organisms to obtain temporal and spatial information about gene expression and genetic loci.

Microarrays They are DNA chips consisting of a collection of microscopic DNA spots attached to a solid surface such as a glass slide. They are used for the comparative measurement of gene expression levels.

Ontogenesis It is the development of an organism including all stages from the time of fertilization of the egg to the mature, reproductive form.

RNA interference (RNAi) It is a naturally occurring mechanism in which RNA molecules inhibit gene expression via the RNAi pathway. Small interfering RNAs can be generated *in vitro* and are used in EvoDevo to reduce or inhibit the expression of specific genes to study their function.

RNAseq (RNA sequencing) RNA sequencing is a technique that uses next-generation sequencing to reveal the presence of transcripts and their quantity in a given tissue/organism at the time of collection.

Introduction

In order to understand the key roles of model systems in EvoDevo, it is important to first define the term model system (or organism which will be used interchangeably here). In developmental biology, a few species are recognized as model organisms, for example, the fruit fly *Drosophila melanogaster*, the worm *Caenorhabditis elegans*, the zebrafish *Danio rerio*, and the mouse *Mus musculus*. They fulfill several requirements that make them suitable for a wide range of developmental questions: (1) They have short generation times and can be cultured in the lab. (2) Embryos are easily accessible and can be manipulated for functional studies. (3) Mutant and transgenic lines are available and the genome is sequenced. (4) Substantial knowledge of the development of the organisms has been collated, including detailed subdivisions of the developmental processes and stages (staging system) and fate maps. (5) Databases are available as repositories of genetic, molecular, and expression data, including descriptions of mutant alleles and phenotypes, gene expression data (e.g., *in situ* hybridizations, RNAseq, microarrays), and anatomical data.

Species that have been developed into model systems over the past decades ('traditional' model organisms) have been – and still are – invaluable for deepening our knowledge of the molecular and morphological processes of development. However, studies in additional vertebrate and invertebrate species have made it clear that the regulation of developmental processes is substantially different not only in different clades of the same phylum but also within clades and even in species of the same order (Choe and Brown, 2007; Krol *et al.*, 2011). For example, variations have been detected in the insect orders Diptera (true flies) and Hymenoptera in the formation of the embryonic midline cells which play an important role in organizing the bilateral symmetry of the nervous system (Zinzen *et al.*, 2006). In the fruit fly *D. melanogaster*, a fixed number of eight midline precursor cells per segment generates individually identifiable neurons and glial cells (Bossing and Technau, 1994). While the midline precursors arise from the same tissue ('mesectoderm') in both species, three times as many precursors develop in the honeybee *Apis mellifera*. This is due to changes in the regulation of *single-minded*, a transcription factor which is involved in the formation of the midline precursors (Zinzen *et al.*, 2006). Although the molecular mechanisms of midline formation have not been resolved in basal representatives of the insect clade (e.g., grasshopper) (Jia and Siegler, 2002), it is clear that their origin must differ from dipteran and hymenopteran midline precursors due to the overall difference in body formation. This example shows that developmental processes are highly variable and findings within a single species – regardless of it being recognized as a model organism or not – cannot be generalized. It is therefore clear that a model organism's value depends on the question in focus. Since the concepts and questions addressed in developmental biology and evolutionary developmental biology (EvoDevo) are fundamentally different, the traditional developmental model organisms can only contribute a small part to the latter research field. However, as we shall see below, they were indispensible at the start of the molecular area of EvoDevo.

The Role of Traditional Model Organisms in Evolutionary Developmental Biology

EvoDevo combines evolution and development and compiles new concepts of how developmental processes link the

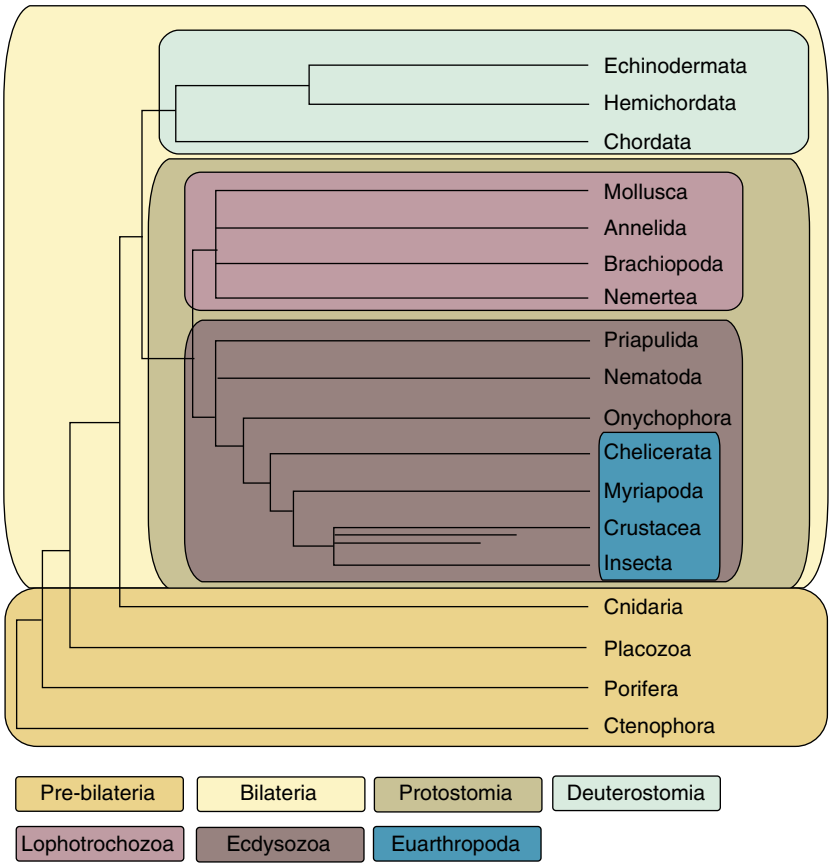

Figure 1 Phylogeny of the animal kingdom. The phylogeny shows the major subdivisions of the animal phyla and some selected taxa within each group. It is based on the Tree of Life web project (http://tolweb.org/tree/phylogeny.html) and references given in the main text. See also references in the main text for alternative scenarios.

genotype and the phenotype in the formation and evolution of traits and organisms (Moczek *et al.*, 2015). Over the past 150 years, the main approach of EvoDevo research was the comparative analysis of developmental processes in a phylogenetic context with the aim of understanding the morphological variations and the origin of structures. In the past decades this approach has been extended to the comparative analysis of developmental genes and their expression and function. Based on the knowledge of the genetic regulation of development in traditional model organisms, EvoDevo research identified homologous genes in a variety of different species across the animal kingdom. An important concept that emerged from these studies is that of the 'toolkit genes,' which means that developmental genes are used over and over again for various developmental processes across the animal kingdom (Shubin *et al.*, 2009). The Hox genes were one of the first toolkit genes that were discovered (Heffer and Pick, 2013). They confer segmental identity along the anterior–posterior (AP) body axis, among others, across the animal kingdom. For example, in vertebrates they determine the structural difference between the cervical, rib, lumbar, and sacral vertebrae and in the fruit fly *D. melanogaster* the identity of the various gnathal, thoracic, and abdominal segments (Horan *et al.*, 1994; Maeda and Karch, 2009). Most importantly, developmental genes are not only used in developmental processes that produce homologous structures but have also been redeployed for the generation of structures that evolved by convergence. For example, a specialized cell type, the neural progenitor cell, which can potentially generate all cells of the nervous system, has evolved by convergence in the invertebrate phylum Arthropoda and in the phylum Chordata, which contains the vertebrates. Yet, in both cases the Achaete-Scute bHLH transcription factors and the Notch signaling pathway regulate early steps of neural progenitor formation and differentiation (Ungerer *et al.*, 2012).

Traditional model organisms were not only used for cloning homologous genes in other species but also as a first reference point for comparing and understanding evolutionary changes in gene function. For example, insects belong to the euarthropods whose bodies are composed of segments (**Figure 1**). In the traditional model organism, *D. melanogaster*, all segments are generated at the same time in the embryo. A hierarchical gene cascade subdivides the embryo into smaller and smaller units (Nüsslein-Vollhard and Wieschaus, 1980). However, in most other euarthropods, the cellular material which is present at the start of the segmentation process only corresponds to the anterior part of the embryo; the remaining segments are generated one by one from a posterior growth zone. Despite this difference, the '*Drosophila*' segmentation genes are expressed in all analyzed euarthropods (Arthur and Chipman, 2005; Cerny *et al.*, 2008; Eriksson *et al.*, 2013). However, significant differences in the expression and function

have been reported, in particular for the so-called pair-rule genes, which are expressed in alternating segments in *D. melanogaster* but in every segment in many euarthropods or even in opposite register as *Tc-sloppy-paired* in the flour beetle *Tribolium castaneum* (Choe and Brown, 2007; Eriksson *et al.*, 2013; Schoppmeier and Damen, 2005).

Are traditional model organisms only useful for comparative purposes or can they be used directly for addressing EvoDevo questions? Several research groups have in fact shown that the traditional model organism *D. melanogaster* is useful for investigating host–microbe evolution (Richardson *et al.*, 2012; Teixeira *et al.*, 2008; Versace *et al.*, 2014). *Wolbachia* is a common bacterial endosymbiont of arthropods and transmitted to the offspring via the egg cytoplasm (Werren *et al.*, 2008). *Drosophila* benefits from *Wolbachia* by increased fecundity and antiviral protection, among others (e.g., Fry *et al.*, 2004; Hedges *et al.*, 2008; Olsen *et al.*, 2001; Teixeira *et al.*, 2008). Various *Wolbachia* strains originating from different climates and grouped into five clades are capable of infecting the flies at the same time (Richardson *et al.*, 2012). The naturally occurring diverse infection patterns are poorly understood, partially due to the complexity of influencing environmental factors. This problem can be approached by experimental evolution. *Drosophila* populations infected with several *Wolbachia* strains were exposed over many generations to single environmental cues in controlled laboratory conditions (Versace *et al.*, 2014). Schlötterer and co-authors found that after 15 generations in a cold environment, *Wolbachia* clades I to III were lost from the *Drosophila* populations, while clade VI was reduced in frequency; however, clade V showed an increase of about 50% (Versace *et al.*, 2014). This does not occur when the flies are exposed to a hot environment. The selective loss can be explained by the origin of the clades. Clades I to III originate from warm climates (the Afrotropics), while clades V and VI derive from colder Eurasian climates.

Evolutionary experiments using *Drosophila* populations have also improved our understanding of longstanding fundamental questions in EvoDevo. In the 1940s Waddington observed that ontogenesis results in the same phenotypic outcome over a wide range of internal and external variations suggesting that internal buffering mechanism canalize the developmental processes (Waddington, 1942). Waddington named this phenomenon 'canalization.' By exposing *Drosophila* to ether, he unveiled hidden ('cryptic') genetic variations resulting in dramatically different phenotypes such as flies with two pairs of wings instead of one (Waddington, 1956). After several generations the flies even showed the phenotypic variation without the environmental cue. The experiment shows that environmentally induced phenotypic variants can become genetically stabilized and thus demonstrates the influence of the environment on morphological evolution. What are the underlying mechanisms? Again the traditional model organism *Drosophila* has provided possible answers. The heat shock protein Hsp90, for example, masks genetic variations due to its role as a chaperone in correcting misfolding of proteins. In Hsp90 mutants phenotypic variations of nearly any adult structure are produced showing that genetic variations for multiple developmental pathways exist (Rutherford and Lindquist, 1998). Recent expression studies show the canalization effect on a genome-wide level (Chen *et al.*, 2015; Levine *et al.*, 2011). For example, the different alleles of two *D. melanogaster* strains show the same expression levels at moderate temperatures (18 °C) but significant differences appear when the flies are exposed to extreme temperatures indicating that extreme environmental changes unmask existing genetic variations ('decanalization') (Chen *et al.*, 2015).

To summarize, traditional model organisms have played a significant role in directing the research at the start of the molecular area of EvoDevo and continue making valuable contributions both as benchmark for comparative developmental studies and as independent EvoDevo model organisms.

The Role of New Model Organisms in Evolutionary Developmental Biology

In the previous section, I briefly addressed the question of how diverse molecular processes can lead to the same morphological outcome. On the other hand, the discovery of the toolkit genes raises the question of how conserved molecular pathways perform their role regardless of major differences in morphology. The integration of evolutionary changes into existing organ systems is another unsolved question in evolution. How are evolutionary changes in a certain organ system integrated with the remaining developmental processes to generate a functional phenotype? And how do novel traits evolve? These questions make it clear that an important criterion for choosing EvoDevo model organisms is their phylogenetic position. Thus, there are strong links between EvoDevo and taxonomy and adjustments in the phylogenetic positions can change our view of how and when characters have evolved.

For example, the question of when the nervous system has evolved requires the investigation of representatives of phyla across the animal kingdom. Comparative analysis of all major bilaterian groups, the deuterostomes and the protostomes which are divided into the supertaxa Ecdysozoa and Lophotrochozoa revealed that all bilaterians have nervous systems, while they are missing in some pre-bilaterian phyla (reviewed by Hartenstein and Stollewerk, 2015; **Figure 1**). Traditionally it was thought that the nervous system evolved once in pre-bilaterians. However, the two pre-bilaterian phyla with nervous systems, the cnidarians and ctenophores, might not be as closely related as was assumed. In a recent molecular phylogeny, the latter were placed close to the origin of the metazoan tree (Moroz *et al.*, 2014). In this scenario, two phyla without a nervous system branch off between ctenophores and cnidarians suggesting that the ctenophore nervous system has evolved independently (but see Ryan *et al.*, 2013; Marlow and Arendt, 2014 for discussion; **Figure 1**).

The presence of a nervous system can be determined by morphological analysis of adult specimen and by genome sequencing or transcriptome analysis to identify neural genes. However, different, more challenging strategies are required for addressing the question of how the various nervous systems have evolved because this requires ontogenetic and functional studies, among others. There is a huge variety in the structure and degree of centralization of bilaterian nervous systems (reviewed by Hartenstein and Stollewerk, 2015). Since

developmental processes ultimately determine the adult structure, a detailed knowledge of the development of the nervous system is required. If we want to analyze the evolution of the nervous system on a larger evolutionary scale, representatives of various phyla with diverse CNS structures have to be chosen such as euarthropods with tripartite brains and metameric ventral ganglia, vertebrates with complex brain centers and dorsal ganglia as well as animals with simple brain structures such as Platyhelminthes (flatworms) and prebilaterians like Hydra which show some degree of centralization (reviewed by Hartenstein and Stollewerk, 2015). In such large-scale studies, we can investigate how the different areas of the CNS are established. The Wnt/β-catenin pathway, for example, patterns the CNS along the AP axis in vertebrates and in planarians (flatworms) which belong to the lophotrochozoans (Niehrs, 2010). In contrast, in arthropods (Ecdysozoa) Wnt signaling regulates posterior elongation and segmentation (Janssen *et al.*, 2010). It is not required for overall AP patterning of the brain, rather it determines AP fate within neuromeres (Bhat *et al.*, 2000). Although many of the genes downstream of the primary patterning mechanisms are conserved across the animal kingdom, such as the Hox genes which subdivide the neurogenic region along the AP axis (e.g., Gummalla *et al.*, 2014; Nordstrom *et al.*, 2006), considerable differences in their function and requirement for the formation of specific parts of the CNS are observed even within the same phylum (e.g., Janssen *et al.*, 2014; Merrill *et al.*, 1989; Posnien and Bucher, 2010; Schaeper *et al.*, 2010). These evolutionary changes might explain evolutionary differences in the functional and morphological subdivisions of the bilaterian CNS.

On a cellular level, the identification of potentially homologous structures can be obscured by multiple evolutionary changes if they are compared over a large evolutionary distance. In order to understand, for example, the finer details of how specific neuronal lineages have changed during evolution to support the function of new or modified morphological structures and behaviors, the comparative analysis of closely related taxa is required. Arthropods are highly suitable for addressing this question because they comprise the largest animal phyla and show a great variety of shapes and behaviors. Again, the traditional model organism *D. melanogaster* is a good starting point since the neuronal lineages in the ventral nerve cord have been described in detail (e.g., Bossing *et al.*, 1996; Doe, 1992; Landgraf *et al.*, 1997; Schmid *et al.*, 1999). However, a comparison of neuronal lineages across all euarthropod phyla (insects, crustaceans, myriapods, chelicerates) is not possible because of the different modes of neurogenesis (**Figure 2(a)**). In chelicerates and myriapods groups of neuroectodermal cells differentiate directly into neurons and glia, while in insects and crustaceans neuroblasts divide asymmetrically to generate fixed neural lineages (Bate, 1976; Dove and Stollewerk, 2003; Eriksson and Stollewerk, 2010; Stollewerk *et al.*, 2001; Ungerer and Scholtz, 2008). The neural precursor groups and neuroblasts are arranged in similar patterns in rows and columns in the ventral nerve cord allowing for the comparison of areas of neurogenesis between euarthropod groups (**Figure 2(a)**). However, the evolutionary comparison of single neurons and precursors is hampered by the differences in their formation (Döffinger and Stollewerk, 2010). Within euarthropod groups, individual, potentially homologous neuronal lineages can be identified and studied as has been shown in insects. The arrangement of neuroblasts is highly conserved across insects and maps have been established for various species (references in Döffinger and Stollewerk, 2010). Recent comparative studies in *D. melanogaster* and *T. castaneum* revealed that the expression profile of presumably homologous neuroblasts varies considerably, which might explain evolutionary changes in the neural lineages (Biffar and Stollewerk, 2014, 2015; **Figure 2(b)**). Despite the molecular changes conserved pioneer neurons are produced which establish the axonal scaffold (Biffar and Stollewerk, 2015; **Figure 2(b)**). This suggests that internal buffering mechanisms exist ensuring that the early part of the neuronal lineages is stable over a wide range of molecular changes.

Ideally, one would like to study evolutionary questions such as those discussed above systematically, filling in the knowledge gaps and generating a complete set of comparative data that would allow for piecing together the evolutionary history of shapes and behaviors across the animal kingdom. This is complicated by the sophisticated techniques that are required, in particular when nontraditional model organisms are included in the study. Methods have to be developed for culturing the animals in the lab as well as collecting and manipulating the various ontogenetic stages. Furthermore, protocols have to be established for morphological, gene expression, and functional studies. In many cases animals in either crucial or uncertain phylogenetic positions have proved to be difficult to work with and functional techniques could not be established despite considerable efforts. For example, myriapods have a crucial position within euarthropods for understanding the evolution of neurogenesis in arthropods. They are a sister group of the pancrustaceans (insects and crustacean; also called Tetraconata), together forming the Mandibulata (but see also Boore *et al.*, 1995; Friedrich and Tautz, 1995; Kusche and Burmester, 2001; Mayer and Whitington, 2009). Myriapods could significantly improve our understanding of the evolution of neuroblasts in arthropods since they show unique mechanisms of neurogenesis that can be interpreted as intermediate between the neural precursor groups of chelicerates and the neuroblasts of pancrustaceans. However, it has neither been possible to culture myriapods in the lab nor to establish functional techniques to address the question of how asymmetric divisions of neural precursor cells have evolved.

Fortunately, however, the continuous progression of molecular techniques has made it possible to perform functional and expression studies in a wide range of nontraditional model organisms. It is outside the scope of this article to discuss the whole range of techniques but here main inventions that had a considerable impact on the EvoDevo field are briefly mentioned. Mutagenesis screens have been used successfully in traditional model organisms (e.g., *D. melanogaster* and the zebrafish *Danio rerio*) to analyze gene function; however, they require selective crosses and reproduction in the lab as well as short generation times – requirements that most nontraditional model organisms do not fulfill. The discovery that RNA interference is a widespread phenomenon and can be induced by injection/uptake of synthetic double-stranded RNA has made it possible to transiently disrupt the expression

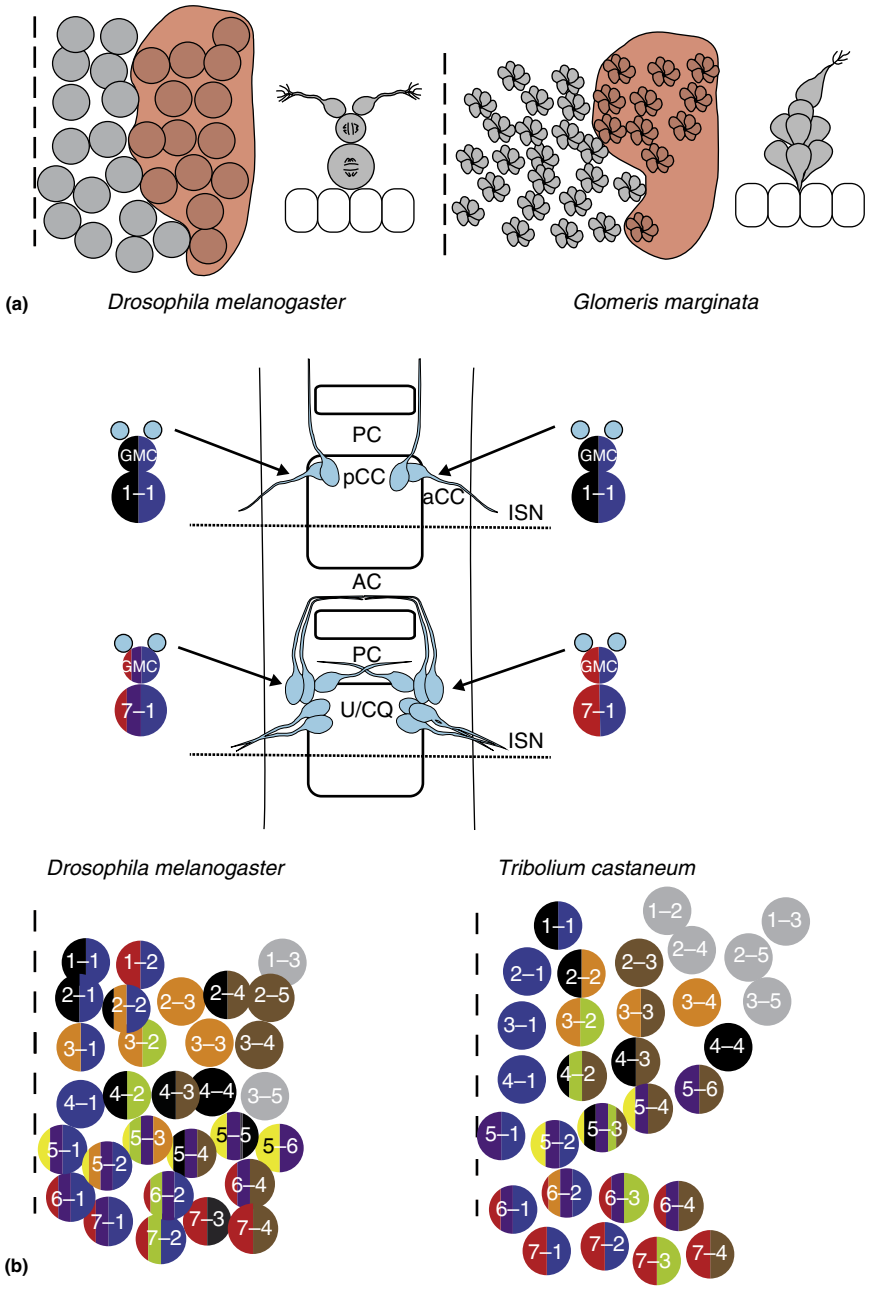

Figure 2 Evolutionary comparisons of areas of neural gene expression and neuronal lineages in euarthropods. (a) Insects (*Drosophila melanogaster)* and myriapods (*Glomeris marginata*) show different modes of neurogenesis. The left panels show horizontal views of hemi-neuromeres; the right panels show transverse views of neural progenitors/precursors. In insects neural progenitors (neuroblasts; gray circles in the left panel) divide asymmetrically to generate ganglion mother cells, which divide once to produce neurons and glial cells (right panel). In myriapods neural precursor groups (gray rosettes in the left panel) directly produce neurons and glial cells (right panel). The red shapes indicate the lateral expression of the dorso-ventral patterning gene *msh*. (b) The bottom panels show the comparative gene expression patterns in the neuroblasts of two insects, *Drosophila melanogaster* and *Tribolium castaneum*. Presumptive homologous neuroblasts show high variations in gene expression between the insects. Color code: brown, msh; green, ind; blue, vnd; black, hkb; orange, run; yellow, wg; purple, gsb; red, en; gray, none. The top panel shows the conserved axonal scaffold of euarthropods and the conserved positions of the *even-skipped (eve)* positive pioneer neurons (light blue). In both insects the eve positive aCC/pCC neurons are most likely generated by neuroblast 1-1 and the eve positive U/CQ neurons by neuroblast 7-1. AC, anterior commissure; PC, posterior commissure; GMC, ganglion mother cell, ISN, intersegmental nerve. The dashed lines indicate the segmental borders.

of genes of interest and study the functional consequences in a wide range of animal and plant phyla (Agrawal *et al.*, 2003; Fire *et al.*, 1998). RNA interference has its limitations since the double-stranded RNA has to be applied either parentally or during early stages so that later functions of the genes of interest might be obscured if their early role is vital.

Furthermore, the RNAi effect decreases over time and the injection procedure itself can lead to developmental defects (e.g., Linne and Stollewerk, 2011; Stollewerk, 2002). In *D. melanogaster*, this problem has been overcome by generating transgenes that contain a fragment of the candidate gene as an inverted repeat (Dietzl *et al.*, 2007). By using the GAL4/UAS system long double-stranded hairpin RNA is produced reducing the function of the candidate gene in a tissue and time-specific manner. This again shows the power of traditional model organisms.

Recently, the CRISPR/Cas9 system has been developed for genome editing in a variety of species including nontraditional model organisms (Gong *et al.*, 2014; Jinek *et al.*, 2012; Overballe-Petersen *et al.*, 2013). Genes are mutagenized by guiding Cas9 nucleases to specific target sequences using small RNAs. The DNA is cleaved and the repair of the lesion can introduce nucleotide substitutions or indel mutations. If the mutagenesis includes germline cells, stable mutant lines can be generated as has been shown recently in the emerging crustacean model organism *Daphnia magna*, for example (Nakanishi *et al.*, 2014). In this species, the regulator for eye development eyeless was targeted and about 8% of the mutagenized adults produced progeny with abnormal eyes.

Furthermore, next-generation sequencing has made many additional organisms accessible for EvoDevo research. Whole genomes can be sequenced and analyzed cost-effectively to study developmental pathways and their regulators (Metzker, 2010). Questions involving the expression of developmental genes and their regulatory networks can be addressed by sequencing transcripts of different developmental stages and mutant organisms as well as transcripts from organisms that have been exposed to different developmental conditions (e.g., Etges *et al.*, 2015; Staller *et al.*, 2015).

Taken together, both traditional and new model organisms have a deeply rooted place in the EvoDevo research field. While nontraditional model organisms are useful for addressing large-scale evolutionary questions such as the origin of body plans, the nervous system and novel structures, the developmental data of traditional model organisms are not only invaluable benchmarks but continuously contribute to collecting detailed knowledge of how developmental processes have been modified during evolution.

See also: Cellular Behaviors Underlying Pattern Formation and Evolution. Developmental-Genetic Toolkit for Evolutionary Developmental Biology. Gene Networks Driving Development, Conservation and Evolution of. Phylogenetic Approach to Studying Developmental Evolution: A Model Clade Approach

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Modularity and Integration

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Glossary

Developmental module Performs a specific role in developmental processes and corresponds to a set of cells, genes, or tissues that are relatively independent with respect to pattern formation and differentiation, or an autonomous developmental signaling pathway.

Evolutionary modules Sets of phenotypic elements evolving in coordinated fashion, because the elements are inherited together or because they are jointly selected.

Functional module Sets of traits or features that interact to perform some discrete function or task.

Genetic architecture Refers to the pattern of genetic effects that underlie the variation for a given set of phenotypic characters and its variational properties. A description of genetic architecture may include statements about gene and allele number, the distribution of allelic and mutational effects, and patterns of pleiotropy, dominance, and epistasis.

Genetic modules Sets of traits that are modular due to pleiotropy or linkage disequilibrium.

Genotype–phenotype map Depicts relationship between genetic variation and phenotypic variation; that is, it specifies which locus or loci affects each trait or traits.

Internal stabilizing selection Stabilizing selection due to the interaction of the phenotype with other internal characteristics of an organism, and is related to the need for coadaptation of traits to one another rather than to the external environment.

Linkage disequilibrium The nonrandom association of alleles at different loci.

Morphological integration Refers to the cohesion or association among morphological traits that are related functionally and/or developmentally. Traits that are integrated tend to covary together, and so this results in higher correlation between these traits when compared to traits that are not integrated.

Pleiotropy A single locus affecting two or more phenotypic traits.

Quasi-independence (quasi-autonomy) A lower than average grade of connectedness, for example, the elements of modules are highly interconnected, while being less connected to other modules. This 'quasi independence' may allow one character to change without affecting others.

Quantitative Trait Loci (QTL) Refers to DNA loci that affect quantitative traits.

Variational modules Set of covarying traits that vary relatively independently of other sets of traits. The reason for this relative independence of different sets of traits, or modules, is that pleiotropic loci with effects on traits belonging to different modules are less frequent than those within modules. These modules are recognized by higher correlations between traits in the same module and lower correlations between traits of different modules.

What Is Modularity and Integration?

Biology is rapidly embracing the challenge of dealing with multidimensional hierarchical systems as a way of moving forward and addressing questions that range from the genetic basis of diseases, behavior, or morphology, the ecological structure of communities, or the evolution of any of these features. To face this challenge we need both theoretical developments and methods capable of dealing with such complexity. At the core of all this lies the concept of modularity. In Biology, modularity refers to the pattern and magnitude of association among elements in a system. This pattern emerges whenever a high connectivity between some elements in the system exists, forming modules, and at the same time these same elements are more loosely associated to other elements that compose other modules. Modularity depends on the ability of a system to organize semi-autonomous parts, or even discrete elements, into a coherent whole. Modularity can be studied at nearly every scale of biological organization; and it has been described in a variety of contexts and observed in many model systems, in a wide range of disciplines and specialties. These include proteins (Han *et al.*, 2004), genes (Litvin *et al.*, 2009), cells (Hartwell *et al.*, 1999; Wagner, 1996), organs (Schlosser and Wagner, 2004), and ecosystems (Montoya *et al.*, 2006).

Here, we address modularity in the context of morphological quantitative traits and discuss the influence of genetic, functional, and developmental factors at this level. In this context, different parts of organisms can behave as modules because they exhibit some degree of independence, and are internally organized, reflecting their developmental origins and functions, as we will see later (Cheverud, 1996; Klingenberg, 2004).

Most of our current understanding of character correlations and on the evolution of complex continuous traits is influenced by the concept of morphological integration (Olson and Miller, 1951, 1958). Olson and Miller (1951, 1958) coined the term morphological integration to describe high levels of phenotypic correlation within subsets of morphological traits. Today, these sets of integrated traits related functionally and/or developmentally are termed modules. In a remarkable work addressing morphological variation and correlation in plants, Raissa Berg (1960) described a similar concept known as 'correlation pleiades.' As with morphological integration, correlation pleiades are based on the presence of high levels of

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correlation between some parts of an organism, and low association between these and other parts of the same organism.

From the above definitions, we can see that modularity and integration are interrelated concepts, as both of them deal with the interdependence between different structures based on developmental, genetic, and/or functional factors, and, moreover, are quantified by the degree of correlation or covariation among traits. In fact, they can be understood as two sides of the same coin. While the concept of morphological integration describes connections among parts of an organism, the concept of modularity stresses its relative independence or autonomy (Schlosser and Wagner, 2004; Wagner, 1996).

In biology, several types of modules have been recognized, including functional, developmental, genetic, evolutionary, and variational (Cheverud, 1996; Wagner *et al.*, 2007). A functional module is composed of characters or features that interact together on performing a task or function and are relatively independent in relation to other functional sets (Cheverud, 1996; Wagner *et al.*, 2007). Developmental modules correspond to set of cells, genes, or tissues that are relatively independent with respect to pattern formation and differentiation, or an autonomous regulatory control (Wagner *et al.*, 2007). Genetic integration occurs when sets of morphological elements are inherited together as a module, due to pleiotropy and/or linkage desequilibrium. These sets of morphological elements are more or less independently of other sets or modules (Cheverud, 1996). An evolutionary module is a set of morphological traits evolving in coordinated fashion, because the elements are inherited together or because they are jointly selected (Cheverud, 1996). Variational modularity is recognized by higher correlations between traits in the same module and lower correlations between traits of different modules, and can have different causes (Armbruster *et al.*, 2014; Grabowski *et al.*, 2011; Melo and Marroig, 2015; Young and Hallgrímsson, 2005).

The Causes of Modularity: Development and Function

A variational modularity is thought to be the outcome of functional and/or developmental relationships between traits (Berg, 1960; Cheverud, 1982; Olson and Miller, 1958; Porto *et al.*, 2009). These two forms of individual level integration are related because development can be viewed as a dynamic process, and functional integration in the adult is likely achieved through developmental integration (Cheverud, 1996). Moreover, the developmental process is the path by which genetic variation is translated into phenotypic variation (genotype–phenotype map *sensu*, Wagner and Altenberg, 1996). Consequently, the study of modularity is crucial to understand these developmental pathways.

Processes of shared function and development can act as an internal stabilizing selection force on the maintenance of the modular structure observed at the phenotypic level (Estes and Arnold, 2007; Porto *et al.*, 2013, 2009; Shirai and Marroig, 2010). This can be seen with a simple example: in almost all mammals the mandible and maxilla need to work together in order to function. Furthermore, these two bones share the same developmental origins. Thus, while a multitude of dietary habits exists, the shared internal development and function keep the two traits highly correlated in all mammals.

If we extend this example a bit further to four traits we can perhaps get a firmer grip on the origins of modularity in functional and developmental factors. Empirically, functional, and developmental integration can be measured by detecting the existence of groups of highly correlated traits. Under the hypothesis of modularity, one would expect that developmentally and functionally related traits would have a relatively higher correlation between them than the correlation among those without shared function or developmental origin/interaction (Cheverud, 1982). To illustrate this concept, we can look to **Figure 1** that presents four cranial traits measured in a bat skull and mandible. The first two traits, maxilla length and mandible length, are related to chewing function. Since both share the same function, it is expected that they present relatively higher correlation. In addition, these traits correspond to bones that share a common cellular origin in the neural crest, which reiterate the expectation of high correlation for both characters. Consider now the other two traits in **Figure 1**: frontal and parietal length. These two are not directly related with mastication but instead are primarily involved in brain protection. Moreover, these two bones share a common embryonic origin in the paraxial mesoderm cells. Accordingly, we would expect to find a high correlation between maxilla and mandible as well as a high correlation between parietal and frontal measurements. On the other hand, we would expect to find a considerably lower, or even absent, correlation between these two groups (**Figure 1**).

The Origin of Modularity and Integration from a Genetic Perspective

From a genetic perspective, pleiotropy and linkage disequilibrium are the two mechanisms behind modularity and integration (Falconer and Mackay, 1996). Linkage disequilibrium is the nonrandom association of alleles at different loci. In other words, it is the presence of statistical associations between alleles compared to what would be expected if alleles were independently, randomly sampled from the population. This process leads to nonrandom co-occurrence of different combinations of trait values associated with different alleles. The relative contribution of linkage disequilibrium for modularity patterns is debatable, since recombination might break these associations. However, linkage disequilibrium can be actively maintained through natural selection (Barton and Turelli, 1989; Templeton, 2006). Pleiotropy, on the other hand, has a more established role on the emergence of modularity (Wright, 1980). Pleiotropy is a common property of many genes, and occurs when a gene affects the phenotypes of two or more traits (Cheverud, 2004; Hodgkin, 1998; Wagner and Zhang, 2011). Traditionally, it was believed that widespread pleiotropy could be prejudicial to the adaptation process. The rationale behind this is that, the more complex an organism is (in terms of a higher dimensional trait space), the less likely a pleiotropic mutation will be advantageous. This is because the larger the number of traits affected by a particular pleiotropic loci, the more unlikely it is for the changes caused by a mutation to be advantageous in all

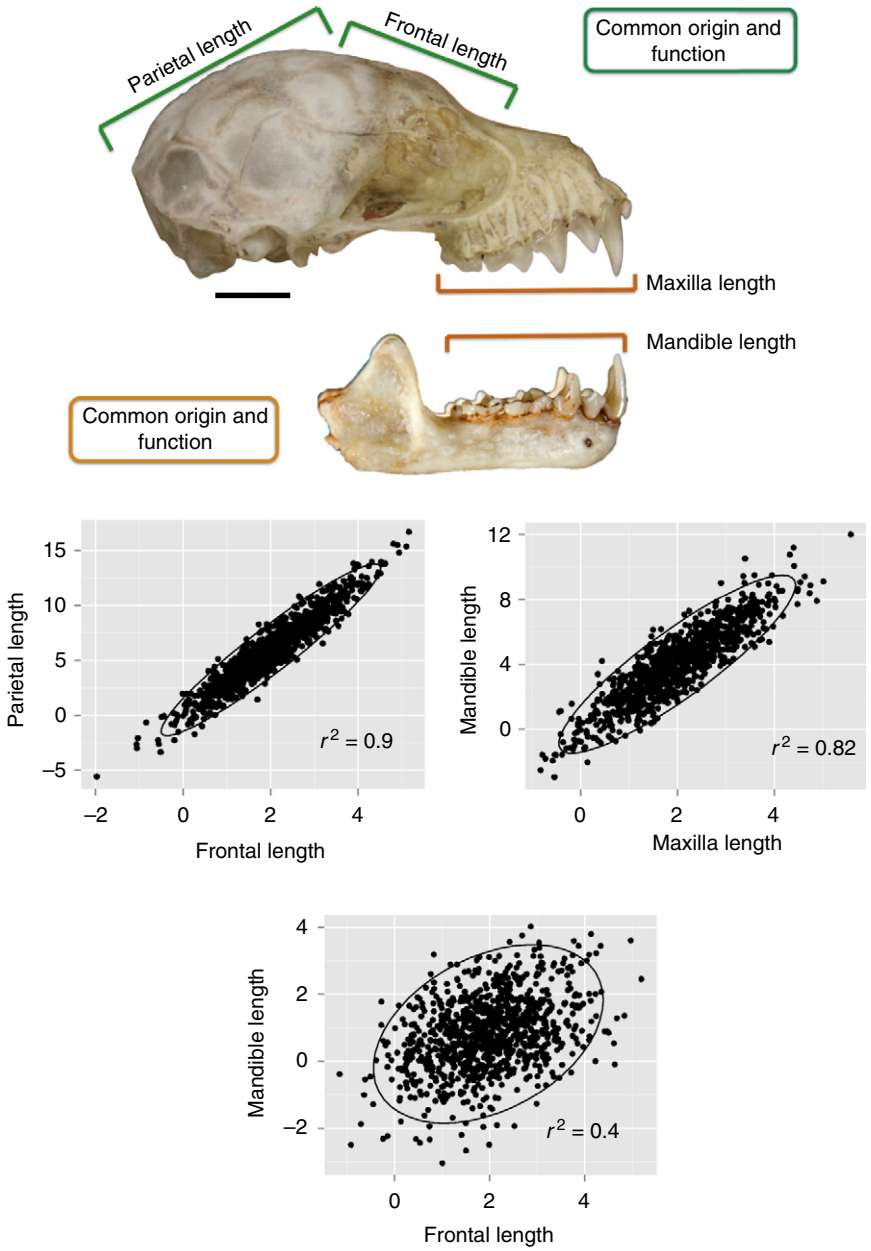

Figure 1 Hypothetical example of four cranial traits in a bat skull and mandible and their correlations. Note that developmentally and functionally related traits exhibited higher correlation. Green: parietal and frontal bones share a common embryonic origin in the paraxial mesoderm cells and are related in protect central nervous system. Orange: maxilla and mandible are related to chewing function and share a common cellular origin in the neural crest. Scale bar (black line) = 5 mm. Picture credits: *Daniela Rossoni.*

traits simultaneously; an issue known as 'the cost of complexity' (Fisher, 1930; Orr, 2000). From this point of view, the more complex an organism is, the more difficult it would be to respond to selection. Wagner (1996) was the first one to propose a model that could circumvent the complexity cost problem. He suggested that pleiotropic effects must be somewhat limited and related to function, creating modules of genetic effects that allow relative independence, in the same vein as the classical Olson and Miller modular organization. Indeed, there is considerable empirical evidence pointing to the emergence of modularity due to pleiotropic gene effects restricted to a set of functionally or developmentally related traits, a pattern known as modular pleiotropy (Cheverud, 2004, 1996; Cheverud *et al.*, 1997; Ehrich *et al.*, 2003; Leamy *et al.*, 1999; Mezey *et al.*, 2000; Pavlicev *et al.*, 2008; Vaughn *et al.*, 1999; Wagner *et al.*, 2007).

Pleiotropy can have both a constraining and a facilitating effect in the evolutionary process. It can be a constraint in the sense that the more traits a gene affects the more unlikely a mutation will be advantageous (Fisher, 1930; Orr, 2000). On the other hand, pleiotropy might be a facilitator of evolution, since populations whose individuals are organized in

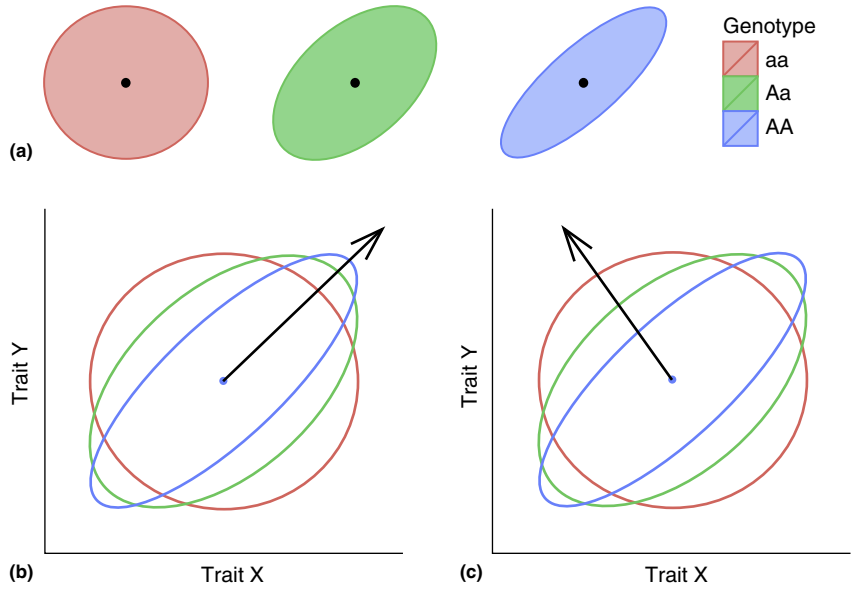

Figure 2 (a) Schematic representation of correlation between two traits for different genotypes. Genotype AA has a high correlation of 0.8 between trait X and Y; genotype *Aa* has a correlation of 0.5 and genotype *aa* has a correlation of zero between traits. The black dot in the center of each ellipse is the mean values for trait X and Y. (b and c) represent the three genotypes plotted together. In (b) we have selection (black arrow) favoring bigger individuals for both traits X and Y. In (c) we have selection favoring individuals smaller for trait X but bigger for trait Y. We might expect population b evolve in such a way to increase the positive covariation between traits and, therefore, we expect an increase of allele *A* frequency. We expect population c to evolve toward a decrease in the covariation between traits and, therefore, a decrease in frequency for allele *A* and increase for allele *a*. Adapted from Wagner, G.P., Pavlicev, M., Cheverud, J.M., 2007. The road to modularity. Nature Reviews Genetics 8 (12), 921–931.

developmentally and/or functionally restricted modules can respond to selection on a set of traits without perturbing the other sets (Griswold, 2006; Pavlicev *et al.*, 2011).

Also, pleiotropy itself can evolve, and for this there must be genetic variation in pleiotropic relations (Pavlicev *et al.*, 2011). This means that the relationship between traits can be genetically variable; and a possible source for this variation is a phenomenon called differential epistasis (Cheverud, 2004; Pavlicev *et al.*, 2011, 2008). Several studies have reported different loci presenting differential epistasis that are responsible for differences in the relationship between continuous traits. These loci are termed 'relationship Quantitative Trait Loci' or rQTL and refer to genomic regions that show variation in epistatic effects, altering pleiotropic relations and the correlation between phenotypic traits (Cheverud, 2004; Pavlicev *et al.*, 2011, 2008; Wolf *et al.*, 2005). To better understand this, let's imagine a rQTL loci with two alleles *A* and *a* (**Figure 2(a)**). Depending on the genotype, the covariation between traits X and Y is different, with a high positive correlation if the genotype is *AA*, a moderate positive correlation for genotype *Aa*, and no correlation between them for genotype *aa* (**Figure 2(a)**). As follows, we can say that the covariance between two traits depends on the genotype of this rQTL locus, although the genotype does not affect the traits means. If directional selection is favoring an increase for both traits (**Figure 2(b)**), we would expect allele A to increase its frequency in the population. This would happen because individuals with higher correlation between traits have higher values for both traits X and Y and would, therefore, be favored. On the other hand, if selection is acting for an increase of trait Y but a decrease of trait X (**Figure 2(c)**) we expect an increase of allele *a* frequency in the population. Again, the mechanistic reason for that would be that individuals *Aa* and *AA* have positive correlation between traits, which is been selected against in this case. It is easy to imagine how modular pleiotropy might appear in a population with this differential epistasis model: as long there are genetic variation in pleiotropy, natural selection can act leading to tighter or looser connections between traits (Pavlicev *et al.*, 2010).

Empirical Studies Investigating Modular Patterns

There is a vast literature on recognizing and characterizing modular patterns, especially concerning the mammalian skull. Thus, we will use the skull as a case study in order to exemplify the points raised earlier in this article. One of the forms of recognizing modules is by comparing correlation matrices from empirical data, and theoretical matrices based on hypotheses of functional/developmental relationships among characters. These theoretical hypotheses are strongly anchored on state-of-the-art literature about mammalian skull development (Cheverud, 1996, 1995; Moore, 1981; Smith, 1996, 1997, 2001). This methodology permits recognizing several different modules in different taxa, studied on a broad (orders and families), as well as on a more limited (as genus), phylogenetic framework (Ackermann and Cheverud, 2004; Cheverud, 1982; Marroig *et al.*, 2004; Marroig and Cheverud, 2001; Porto *et al.*, 2009, 2013; Shirai and Marroig, 2010).

From a developmental perspective, metatherian mammals (marsupials) present a similar modular pattern. Porto *et al.* (2009), analyzing five different orders of metatherians, reported a strong integration among facial traits, especially oral and nasal subregions. In contrast, the other 10 orders of eutherians mammals also evaluated by these authors exhibited a more variable modular pattern, with the vast majority of orders displaying a significant oral integration, but also with a broad contrast between neural and facial integration. These contrasting results between two mammals' infraclasses may reflect their developmental history, as metatherians present an early development of the facial traits because newborn survival depends directly on its ability to suckle (Smith, 1996). Differently, eutherians have more variation in neonatal states (having both highly altricial and precocial neonates) and a longer intrauterine growth (Smith, 2001, 1997). All these results indicate that shared development and function structures the current diversity of mammalian patterns of modularity/integration as expected by Olson and Miller (1958) hypothesis.

Another approach to the study of modularity is mapping QTLs in genomes. Along this line, one of the first direct evidences of modular genetic architecture organization came from the study of QTLs affecting different regions in the mouse mandible. Cheverud *et al.* (1997) used crossings between two inbreed lines of mice with very different sizes and a set of known markers to map genomic regions that affected linear distances measured on the mouse mandible. Using these experiments, they were able to show that most pleiotropic effects were restricted to one of the two regions in the mandible, the alveolar region and the ascending ramus, each related to different functions. The teeth are inserted at the alveolar region, while the ascending ramus is home to most muscle insertions. While both regions are related to mastication, the genetic effects are somewhat independent, with only 23% of the observed QTLs affecting both regions at the same time.

More recently, working with the same mouse strains as Cheverud, Kenney-Hunt *et al.* (2008) measured the number of shared QTLs between a series of skeletal traits. They expand the scope to include 70 traits, both in the skull and post-skull, painting a remarkable picture of the pleiotropic structure controlling skeletal development in mice. A total of 798 QTLs were identified, with many of the QTLs affecting more than one trait, indicating frequent pleiotropy. The authors used the information on pleiotropy to create a genetic effects adjacency matrix between traits, where traits that shared more pleiotropic QTLs were more related. This adjacency matrix was then compared to the correlation matrix between traits, and this showed a relatively high and significant correlation. These results suggested that phenotypic correlations are in part determined by shared pleiotropic effects.

On a larger scale, Wang *et al.* (2010) used a large dataset of yeast, nematode, and mouse mutants to show that the gene-trait relationship is highly modular, with most genes having small localized effects, and only a few genes having widespread pleiotropic effects and large effect sizes. This leads to an offset of Orr's cost of complexity, and allows for intermediate levels of complexity to exist via modularity.

Evolutionary Implications and Some Caveats

Studying the modularity, or the morphological integration of organisms, is fundamental to understand the evolution of complex features, as the modular structure influences multivariate evolution. The relationship between the inherited patterns of modular covariation and directional selection may, for example, restrict or facilitate certain evolutionary paths for a population. One way to appreciate the effects of genetic covariance upon the magnitude and direction of evolution is portrayed in **Figure 3** (adapted from Arnold *et al.*, 2001; Marroig and Cheverud, 2010). In this hypothetical adaptive landscape, three populations (a, b, and c) differ in their current position in relation to the adaptive peak. All three populations share the same basic genetic covariance pattern. Selection will push all three populations to the nearest adaptive peak, but the orientation of the selection gradient will differ due to their differences in current position on the landscape. While selection in all three populations specifies the shortest linear path to the peak, the realized evolutionary trajectory (Δz) from generation to generation may be quite different. In fact, if the two axes of major genetic variance are not aligned (population c) with the direction of selection, the evolutionary response to selection will be curvilinear (**Figure 3**, right panel). Furthermore, this curvilinear trajectory would be biased by the line of least resistance (defined as the linear combination having maximum genetic variance within a population, see (Schluter, 1996) embodied in the G-matrix. Because in this simple example (with only two dimensions) the first line of least resistance holds almost twice as much variation as the second one, the initial response in population c would be strongly biased in the direction of the largest genetic variance. It is also important to note how the line of least resistance influences not only the direction but also the magnitude of the evolutionary response along the path of selection. This point is made clear when comparing populations a and b. The magnitude of the response in population a is much larger than in population b. This reflects the fact that in population a, the first line of least resistance is aligned with the selected dimension; while in population b, the second line of least resistance is the one aligned with the direction of selection (β). The difference in response magnitude between a and b due to the variance differences even overcomes differences in strength of selection due to the fact that the path between a and the peak has a shallower slope than the path between b and the peak that is steeper and therefore reflects stronger selection. This example clearly shows that correlation among traits would bias the direction, influence the magnitude and pace of evolution on a microevolutionary (few generations) scale. Whether or not these modularity/integration patterns affect at macroevolutionary scales (species groups, generas, families, and so on) is an open question, but most biologists agree that such influence should decrease with time.

Finally, it is important to keep in mind that when looking at modularity/integration patterns at a given age (adults are by far the most commonly studied) we should interpret patterns as a product of a continuous development. Thus, the covariance observed in a population might not be a simple result of separate or discrete developmental factors. In fact, the development process influencing the covariance between characters

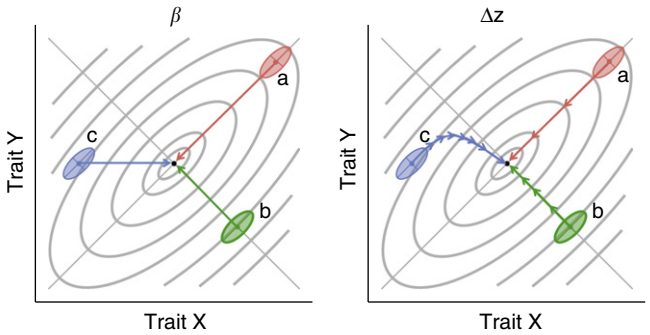

Figure 3 A hypothetical adaptive landscape for two traits (X and Y) with the black dot marking the adaptive optima (peak), and gray ellipses indicating isoclines of subsequent smaller fitness. Three populations (a, b, and c) are shown with their corresponding patterns of variance and covariance for both traits. The points inside each ellipse represent the mean value for the traits in that population. Left panel shows the direction of selection (β), and right panel the evolutionary trajectory (Δz). As we can see at the left panel, selection (β) is acting linearly in the three populations attracting them to the adaptive peak. Because each population is at a specific place in the adaptive landscape, the response to selection will be different in each population (right panel). Population a, which has its major axis of variance (represented by the longest axis of the ellipse) aligned with the adaptive landscape, will have a linear and fast response to selection. In few generations (represented by the number of arrows in the graph) it will reach the adaptive peak. Population b has its second axis of variance aligned with the adaptive landscape. Therefore, the response to selection will also be fast and linear, although not as fast as in population a. On the other hand, population c, which axis of variation is not aligned with the adaptive landscape, will take more generations to reach the adaptive peak and the trajectory will be deflected by the pattern of covariance. Adapted from Arnold, S.J., Pfrender, M.E., Jones, A.G., 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112–113, 9–32; Marroig, G., Cheverud, J.M., 2010. Size as a line of least resistance II: Direct selection on size or correlated response due to constraints? Evolution 64 (5), 1470–1488.

observed at the population level is variable over time and space during ontogeny and, hence, later factors can overlap and obscure the signal of earlier factors affecting the covariance structure. The combined effect of these developmental processes suggests viewing the covariance as a palimpsest (Hallgrímsson *et al.*, 2009) where the underlying determinants of integration and modularity cannot be easily decipherable from the covariance or correlation data. Furthermore, distinct developmental factors per se are most likely not independent but instead might present various degrees of correlation among them in a hierarchical way, and thus one should not expect that modularity patterns in a population of adult organisms would be a simple amalgamate or sum of individual components parts. Thus, modules still need to be integrated in larger hierarchical functional complex structures (like the mammalian skull) and the process of growth during development is one, if not the most important, agent of such integration (Porto *et al.*, 2013). Yet, the study of variational modularity patterns can give us clues of these underlying developmental factors, since it is informative for many aspects of its underlying genetics and is critical for our comprehension of organismal evolution.

See also: Modularity and Integration in Evo-Devo. Multivariate Quantitative Genetics. Quantitative Trait Variation, Molecular Basis of. Systems in Evolutionary Systems Biology

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Modularity and Integration in Evo-Devo

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Glossary

Covariation Correlated variation of two or more variables.

Homology Similarity between organisms in DNA sequence, protein, or structure due to common ancestry.

Module A distinct unit that can be combined with other units to form a larger system.

Pleiotropy The phenomenon occurring when one gene affects more than one phenotypic trait.

Regulatory network A collection of regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins. The regulator can be DNA, RNA, protein, and their complex.

Organisms are comprised of a series of relatively independent anatomical modules, which are internally closely linked but interact less consistently with other modules. Modularity is thus essentially the result of integration among subparts of the system and therefore modularity and integration are closely related concepts (Olson and Miller, 1958). In organismal biology, study of the factors that shape these modules during development, and the impact of these modules on morphological evolution, has emerged as a primary research area in evolutionary developmental biology.

The integration among adult structures is determined by the many complex biological processes that produce the structures, therefore analysis of modularity and integration has been approached from different perspectives focusing on different levels of integration or particular developmental or functional processes. As such, the concept is rather flexible in the literature and several definitions of modularity are used commonly. Structural modularity refers to anatomical units that are cohesive tissues located close together. Functional modularity refers to body parts that interact to carry out a particular function but may not be located adjacent to each other. Developmental modularity refers to a part of the embryo that differentiates from the same tissue during development. Sometimes these modules coincide; for example, anatomical units are produced by developmental processes, therefore developmental integration may influence the patterns of anatomical covariation later in life (**Figure 1**). Modularity can also refer to integrated systems at the molecular level, through conserved interacting gene or protein networks (Raff and Sly, 2000).

Indeed, by linking different levels of biological organization, the concepts of integration and modularity aid in understanding of the developmental basis of morphological evolution (Klingenberg, 2008). Integration among developing parts leads to biased production of certain variants (e.g., different bone shapes or organ morphologies) in a population, which restricts the variants available for selection and thus biases evolutionary patterns. The concept of homology may be linked to modularity, as identifying homologous organs, for example, requires that an integrated unit is detectable as a homologous feature in different organisms (Minelli, 2015; Reippel, 2005).

Measuring Integration and Modularity

While the concepts of modularity and integration are ubiquitous in the field of evolution, there is no general consensus on either the meaning or the measurement of morphological integration. All the techniques aim for a similar goal – assessing covariation. Many different approaches have been used, but a general strategy to discover modules comes from comparisons among different subsets of the total set of traits under scrutiny. Trait subsets that show stronger covariation can be considered part of the same module. Integration and module detection is thus a matter of degree, not absolute linkage or independence.

Variational modules have been detected using a number of statistical methods, primarily involving comparing magnitude of correlations and covariance in identified traits (Klingenberg *et al.*, 2003; Leamy *et al.*, 1999; Marquez, 2008; Mitteroecker and Bookstein, 2007; Polly *et al.*, 2001; Klingenberg 2009; Hallgrimsson *et al.*, 2009). Differences among methods are partly due to choice of clustering and filtering methods by the researcher seeking to emphasize particular processes (Klingenberg, 2008).

Important model systems that have been used in the development of theory and technique for detecting modularity and integration include the mouse lower jaw (Zelditch *et al.*, 2008; Cheverud, 1982), mammal skulls (Goswami *et al.*, 2009, 2014), appendages/limbs (Minelli, 2000; Shubin *et al.*, 2009; Kavanagh *et al.*, 2013), butterfly wingspots (Brakefield, 2003), and vertebral regions (Polly *et al.*, 2001; Burke *et al.*, 1995; Buchholtz, 2014).

Because many of these measures require a priori division of the organism into potential modules, there is often some level of investigator bias in these methods of estimating modularity. In any measure, modularity is a sum measure of the degree of integration across the selected structures in these techniques.

The direct causative mechanism or regulatory network is most often unknown in these comparative morphological studies and indeed may differ among structures. However, in a few cases, *in situ* experimental measurement of integration has been attempted.

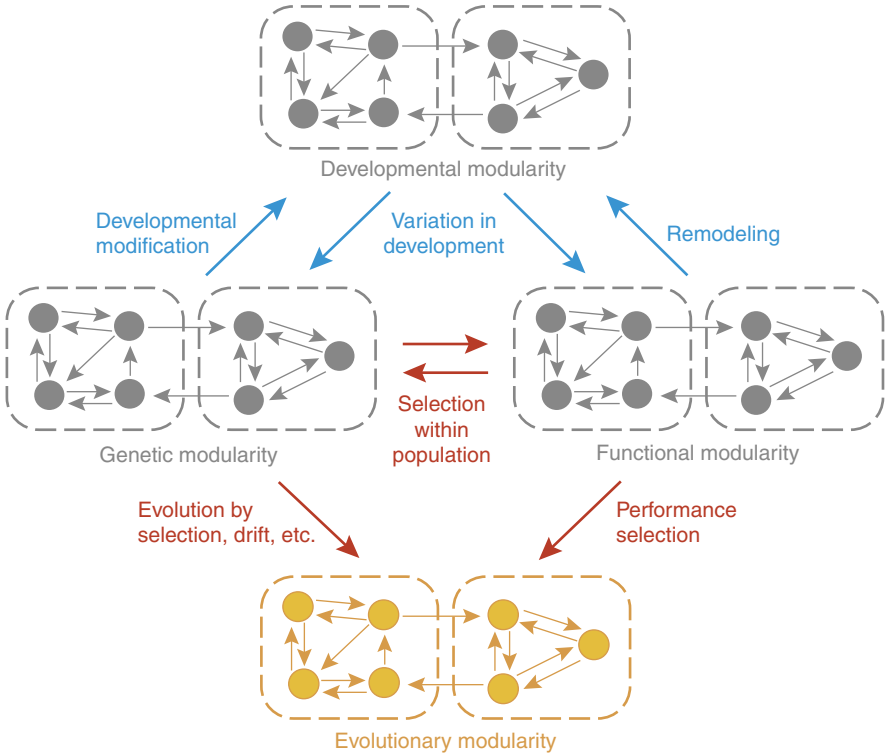

Figure 1 Types of modularity that concern morphological variation and the connections between them. Developmental, genetic, and functional modularity are based on processes taking place within extant individuals or populations (gray diagrams), whereas evolutionary modularity results from the history of divergence among evolutionary lineages in an entire clade (orange diagram). These types of modules are mutually influencing each other through various processes within individuals (blue arrows) or within populations (red arrows). Developmental modularity has an effect on both genetic and functional modularity by modulating the available morphological variation. Genetic modularity feeds back to developmental modularity through the genetic control of development, whereas processes of tissue remodeling (e.g., bone remodeling under mechanical load) provide a feedback from function to developmental processes. At the population level, the process of natural selection establishes a two-way interaction between genetic and functional modularity. These effects of selection, jointly with the additional effect of genetic modularity on changes by drift, accumulate through time to constitute the evolutionary modularity of an evolving lineage or clade (from Klingenberg, 2008).

Experimental Analysis and Direct Measurement of Integration

More direct measurements of integration have been attempted through developmental experimental manipulations that perturb one part of a system and observe the consequent effects on downstream elements or structures. If the perturbation is genetic, as in a genetically modified mouse, for example, the effect could indicate pleiotropy (Cheverud, 1996). Another method used is direct embryonic manipulation at particular stages or times. Protein bead or foil barrier interference experiments within modules may show coordinated effects. For example, foil barriers that perturb proximal joint-positioning fields within the phalanges were shown to affect positions of later-developing distal joints downstream, indicating a system-wide or modular effect (Kavanagh *et al.*, 2013).

Selection experiments are another type of experimental method to detect modular structure. If selection for one trait causes coincident evolution of another trait, then it may be interpreted as evidence of modularity in regulatory structure. To get a sense of the level of integration, experimental selection to break apart modules can be measured as more or less difficult. For example, butterfly wingspot sizes in fore and hindwings evolve in coordination in nature, modularly, and it was only possible after many generations of disruptive selection in the laboratory for the wingspot sizes on the forewing and hindwing to be independently selected (Beldade and Brakefield, 2003).

Interpretation is similar in all of these cases, where alteration of an element within the module (by evolution or developmental perturbation) has greater effects on the rest of the elements in the module than on elements of other modules. Furthermore, organisms can show a nested hierarchical modularity, such that there can be modules within modules with varying degrees of integration, for example, digits within limbs (Young *et al.*, 2015).

Evolution May Favor Modularity; Modularity May Bias Evolution

Modularity itself may be a consequence of biological evolution; it is observed that other types of networks such as electrical or computational networks do not necessarily evolve modularity, while biological systems inevitably do (Kashton and Alon, 2005). Evolution proceeds primarily by tinkering with the same conserved genes, genomic regions, protein interactions, developmental networks, dynamic patterning

modules (Newman and Bhat, 2008), and tissue mechanics from generation to generation. Because it is the 'path of least resistance,' biological evolution appears to be biased toward conserving these functional integrated connections. Therefore, modular differential regulation of body parts may be an inevitable result of organisms under selection.

Viewed from another perspective, such modular construction may have consequences on how selection shapes adaptations to changing environments. Because individual modules can be selected semi-independently, the entire organism can remain functional while selection acts on particular networks, organs or structures. For example in birds, forelimbs have been modified independently of hindlimbs to evolve wings while still maintaining walking legs. Since modularity and integration occurs at many organizational levels, adaptations can be many and varied, yet still biased along lines of least developmental resistance. As such, it is no wonder that the concepts and consequences of modularity and integration pervade all aspects of evolutionary biology (**Figure 1**).

See also: Gene Networks Driving Development, Conservation and Evolution of. Modularity and Integration. Systems in Evolutionary Systems Biology

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Molecular Evolution, Functional Synthesis of

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Glossary

Adaptive landscape The relationships between genotype, phenotype, and fitness.
Ancestral sequence reconstruction The inference of ancestral gene sequences at the nodes in a phylogenetic tree from the gene sequences at the tips.
Clinal polymorphisms Allele frequencies change over geographic space.
Comparative method The inference of process based on observational data collected from different taxa across space and time.
Directed evolution Process of goal-oriented laboratory adaptation through repeated rounds of mutagenesis and selection.
Dykhuizen–Hartl effect The phenomenon whereby mutations that are selectively neutral in one environment, or in one genetic background, are subject to selection in others.
Ecological genetics The study of adaptation in natural populations.
Epistasis Nonadditive interactions among mutations.
Functionalism The experimental study of mechanistic proximate causes.
Maximum parsimony A method for constructing phylogenetic trees using characters that are both shared (two or more species/genes have it) and derived (have arisen by mutation within the phylogenetic tree).
Subfunctionalization The process by which specialist genes arise following duplication of a generalist ancestor through the complementary degenerate loss of ancestral functions.

Background

There exists a deep rift within biology, evident even in Darwin's time, between functional biology (which studies the 'how?') and evolutionary biology (which studies the 'why?') (Mayr, 1961). Functionalists are interested in proximate causes (how does a bird's wing generate lift?), whereas evolutionists are interested in ultimate causes within a historical context (what is the origin and adaptive significance of a bird's wing?).

The rift is deepened further by the very different approaches taken to resolving scientific questions. Functionalists consider the relationship between cause and effect to have been established only when all other variables in an experiment have been held constant. Necessarily, their studies are conducted in controlled laboratory settings to eliminate extraneous noise. Though causality can be established in these unnatural circumstances, its biological relevance is often unclear to evolutionists.

Evolutionists forgo the rigors of functional biology and embrace nature's noise – variability within and between individuals, species, and habitats – as the stuff that needs to be explained. Their approach is comparative, identifying patterns and associations in data gathered from field surveys. Unfortunately, statistical associations alone cannot establish causality and predictions from divergent hypotheses often cannot be distinguished. This ambiguity leads many functionalists to regard evolutionary biology as unscientific.

The first half of the twentieth century saw the rise of a general mathematical theory of evolution, one consistent with known facts and data, yet one necessarily detached from the then unknown relationships between gene structure and function, between genotype and phenotype. By midcentury, many evolutionists had concluded that functional biology could only add detail to the big picture already painted by evolutionary biology (Morange, 2011).

That soon happened – spectacularly – as new molecular approaches swept through biology to reveal the structure of the gene, the genetic code, protein structure–function relationships, metabolic and intracellular architecture, etc. Ignored in the stampede for proximate mechanisms were larger questions of evolutionary causation. Dobzhansky (1973), in particular, recoiled at the rampant reductionist thinking and declared, "Nothing in biology makes sense except in the light of evolution ..."

Evolutionary biology was not untouched by these developments. Molecular biology provided first protein and then gene sequences to revolutionize phylogenetics and population genetics (Zuckerkandl and Pauling, 1965). Yet even as they adopted the techniques of molecular biology, evolutionists rejected functionalism in favor of their comparative approach (Graur and Li, 1999). From the 1980s onwards, statistical analyses of sequence data, and today of entire genomes, would come to dominate studies of molecular evolution.

Setting the Stage

Functionalism was not entirely absent from evolutionary biology. In the 1950s, Kettlewell provided direct experimental evidence that differential predation by birds was responsible for maintaining the melanic (dark) and peppered forms of the moth *Biston betularia* (Kettlewell, 1973). During the 1970s, a small band of ecological geneticists pursued functional studies on clinal enzyme polymorphisms, attempting to establish relationships between kinetic activity, physiology, and fitness components (reviewed in Hedrick *et al.*, 1976,

 doi:10.1016/B978-0-12-800049-6.00233-X

1986). Their results were tantalizing, but technical limitations severely hampered progress. Laboratory experiments with *Drosophila* faced similar difficulties.

The ability to construct strains of *Escherichia coli* that differ only at specific loci enabled Dykhuizen and Hartl to demonstrate that enzyme polymorphisms might be selectively neutral in some genetic backgrounds and environments and yet selected in others (Dykhuizen and Hartl, 1980; Hartl and Dykhuizen, 1981). When combined with metabolic control theory (Kacser and Burns, 1981; Hartl *et al.*, 1985), their approach culminated in an exact mechanistic model of fitness, one that could be used to predict the direction and intensity of selection solely from knowledge of enzyme kinetics (Dean, 1989). The old criticism that 'the survival of the fittest' is a mere tautology could be laid to rest. Darwinian fitness can be predicted from biochemistry alone (**Figure 1**).

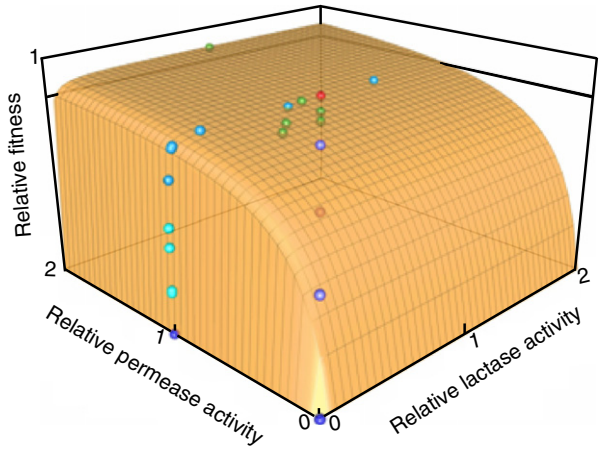

Figure 1 The adaptive landscape of *lac* operon. The surface is not fitted to the fitness data, but is instead the prediction based solely on knowledge of biochemical kinetics. Wildtype K12 *lac* operon (red), laboratory mutants of β-galactosidase (blue), and evolved β-galactosidase (light blue), varied expression of wildtype in competition with a constitutive *lacI*$^-$ *lac* operon (purple) and six operons from natural isolates (green).

However, the precise connection between gene sequence and biological function was still tenuous. In the early 1980s, Hall (1984) showed that either of two single amino acid replacements conferred sufficient activity on evolved β-galactosidase, a secondary β-galactosidase in *E. coli*, to allow growth on lactose. In another study, a comparison of protein structures led Holbrook and coworkers to identify a single amino acid replacement that, when introduced into the active site of *Bacillus stearothermophilus* lactate dehydrogenase (using the then new technique of site-directed mutagenesis), produced a malate dehydrogenase as active and as specific as any natural homologue (Wilks *et al.*, 1988). That such massive functional changes could be wrought by single amino acid replacements suggested that functional changes during molecular evolution might proceed in similar fashion. The classical evolutionary idea of gradualism, enunciated by Darwin himself, that adaptation proceeds slowly, was dead.

Rise of the Functional Synthesis

The 1980s had seen the tools of phylogenetics and molecular biology develop to the point that hypotheses generated by comparative sequence analyses could be tested decisively using functionalist experiments. Work during this period had undermined much conventional wisdom and, with the straightjacket of NeoDarwinian dogma ripped apart, the Functional Synthesis was free to emerge in the 1990s as a means to explore the mechanistic foundations of molecular evolution.

Benner and coworkers (Stackhouse *et al.*, 1990) were the first to resurrect ancestral proteins (**Figure 2**). They chose to study RNase A, a digestive enzyme abundant in certain artiodactyls. Using parsimony, they inferred the ancestral sequences of *Pachyporrux lutidem*, the extinct ancestor of modern ox and buffalo, and several other ancestors. The ancient genes were synthesized by cassette mutagenesis, expressed in *E. coli*, and purified. Consistent with neutral evolution, the kinetic properties and thermostabilities of these ancient enzymes were indistinguishable from their modern counterparts.

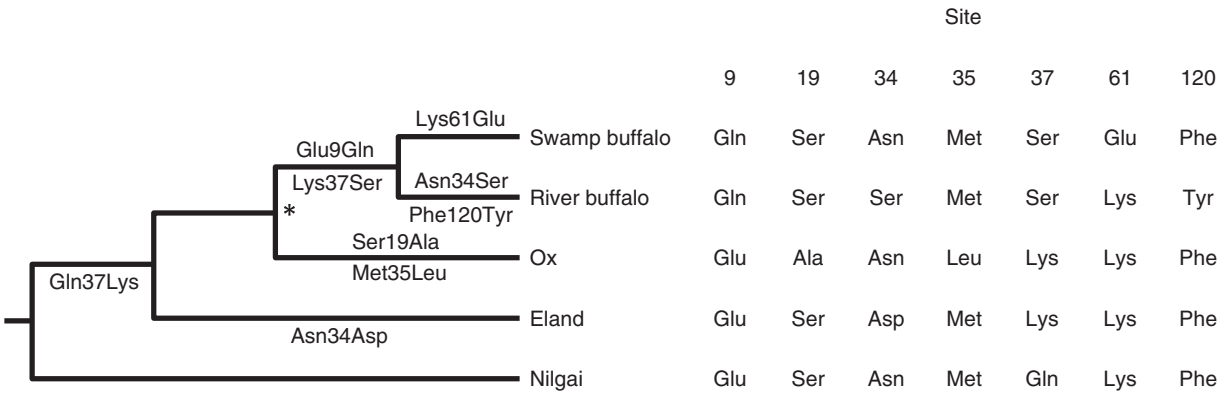

Figure 2 Maximum parsimony phylogeny and amino acids at variable sites in artiodactyl RNAses studied by Stackhouse *et al.* (1990). The marked node (*) was the first ancestral gene ever to be resurrected: Gln9, Ser19, Asn34, Met35, Lys37, Lys61, Phe120.

This article brought to fruition the dream of Zuckerkandl and Pauling (1965) that,

> By, furnishing probable structures of ancestral proteins, chemical paleogenetics will in the future lead to deductions concerning molecular functions as they were presumably carried out in the distant evolutionary past.

The Functional Synthesis was born.

Later, Jermann *et al.* (1995) would resurrect and express 13 ancestral genes using a broader artiodactyl phylogeny. Results showed a modest increase in thermostability and a fivefold decrease in activity toward double stranded RNA associated with the Gly38Asp replacement that appeared approximately 40 million years ago when ruminant digestion first arose.

At about the same time Yokoyama and Yokoyama (1990) used phylogenetic analysis to show that red visual pigments had evolved independently in both humans and Mexican cavefish, following duplications of their respective ancestral green visual pigments. Three amino acid replacements, Ala180Ser, Phe227Tyr, and Ala285Thr, were identical in both lineages (**Figure 3**). The probability of such a parallelism occurring by chance is so remote that the replacements were almost certainly adaptive. Yokoyama and Yokoyama (1990) wrote:

> Molecular characteristics of the visual pigment genes in different species provide valuable information as to which residues are important for specific wavelength absorption. Fortunately, it will be possible to test such hypotheses of adaptive evolution by using site-directed mutagenesis at specific residues (such as 180, 277, and 285), expressing them in cultured cells and measuring their absorbance …

A year later, Neitz *et al.* (1991) determined the gene sequences and spectral properties of several closely related primate visual pigments and concluded that replacements Ala180Ser, Phe227Tyr, and Ala285Thr accounted fully, and additively, for the spectral shift. The following year Chan *et al.* (1992) used site-directed mutagenesis to show that a similar spectral shift could be engineered into rhodopsin and confirmed that the contributions were additive. Asenjo *et al.* (1994) constructed chimeric proteins and additional site-directed mutants to show that four additional replacements, each of small functional effect and not found in the evolution of cavefish visual pigments, were needed to fully interconvert the human red and green visual pigments. A strictly additive model accounted for 98% of the variation in maximum absorbance. The genetic basis of an adaptive shift in function had been established.

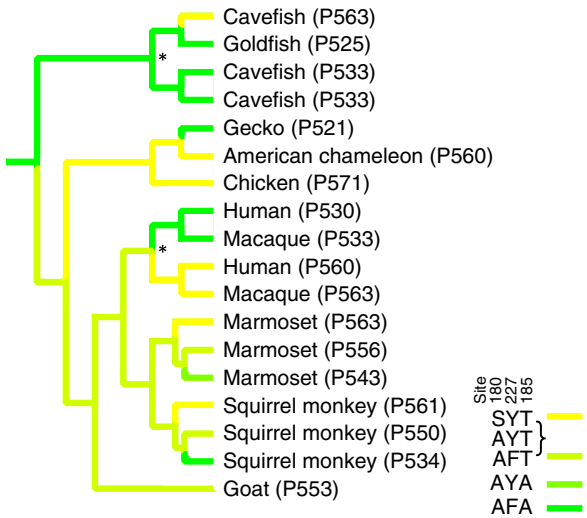

Figure 3 Spectral tuning in vertebrate medium wavelength visual pigments. Amino acid replacements occur in parallel and in reverse at sites 180, 227, and 185 are associated with specific shifts in the maximum absorption (in parentheses) of cone opsins. Such repeated changes, highly improbable due to chance alone, indicate that adaptive responses can be highly constrained. Asterisk denotes gene duplications.

The accuracy with which ancient gene sequences can be inferred declines with every inference made, with every site that changed and with every insertion and deletion incorporated. The nicotinamide adenine dinucleotide phosphate (NADP)-dependent isocitrate dehydrogenase of *E. coli* (EcIDH) differs from its NAD-dependent ancestor at 341 of 429 sites (Dean and Golding, 1997). Resurrecting this ancestor would be a dubious exercise.

X-ray crystallography identified the modes of coenzyme binding in EcIDH and in the distantly related, though structurally similar, *Thermus thermophilus* isopropylmalate dehydrogenase (TtIMDH) (Hurley *et al.*, 1991; Hurley and Dean, 1994). Six conserved amino acids bind the 2'-phosphate of NADP in EcIDH. At the same sites in TtIMDH three conserved amino acids bind the adenosine ribose hydroxyls of NAD, while three others play no role in binding NAD and vary freely in related NAD-dependent enzymes (**Figure 4**). Site-directed mutatgenesis was used to engineer EcIDH into an NAD-dependent enzyme (Chen *et al.*, 1995), one as active and as specific as any known NAD-dependent family member. TtIMDH was then engineered to use NADP (Chen *et al.*, 1996). Engineering in both directions eliminated 246 amino acid replacements at the 334 sites in common, and 91 sites comprising 13 insertions and deletions, as making any net contribution to coenzyme use. Identifying sites critical to a 3.5 billion year old functional change in isocitrate dehydrogenase (IDH) opened up the entire history of life to experimental investigations of biochemical evolution.

Epistasis and Ancestral Sequence Reconstruction

Epistasis was evident in early experiments in the Functional Synthesis. If introducing the active site Gln102Arg replacement converted *B. stearothermophilus* lactate dehydrogenase into a malate dehydrogenase, the reciprocal replacement in *E. coli* lactate dehydrogenase failed to produce an active enzyme (Nicholls *et al.*, 1992). Epistatic interactions with the genetic background can thwart identifying functionally important residues.

Epistatic interactions are common (Lunzer *et al.*, 2010) and arise within the complex web of physical interactions underlying protein architecture, solubility, conformational fluidity, specificity, and activity. Most amino acid replacements compromise protein stability and those causing functional changes are no exception (Tokuriki *et al.*, 2008; Soskine and Tawfik, 2010).

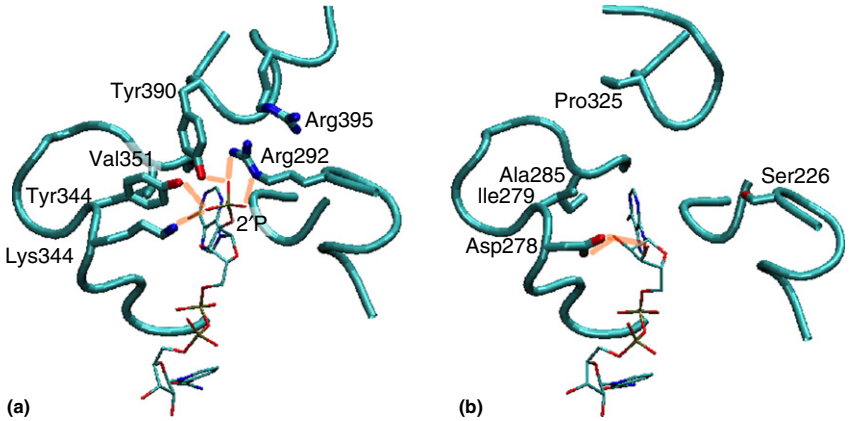

Figure 4 Coenzyme binding sites of (a) isocitrate dehydrogenase with bound NADP and (b) isopropylmalate dehydrogenase with bound NAD. Amino acids hydrogen bonding (transparent orange) to the coenzyme 2′-phosphate (2′P) in IDH are replaced in IMDH where Asp278 instead hydrogen bonds to the 2′ and 3′ hydroxyls of the adenosine ribose. Repulsion between the negatively charged Asp278 and the 2′-phosphate serves to further reduce specificity toward NADP in IMDH. Color code: carbon (cyan), oxygen (red), nitrogen (blue), and sulfur (yellow).

Indeed, replacements necessary for a functional change can be so destabilizing that no folded protein is produced. Using directed evolution, Bloom *et al.* (2006) showed that prior selection for increased stability of cytochrome P450 BM3 was essential to the subsequent acquisition of novel activities.

Ancestral sequence reconstruction provides a powerful approach to identify causal mutations when background epistasis confounds our engineering modern proteins. The idea is to recapitulate the evolutionary course that nature itself took. First, the branch in the phylogenetic tree on which the functional change occurred is identified. Then the ancestral gene at the base of the branch is synthesized. Mutations on that branch are then introduced singly, and in combination, into the ancestral gene. Finally, the proteins are expressed, assayed, and the mutations causing the functional change identified.

Using this approach Thornton and coworkers (Bridgham *et al.*, 2006) explored how subfunctionalization in an ancient steroid receptor led to the modern glucocorticoid receptors. They identified two replacements, Ser106Pro and Leu111Gln, that together restricted activation of the ancestral receptor to cortisol alone (**Figure 5**). Introducing the same pair of replacements into a modern paralogous receptor abolished function. Functionally important replacements are best identified in the genetic background on which they first arose and for this very reason ancestral gene reconstruction has become *de rigueur* when reconstructing the history of functional changes.

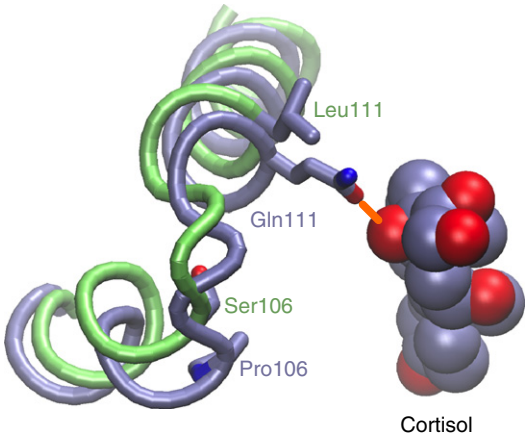

Figure 5 Snapshots of molecular dynamics simulations of cortisol bound to homology models of ancestral (green) and descendent (gray) glucocorticoid receptors showing the hydrogen bond between Gln111 and cortisol which is retained despite Pro106 occupying a variety of positions. Color code for side chains and cortisol: carbon (gray), oxygen (red), nitrogen (blue), and sulfur (yellow).

Some Consequences of Epistasis

Sign epistasis, in which the same mutation has opposite effects in different genetic backgrounds, limits the number of mutational pathways to high fitness (Weinreich *et al.*, 2006). Resistance by *E. coli* to high levels of the antibiotic cefotaxime is conferred by five replacements in TEM-1 β-lactamase, many of which interact in nonadditive fashion. For example, the Met182Thr replacement reduces resistance in wildtype TEM-1, but increases it when the Gly238Ser replacement is present. Weinreich *et al.* (2006) constructed all $2^5 = 32$ combinations of residues and showed that epistasis limited the number of pathways where resistance was increased at each step to just 18 out of the possible $5! = 120$.

Structural studies revealed the mechanism generating epistasis among mutations leading to the glucocorticoid receptors (Ortlund *et al.*, 2007; **Figure 5**). Alone, replacement Leu111Gln has little effect on structure and function and the mutated ancestor remains sensitive to both aldosterone and cortisol. Alone, replacement Ser106Pro severely impairs sensitivity toward both steroids, repositioning a helix in the binding cleft to impede steroid binding. In the double mutant, the repositioned helix enables the amide of the Leu111Gln replacement to hydrogen bond to the hydroxyl unique to cortisol, restoring sensitivity to this steroid alone.

Sign epistasis can delay an adaptive response. The neutral Leu111Gln replacement must have occurred first and drifted for some time before the Ser106Pro replacement produced the

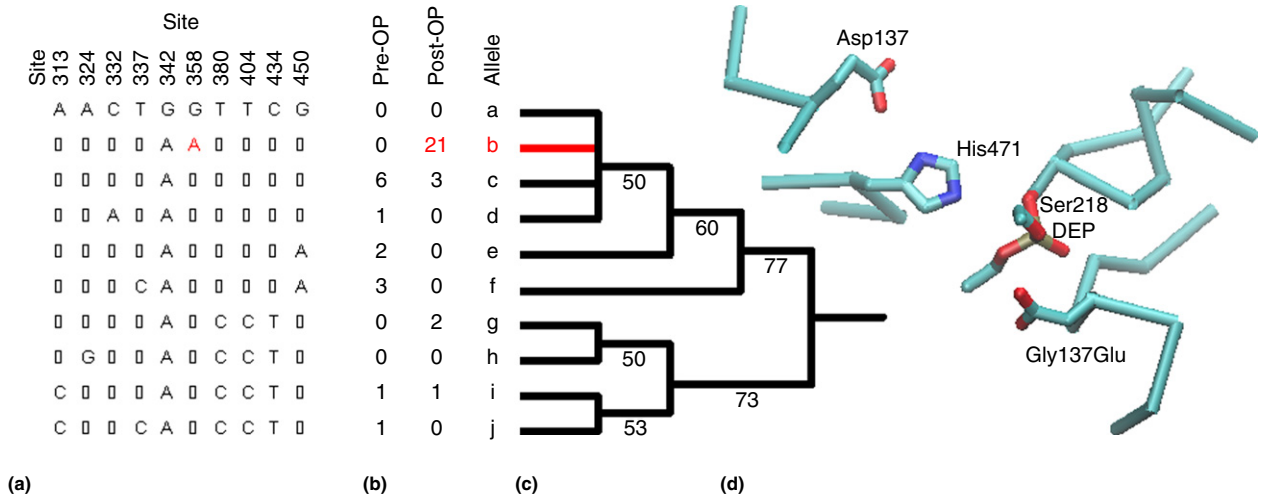

Figure 6 Evolution of blowfly insecticide resistance. (a) Variable sites in the gene sequences of *Lucilia cuprina* α-esterase 7. The diazinon resistance conferred by amino acid replacement Gly137Asp is caused by base substitution G358A (red). Amino acid replacements caused by base substitutions at sites 313, 324, 337, and 450 have no effect on the ability to hydrolyze organophosphates. (b) Allele frequencies before and after diazinon was introduced to Australasia. (c) Genealogy of alleles shows that diazinon resistance (red) arose recently. (d) The active site of organophosphate inhibited blowfly α-esterase 7 showing the catalytic triad (Asp137, His471, and Ser218) with diethylphosphate covalently attached to the catalytic Ser218 (Jackson *et al.* 2013). Modeled is the Gly137Asp replacement that converts fatty acid methyl esterase activity into an organophosphate hydrolase activity to confer resistance to the organophosphate pesticide diazinon, perhaps by activating a water (not shown). Color code: carbon (cyan), oxygen (red), nitrogen (blue), and phosphorous (yellow).

first glucocorticoid receptor. In the conger eel visual pigment RH1 each of three amino acid replacements barely affects maximum absorbance, yet together they cause a shift from green to blue (Yokoyama *et al.*, 2008). Here, epistasis need not have affected the order in which the replacements accumulated but, as in the steroid receptor example, the selective pressure for functional change probably arose long before an adaptive response was made possible.

Zuckerkandl and Pauling (1965) first suggested that selectively neutral mutations might be needed to bring a gene to the point where a functional change is possible. Dykhuizen and Hartl (1980, 1981) demonstrated that many selectively neutral alleles possess a latent potential for selection that is expressed in certain genetic backgrounds and/or in certain environments. Known as the Dykhuizen–Hartl effect (Kimura, 1983), this phenomenon is key to several models of functional adaptation. The innovation period of the innovation–amplification–divergence model of gene duplication (Bergthorsson *et al.*, 2007) assumes weak promiscuous enzyme activities drift in a neutral fashion until a mutant arises with sufficient activity that selection can act. The same idea of a neutral walk applies when replacements that stabilize protein structures beyond current needs are required for future functional changes, as demonstrated with P450 BM3 (Bloom *et al.*, 2006) and TEM-1 β-lactamase (Bershtein *et al.*, 2006).

Causes of Selection

The Functional Synthesis identifies mutations causing functional changes. Most often the functional changes are assumed to be adaptive. However, *demonstrating* that a functional change is adaptive is rather more involved.

Ecological geneticists have the advantage of being able to observe selection in action, and hence the possibility of decisively assigning a selective cause. For example, Oakshott and coworkers (Newcomb *et al.*, 1997) showed that the Gly137Asp replacement converts the E3 esterase of the blowfly *Lucilia cuprina* into an organophosphate hydrolase that confers resistance to diazinon (**Figure 6**). In the absence of diazinon, the Gly137Asp replacement is costly owing to pleiotropic developmental instability (Batterham *et al.*, 1996). Indeed, the Gly137Asp replacement only reaches high frequencies in the presence of a modifier locus that alleviates the developmental instability. PCR analysis of museum specimens of *L. cuprina* collected in Australia before 1958, when diazinon was first introduced, found no evidence of the Gly137Asp replacement (Hartley *et al.*, 2006). The first cases of resistance were detected in 1965 (Shanahan and Hart, 1966). By the early 1970s resistance had reached 95%. Consistent with the rapid sweep of diazinon resistance is the low genetic diversity and strong linkage disequilibrium evident at the E3 locus in modern populations (Rose *et al.*, 2011). These and other studies (Linnen *et al.*, 2013; Barrett *et al.*, 2008; Schluter *et al.*, 2010; Zera, 2011) provide coherent pictures of selection in action, having established chains of causality between mutations, molecular phenotypes, organismal fitness, and the resulting impact on the genetic architectures of extant populations.

Assigning a selective cause to an ancient functional change is challenging. We cannot observe the selection as it happened. The organisms in which the change arose are extinct and the habitats and ecosystems in which they lived no longer exist.

Studies with IDH push the limits of the Functional Synthesis in their attempt to assign an ancient selective cause (Zhu *et al.*, 2005). The engineered NAD-dependent IDH is selectively inferior to the wildtype NADP-dependent IDH

during competition for acetate, an oxidized carbon source where additional reducing power, in the form of reduced NADPH, is needed for biosynthesis. As expected, selection at IDH intensifies as other sources of NADPH are deleted from the genetic background. Genome surveys show that all species capable of growing on acetate have an NADP-dependent IDH. Those that have only an NAD-dependent IDH cannot grow on acetate.

Was the shift in coenzyme use from NAD to NADP in an ancient eubacterial IDH an adaptation to growth on acetate? Short of a means to travel back 3.5 billion years in time we can never know for certain. What we can say is that results from phylogenetics, genomics, structural biology, microbial metabolism, protein engineering, enzyme kinetics, and experimental evolution are consistent with the hypothesis.

Adaptive Constraints

The engineered TtIMDH is as active and specific toward NADP as the wildtype enzyme is toward NAD. Why then has no natural isopropylmalate dehydrogenase (IMDH) ever used NADP? The question is made all the more intriguing by the fact that the related IDHs evolved NADP use on at least two occasions (Dean and Golding, 1997).

Characterization of the adaptive landscape controlling coenzyme use by EcIMDH (**Figure 7(a)**) indicated that the reduced fitness of the engineered NADP-dependent EcIMDH might be attributable to product inhibition by abundant cellular NADPH (Lunzer *et al.*, 2006). Directed evolution experiments recovered mutations that weakened NADPH binding, with NADP binding weakened as a correlated response (Miller *et al.*, 2006). Changes in NAD and NADH binding were small and random. Increased growth rates always corresponded to decreased product inhibition by NADPH even at the expense of activity. Inhibition by NADPH alone reduced the fitness of the engineered NADP-dependent enzyme.

The close proximity of the reduced nicotinamide rings of the coenzymes to the γ-isopropyl of the substrate/product (**Figure 7(b)**) renders NADH and NADPH potent inhibitors of IMDH (Dean and Dvorak, 1995). Cellular NADH is sufficiently scarce that wildtype IMDH retains high activity *in vivo*. Cellular NADPH is so abundant that virtually all the engineered NADP-dependent EcIMDH is inhibited (Lunzer *et al.*, 2006; Miller *et al.*, 2006). This inhibition explains why no IMDH has ever evolved the capacity to use NADP. In contrast, NADPH is a weak inhibitor of IDH because there is no attractive force between the uncharged ring and the adjacent negatively charged γ-carboxylate of the substrate/product (Dean and Koshland, 1993). Hence, IDH has always been free to evolve NADP use. The pattern of functional constraint and evolution across 3.5 billion years of life finds its explanation in terms of just two physiochemical interactions (the one hydrophobic and the other electrostatic) and a metabolic demand for additional reducing power during growth on acetate.

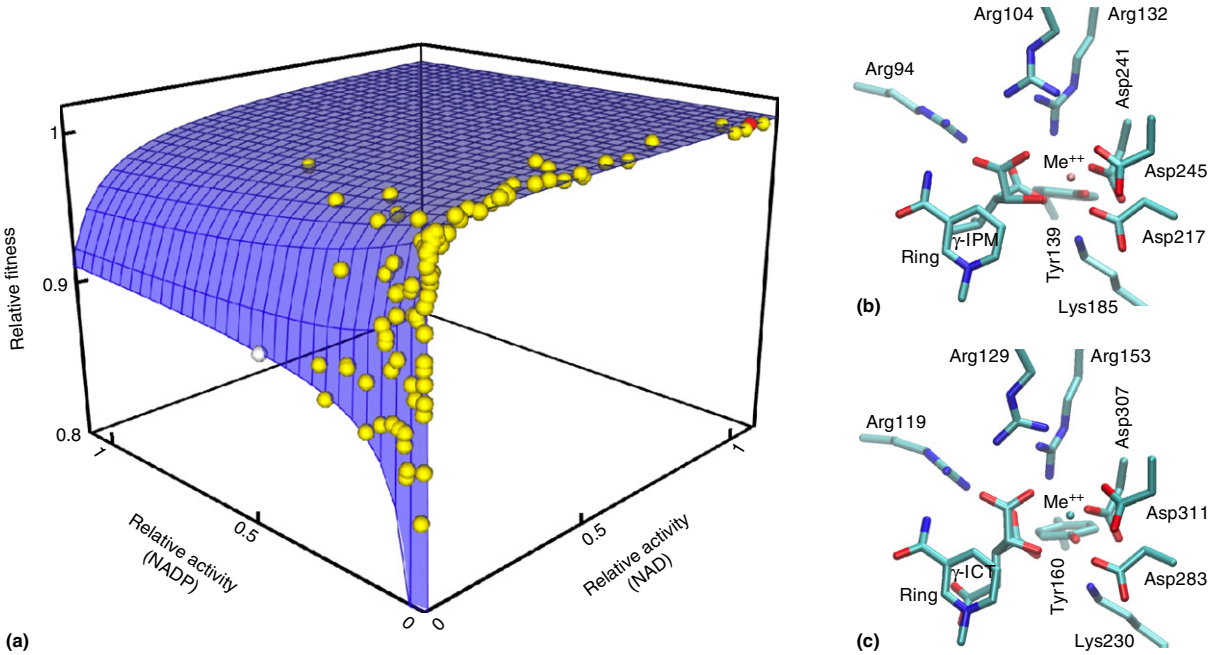

Figure 7 Constraint in the IMDH adaptive landscape. (a) The adaptive landscape controlling coenzyme use in IMDH shows that NADP use (white) confers lower fitness than NAD use (red) with genetic intermediates (yellow). The active sites of (b) *Thermus thermophilus* isopropylmalate dehydrogenase and (c) *Escherichia coli* isocitrate dehydrogenase with bound substrates and coenzymes (Pallo *et al.*, 2014; Gonçalves *et al.*, 2012). In both views the coenzyme nicotinamide rings are poised 'above' the γ-groups of the substrates. Catalytic reduction to NAD(P)H eliminates the charge on the ring. The resulting hydrophobic interaction with the γ-isopropyl renders the reduced coenzymes potent inhibitors of IMDH. In IDH, ring reduction eliminates the salt bridge to the γ-carboxylate. The reduced coenzymes now act as weak inhibitors (Dean and Koshland, 1993).

Mechanisms of Gene Duplication and Functional Divergence

How enzyme families arise through gene duplication and functional divergence is a classic problem in molecular evolution. Ohno (1970) popularized the 'mutation during nonfunctionality' model (Hughes, 1994). A new gene copy, freed from the constraints imposed by purifying selection, would accumulate 'forbidden mutations' during a period of 'nonfunctionality' until it fortuitously acquired a new beneficial function (**Figure 8(a)**). The problem is that during the period of 'nonfunctionality' a new copy would most likely be lost to drift or else inactivated by mutation.

In the subfunctionalization model (**Figure 8(b)**), specialist descendants are produced by the neutral loss of one or more functions following amplification of a multifunctional ancestor (Stoltzfus, 1999; Force *et al.*, 1999). In the escape from adaptive conflict model (**Figure 8(c)**; Hughes, 1994) selection maintains multiple copies of an ancestral gene that encodes an inefficient enzyme of broad specificity. In the innovation–amplification–divergence model (Bergthorsson *et al.*, 2007), selection for weak promiscuous functions in a highly active specialized enzyme stabilizes any gene duplications that arise (**Figure 8(d)**; gene amplification is far more frequent than a beneficial point mutation). In both models, trade-offs ensure selection for improved activities produces specialized enzymes, while selection against excess protein expression eliminates unneeded copies. In each case the outcome is the same; a family of specialized genes.

Näsvall *et al.* (2012) explored the role of transient gene duplication during the evolution of efficient specialists from an inefficient generalist ancestor. Homologous enzymes HisA and TrpF catalyze the same isomerization in their respective substrates during the syntheses of histidine and tryptophan. The two enzymes are so closely allied that to this day Mycobacteria and Streptomyces use a single efficient generalist enzyme, PriA, to catalyze both reactions. A *Salmonella enterica* strain lacking *hisA* and *trpF*, and expressing an inefficient bifunctional *hisA* mutant cotranscribed with a reporter protein from a constitutive promotor, was propagated in serial transfer on minimal medium (no histidine, no tryptophan). Within a few hundred generations faster growing strains had evolved. Consistent with gene amplification, expression of the reporter protein had increased twofold. Both copies encoded an improved version of the bifunctional *hisA* mutant. Continued selection produced an isolate at 1000 generations with one of the copies having evolved into a specialist HisA. Lineages carrying single copies each of a specialist HisA and a specialist TrpF were isolated at 2000 generations. Another lineage carried a single copy of a highly efficient PriA-like generalist (**Figure 8(f)**). Still other lineages carried multiple copies (up to 20) of the original bifunctional *hisA* mutant, with or without a derived specialist *hisA*. This work demonstrates that the same selective force that stabilizes gene duplications of an inefficient generalist can also drive the functional divergence of efficient specialists. Moreover, the recovery of efficient generalists shows that evolution of specialists does not arise from a fundamental trade-off between competing functionalities, but rather from a trade-off between the properties of the particular amino acid replacements concerned.

On the Use and Abuse of Statistics

A number of statistical methods (reviewed in Yang and Bielawski, 2000; Nielsen, 2005; Vitti *et al.*, 2013) have been developed to detect signatures of selection in the patterns of substitution among homologous sequences. For example, an elevated ratio of nonsynonymous (dn) to synonymous (ds) substitution rates is often interpreted as evidence for positive selection. These methods are so popular that "Thousands of papers have been published every year claiming evidence of adaptive evolution on the basis of computational analyses alone" (Hughes, 2008). In truth, rejecting a naïve neutral model is no guarantee that selection shaped the patterns of substitution, only that it might have shaped them. Given the complexity of large data sets, the subtlety of the statistical issues involved, and the ready availability of alternative biological explanations (Hughes, 2007; Friedman and Hughes, 2007; Hughes and Friedman, 2008; Nozawa *et al.*, 2009a,b; Yang *et al.*, 2009; Yang and dos Reis, 2011; Zhai *et al.*, 2012), one should consider statistical evidence for selection as a hypothesis in need of empirical verification.

In a number of studies elevated dn/ds ratios are associated with functional changes (Long and Langley, 1993; Ivarsson *et al.*, 2003; Zhang *et al.*, 2004; Levasseur *et al.*, 2006; Georgelis *et al.*, 2009; Briscoe *et al.*, 2010; Moury and Simon, 2011; Huang *et al.*, 2012; Loughran *et al.*, 2008). On the other hand, functional changes are no guarantor of the action of positive selection (Hartl *et al.*, 1985; Nozawa *et al.*, 2010). This criticism is especially pertinent where functional changes lack any direct connection to fitness.

Several studies have gone further and reconstructed ancestral sequences in combination with site-directed mutagenesis to test experimentally if elevated dn/ds ratios are reliable indicators of functionally important sites. Bielawski *et al.* (2004) identified five sites with elevated dn/ds ratios associated with a shift in maximum absorbance from green to blue in an ancestral bacterial light driven proton pump. Site-directed mutagenesis showed replacements at three of these sites explained the shift (Man-Aharonovich *et al.*, 2004; **Figure 9(a)**). In contrast, dn/ds ratio tests failed to identify sites controlling maximum absorbance in ancestral fish rhodopsins. Of those sites identified as selected, none were involved in spectral tuning (Yokoyama *et al.*, 2008; **Figure 9(b)**). Nor were sites modulating sensitivity to volatile sex-steroid-derived odors in ancestral primate odorant receptors identified by analyses for positive selection (Zhuang *et al.*, 2009). The failure of statistical tests to reliably identify sites where functional changes were likely selected (sight and sex have obvious adaptive significance) serves to emphasize that experimental verification of statistically derived hypotheses is essential to secure firm biological conclusions.

Epilogue

From initial hesitant steps, the Functional Synthesis has grown to become vibrant field of inquiry in its own right, focused on mechanisms of adaptation, both ancient and modern. Today, it finds applications in phlyogenetics (Bloom, 2014), ecological genetics (Barrett and Hoekstra, 2011), evolution of

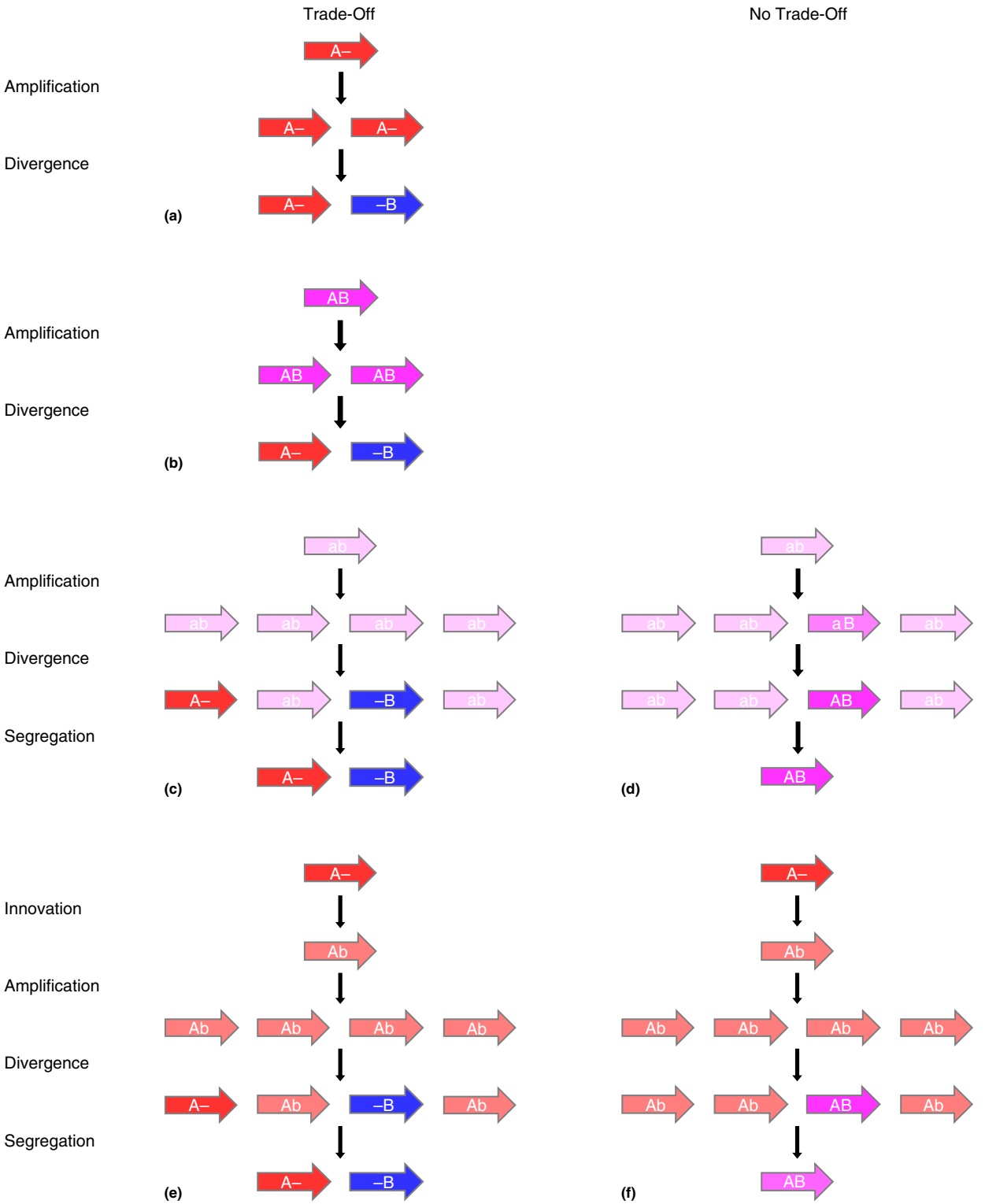

Figure 8 Models of gene duplication and divergence. (a) Ohno's original proposal. (b) Subfunctionalization of ancestral functions into separate genes. (c) Escape from adaptive conflict engendered by a trade-off between functions. (d) Evolution of an efficient generalist from an inefficient ancestor. (e) Evolution of specialists engendered by a trade-off between functions in the innovation, amplification, divergence, and segregation model. (f) Evolution of an efficient generalist in the innovation, amplification, divergence, and segregation model.

disease (Russell *et al.*, 2014; Chou *et al.*, 2014; Lynch *et al.* 2014), development (Lynch *et al.*, 2011), and design of industrial catalysts (Humble and Berglund, 2011; Wang *et al.*, 2015). In fusing functionalism and comparative biology, the Functional Synthesis provides a rigorous means to explore phenotypic evolution at the molecular level.

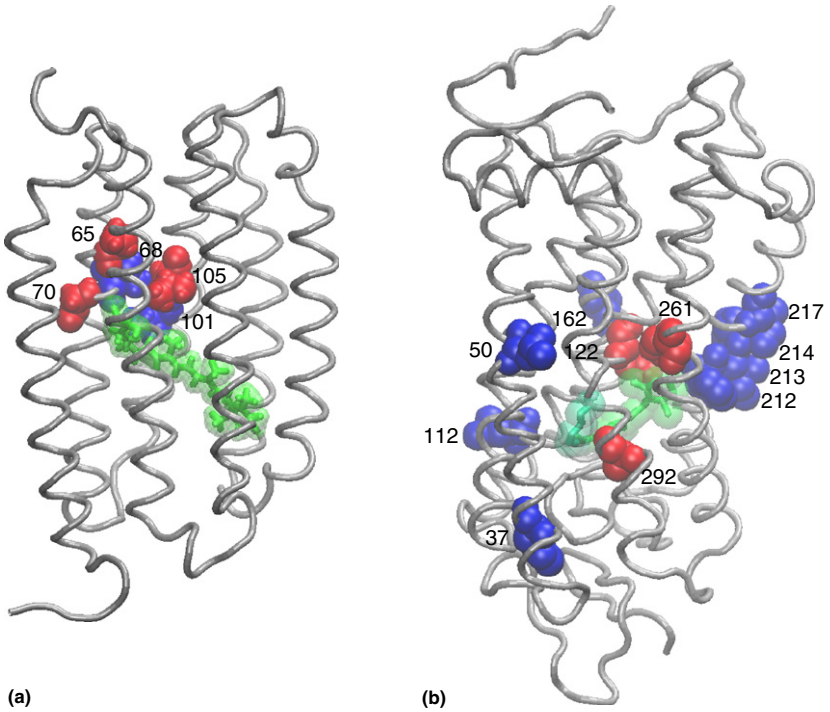

Figure 9 Success and failure of statistical 'tests for selection.' (a) Three (red) of five sites identified as subject to positive selection by statistical methods affect maximum absorbance in marine proteorhodopsin. (b) Statistical methods identified eight sites in squirrelfish rhodopsins as subject to positive selection but missed the three sites demonstrated to control maximum absorbance. The chromophores are shown in green.

See also: Ancestral Reconstruction: Theory and Practice. Genome Organization, Evolution of. Protein Biophysics and Evolution

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Molecular Evolution, History of

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Introduction

Before molecular biologists had even cracked the genetic code and articulated the 'Central Dogma,' biochemists, anthropologists, geneticists, and evolutionary biologists were gathering to discuss how macromolecules at the heart of heredity and metabolism could have evolved (Dietrich, 1994, 2008). In the 1960s, as comparative and variability analysis of proteins developed, the idea that molecular evolution took place at a different pace with different mechanisms from those of organismic evolution gradually emerged. Heated controversies soon enveloped molecular evolution as it struggled to become widely recognized as a biological discipline (Smocovitis, 1996; Dietrich, 1998; Hagen, 1999). The interplay between molecular biology and evolutionary biology that shaped the history of molecular evolution is complex. New methods of experimentation in biochemistry and molecular biology produced new kinds of data regarding molecular phenomena (Suarez and Barahona, 1996). This spurred new reasoning about evolutionary processes at the molecular level. New concepts such as the molecular clock and new theories such as the neutral theory allowed biologists to argue for the distinctiveness of the processes of molecular evolution from organismal evolution (Dietrich, 1994; Morgan, 1998). While these new concepts and theories were controversial, by the 1980s and 1990s the increased availability of DNA sequence data allowed biologists to devise new statistical tests that would help depolarize earlier controversies, and bring the techniques and theories of molecular biology into the organismally oriented evolutionary biology.

Experimenting with Molecular Evolution

The history of systematic research of evolution at the molecular level began in 1904, when bacteriologist George H. F. Nuttall working at University of Cambridge used the precipitin reaction to measure affinities between the blood sera of different animal species. The strength of the reaction was taken as an indication of the genetic relation between species (blood groups had been recently shown to behave according to Mendelian laws, by Karl Landsteiner). Others followed suit, in particular the zoologist Alan A. Boyden, who for almost four decades aimed at improving the serology tests in order to make a more 'objective' systematics (Strasser, 2010a). Well into the 1970s, serology and immunology had a number of followers in systematic anthropology, including John Buettner-Janusch and Morris Goodman (Marks, 1996; Hagen, 2010).

A different, early approach was that of comparative biochemistry, which compared products of secondary metabolism (in plants) and metabolic pathways along biological groups. In 1937, Ernest Baldwin published *An Introduction to Comparative Biochemistry*, and in 1944 the Belgian biochemist (also a historian of the field) Marcel Florkin published his work *L´evolution biochemie*. Florkin´s book (English translation of 1949) included chapters devoted to biochemical adaptations in different physiological domains like respiration, digestion, photoreception, and osmoregulation; he also considered the systematic value of biochemical characters in different animal groups like vertebrates, tunicates, or insects.

The comparison of crystallization patterns, and the analysis of the tertiary (three-dimensional) structure of proteins were part of the biochemical study of proteins since the interwar period. After World War II, the idea that proteins had a stable structure (and were not aggregates of amino acids) practically dominated biochemistry, leading to an increased interest in tertiary structure analysis, which combined X-ray diffraction experiments with early computer calculations, particularly in the work of Max Perutz and Andrew Kendrew at the Medical Research Council laboratory in Cambridge, UK (de Chadarevian, 2002). Meanwhile, biochemist Alfred Sanger, at the Dunn Institute of Biochemistry at Cambridge, had published the first primary sequence of a protein, insulin. The Dunn Institute was directed by Charles Chibnall, who applied the methods of organic chemistry to break the proteins in its constituents, and then analyze the amino acid composition (Garcia-Sancho, 2010). Chibnall invited Sanger to his insulin analysis project, who in the following years developed a method using dinitrofluorobenzene, which reacted with the last amino acid of the insulin chain. Patiently, Sanger identified the amino acid peptides, which he called 'sequences,' and determined the complete sequence of both insulin chains between 1951 and 1955 (Garcia-Sancho, 2010). Soon, Sanger´s method was taken up by other biochemists, and modified by Pehr Edman, a Swedish biochemist who invented the so-called degradation technique, later on taken as the basis for sequencing automation in proteins by William Stein and Stanford Moore. Between the 1950s and 1960s, these methods allowed the determination of sequences of biologically relevant molecules (such as hemoglobin and cytochrome *c*). By the mid-1960s there were enough sequences available for Margaret Dayhoff to publish her first *Atlas of Protein Structure* (Strasser, 2010b).

Using these new protein sequences, in 1962 Emile Zuckerkandl and Linus Pauling articulated what was later referred to as 'the most significant result of research in molecular evolution' (Zuckerkandl and Pauling, 1962; Wilson *et al.*, 1977). After comparing the amino acids sequences of hemoglobin from different lineages, Zuckerkandl and Pauling discovered that the differences in amino acid sequence were 'approximately proportional in number to evolutionary time' (Zuckerkandl and Pauling, 1965, p. 148). In other words, the rate of amino acid substitution was approximately constant. Zuckerkandl and Pauling christened this constancy the molecular clock (Zuckerkandl and Pauling, 1965; Morgan, 1998).

Other experimental contemporary approaches also sought new ways to measure molecular similarity and difference. After the publication of Francis Crick and James Watson´s double-helix model of deoxyribonucleic acid (DNA), and their suggestion that the structure suggested a possible replication model, doubts arose concerning how the double helix could be disentangled (Holmes, 2001). Biophysicist Julius Murmur and biochemist Paul Dotty, at Harvard University, realized that DNA could be dissociated ('denatured') into its two constitutive chains, and regain its double structure ('renatured'), if the solution was heated and then slowly cooled. In 1958, Ellis T. Bolton and Roy Britten (at the Carnegie Institution at Washington) used this phenomenon as a tool to measure the degree of reassociation between two single DNA chains, each coming from different species. The amount of DNA reassociation was taken as a measure of the genetic distance between the two species and used to reconstruct evolutionary relationships. More importantly, hundreds of hybridization tests eventually led Britten to the discovery of a fraction of highly repetitive sequences in eukaryotic genomes ('satellite-DNA'), a non-expected phenomenon within the Darwinian prevailing framework. Together with the molecular clock, and the conviction that molecules provided a new type of evolutionary evidence, the field was set for controversy.

A Troubled Beginning

Indeed, the molecular clock immediately attracted interest and controversy. Some biologists, such as Richard Dickerson, realized that the clock was a powerful tool that could be used to date the evolutionary divergence of different species. Moreover, since molecules like cytochrome *c* were widely shared, for the first time evolutionary trees dating divergences from sponges to human could be constructed based on a set of shared molecular traits. While many evolutionary biologists appreciated the power of molecular clock as a tool for systematics, when Allen Wilson, Vincent Sarich, and Morris Goodman began using immunological comparisons to reconfigure the evolutionary history of primates controversy soon followed. More organismally focused biologists, such as the paleontologist George G. Simpson, thought that the idea of a constant rate of evolution at the molecular level was nonsense. Morphological features evolved in response to the environment and the resulting effects of natural selection. This process was irregular and thought to be irreconcilable with rate constancy at the molecular level. Even among those using molecular techniques, there was controversy as Wilson and Goodman worked to explain why the hemoglobin of higher primates seemed to evolve at a slower rate than they did in other mammals (Wilson *et al.*, 1977; Hagen, 1999; Aronson, 2002).

The rate estimates for protein evolution provided by Zuckerkandl and others fueled a second division between molecular and organismal evolution when in 1968 Motoo Kimura used them to argue that most observed changes at the molecular level were selectively neutral (Kimura, 1968; Provine, 1990; Smocovitis, 1996). Evolutionary biology in the late 1960s was gripped by panselectionism: natural selection was the dominant factor invoked in evolutionary explanations (Gould, 1983). Kimura used the high rate of protein evolution to argue that such a high rate would create too high a selective cost. In effect, all mammals should be extinct if evolution was occurring so quickly because too many deleterious mutants would have accumulated. Kimura's solution was to claim that many of those accumulating mutations were neutral, which rendered the observed rate innocuous. The next year, Jack King and Thomas Jukes extended Kimura's case for neutral evolution at the molecular level under the deliberately controversial name, 'Non-Darwinian Evolution' (King and Jukes, 1969). The controversy that followed between neutralists and selectionists persisted for at least 30 years (Dietrich, 2006).

Only 2 years before Kimura made his argument for neutrality, Jack Hubby and Richard Lewontin and Harry Harris had brought classical evolutionary genetics to molecular biology through the technique of electrophoresis (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Harris, 1966). Gel electrophoresis is a technique that separates molecules by charge and size. This analytical separation method was developed during the interwar period by Arne Tiselius (Kay, 1988). Tellingly, the technique was originally developed to separate blood serum proteins, thus it intersected with contemporary immunochemical studies. The rise of electrophoresis was part of important biomedical advances, such as the study of anomalous hemoglobin (falciform anemia) and other serum components. By the early 1950s, the technique evolved from 'boundary' to 'zone' gel electrophoresis, which finally allowed complete molecular segregation (Chiang, 2009); by the 1960s, researchers adopted polyacrylamide gel as the stabilizing medium, thus transforming electrophoretic separation in one of the most successful, cheaper, and movable experimental tools available in biomedical research. By the mid-1960s, different research teams adopted gel-zone electrophoresis in order to measure the amount of genetic variation available in natural populations. In Great Britain, anthropologist Harry Harris used it to measure enzyme polymorphisms in human populations; while in the United States, Hubby and Lewontin at the University of Chicago, and Wilson Stone and his collaborators in Texas did the same for *Drosophila* populations (Lewontin, 1974; Suarez and Barahona, 1996). Such research was framed by debates regarding the number and kind of mutations caused by atomic radiation (see Beatty, 1987a), and seemed to support the view that argued for high levels of variation and a significant role for balanced polymorphisms. The rise of the neutral theory fundamentally changed the terms of the debate within evolutionary genetics by introducing neutral variation and genetic drift into the picture as real alternatives. That is not to say that the neutral theory was not controversial.

Initial responses to the neutral theory by evolutionary biologists were largely negative. Steeped as they were in selectionist explanations, evolutionary biologists were not convinced by Kimura's cost of selection argument and were skeptical that the theory could be easily tested. Biochemists, in contrast, were much more open to the neutral theory in part because they were less invested in natural selection and more easily moved by the prevalence of synonymous mutations. Because of redundancy in the third position of many codons, changes in the nucleotide at that site produced no corresponding change in which amino acid was coded for. This

meant that many third position substitutions would be synonymous and so not subject to selection. This biochemical argument was much more compelling, and meant that nucleotides in third positions were more likely to be governed by a process of genetic drift (Dietrich, 1994).

The prevalence of drift at the molecular level meant that the constancy of the molecular clock could be explained in terms of neutral rates of molecular evolution. According to the neutral theory, at neutral sites the rate of substitution was equivalent to the rate of mutation. Since the rate of mutation was more or less constant over long periods of time, the constant tick of the molecular clock could be explained as a result of the presence of neutral sites in the DNA coding for the molecule. Molecules had different rates of change depending on how many sites were constrained by selection (and so do not change) and how many were neutral (and free to change). Mary-Claire King and Allan Wilson invoked the prevalence of drift at the molecular level, but not at the organismic level in 1975, to explain how constancy at the molecular level could be reconciled with fluctuating rates at the organismic level (King and Wilson, 1975; Dietrich, 1998). Moreover, it implied a rupture of the 'extrapolationism' of the evolutionary synthesis: instead of one single mechanism (natural selection) explaining evolutionary processes at all levels of biological organization, the neutralists pushed for a differentiated explanation of evolution at the molecular and organismal level.

Making Molecular Evolution

Intellectually, molecular evolution was established as a separate field of study by biologists, such as Allan Wilson and Motoo Kimura, who argued that there were significant differences between evolution at the molecular level and at the organismic level. Tellingly, some evolutionary biologists, such as Ernst Mayr, Theodosius Dobzhansky, G. G. Simpson, and many others, noted a growing opposition between molecular and evolutionary biology. The 'molecular wars,' as E. O. Wilson called them, pitted molecular and organismal evolutionary biologists in a struggle for authority and resources that drew on wider tensions regarding the unity of science, the cultural standing of evolution, and the autonomy of biology as a discipline (Smocovitis, 1996; Wilson, 1994; Hagen, 1999; Dietrich, 1998). Molecular evolution was caught in this complex dispute as it tried to establish a disciplinary identity that drew from both evolutionary and molecular biology (Dietrich, 1998). Institutionally, Emile Zuckerkandl helped the new field come together through a new journal, the *Journal of Molecular Evolution*. Begun in 1974, Zuckerkandl's journal provided a venue for publication for this newly emerging field. Given the hostility of some organismic evolutionary biologists, the *Journal of Molecular Evolution* was an important outlet for early work in this field, especially since some of the first molecular evolutionists, such as Allan Wilson, were biochemists with little training in evolution. However, not everybody felt connected to Zuckerkandl´s journal. In 1982, Masatoshi Nei and Walter Fitch, in the context of a symposium on 'Evolution of Genes and Proteins' held at State University of New York in Stony Brook, launched a second journal for the field, *Molecular Biology and Evolution*. A certain amount of debate was clearly running through the nascent community concerning the best ways to foster communication and transmission of knowledge within the field. In 1992, Fitch and Nei ignited the creation of the Society for Molecular Biology and Evolution. In 1992, a third journal was created, *Molecular Phylogenetics and Evolution*, focused on problems of systematics, and whose editor-in-chief then was Morris Goodman (Suárez-Díaz, 2009).

Moreover, the 1970s and 1980s were challenging times for the study of molecular evolution. At the level of theory, the neutral theory, as applied to proteins, remained controversial. Initial tests of the neutral theory were either statistically problematic, or did not support the neutral theory (Ewens, 1972; Lewontin, 1991). For instance, Francisco Ayala's group used data about electrophoretic variability in populations of the fruit fly, *Drosophila*, to test a range of neutralist predictions. Ayala and his coworkers noted that the frequency distribution of heterozygous loci was significantly different from that predicted for neutral alleles in the infinite alleles model. Instead of the predicted distribution clustering, Ayala's distribution of heterozygous loci was fairly even, but tellingly had an excess of loci with very little heterozygosity. This high number of rare alleles was held against the neutral theory (Ayala *et al.*, 1974, p. 378). Neutralists, such as Jack King, located the problem, not in the neutral theory, but in assumptions made in the infinite alleles model. King suggested that infinite alleles model was not appropriate for electrophoretic data, since electrophoretic classes probably encompassed many allelic differences (King, 1974). Ayala and others were sensitive to these criticisms and introduced mutation models for electromorphs that better fit the data sources (Ewens, 1979). Neutralists, such as Tomoko Ohta and Masatoshi Nei, began to articulate alternatives to Kimura's strictly neutral theory. Ohta suggested a broader class of nearly neutral alleles that would still be largely subject to processes of drift, and could explain the observed numbers of rare alleles (Ohta, 1992; Ohta and Gillespie, 1996; Steen, 2008). Nei proposed that population dynamics, such as bottlenecks, could explain phenomena that confounded the strictly neutral theory (Nei, 1987).

As statistical testing of neutralist and selectionist hypothesis proved more difficult than anticipated in the 1970s, the molecular clock became a proxy for the neutralist–selectionist dispute. From its inception, Zuckerkandl and Pauling had insisted that the molecular clock was stochastic, and so exhibited both constancy and variability. The question was how much variability is reasonable in a clock. In 1971, Ohta and Kimura measured significantly higher variation in the molecular clocks associated with beta hemoglobin and cytochrome *c*. However, they did not interpret this variability as a problem for the neutral mechanism that produced the overall constancy of the rate of substitution. Rather, they held that the overall constancy had been thrown off by a small number of advantageous mutations: it was the balance of selected and neutral sites that was understood to give each molecule its characteristic rate of change. Others, like Morris Goodman, were less convinced, and argued that the slowdown in rates of evolution in hemoglobin in primates was evidence against a hemoglobin-based molecular clock. For clock advocates, like Kimura and Allan Wilson, there were going to be exceptions to the rule of the molecular clock, but what was important was seeing them as exceptions to an intrinsic rate rather than as

evidence that the clock did not exist (Wilson, *et al.*, 1987; Aronson, 2002; Hagen, 2010).

The Coming of the DNA Era

In 1962, Sanger was invited to the newly created Laboratory of Molecular Biology by Sydney Brenner and Francis Crick to develop a method for nucleic acid sequencing. By the late 1960s and early 1970s, Sanger had sequences small transfer and ribosomal RNA molecules, but the degradation method was not suitable for DNA sequencing. It was until the late 1970s that Sanger, using DNA polymerase, developed the plus, minus, and dideoxy methods. Allan Maxam and Walter Gilbert, almost simultaneously, developed their DNA sequencing method between 1976 and 1977 (Garcia-Sancho, 2010). The growing availability of DNA data in the late 1980s and 1990s sparked an important cascade of changes for molecular evolution. These changes were accompanied and made possible by the readily access to computer technologies and the creation of the first databases (Garcia-Sancho, 2010, 2012). They had an impact both on the development of comparative or phylogenetic analysis, a field that has been completely transformed by the advent of the molecular approach (Suárez-Díaz and Anaya-Munoz, 2008; Suárez-Díaz, 2014), and on the testing of the neutral hypothesis as evolutionary mechanism.

In the latter case, DNA sequence data promised direct quantitative comparisons. The idea was not new (Zuckerkandl and Pauling, 1965), and it was built up from previous developments in protein sequence comparison that took place during the 1960s. As the accumulation of hemoglobin and cytochrome data sequences progressed (see above), it opened the door to comparisons of amino acid sequences from different species and gives an estimate of their similarity. Algorithmic tools and the introduction of personal computers in biological research allowed for the construction of the first evolutionary trees from molecular–protein-data (Hagen, 2001; Eck and Dayhoff, 1966; Fitch and Margoliash, 1967; Dayhoff, 1969). The shared idea among these early practitioners was that molecular data provided a quantitative and non-idiosyncratic approach to systematics (Hagen, 2003; Strasser, 2010b; Suárez-Díaz, 2014). Thus, Fitch, for instance, defined similarity as 'minimum (mutational) distance.' Using an IBM of the 360 series he performed a pairwise comparison of pairs of sequences using Margoliash´s set of cytochrome *c* sequences to build a tree upon measures of minimum distance.

Starting in the 1980s, as DNA sequences accumulated in new databases, their use revolutionized the field of systematics. More powerful algorithms to explore similarities between sequences had been put to work and improved (Needleman and Wunsch, 1970; Sellers, 1974). However, as DNA datasets flooded, the number of problems and debates arose. Besides debates on aspects of systematics, including homology, phonetics, and parsimony (Sober, 1988), many methodological decisions need to be taken before a molecular tree is built. These include various approaches to sequence alignment, the multiple hit problem and the different methods for constructing trees (including distance-based methods, maximum parsimony, and maximum-likelihood methods), just to name the most important ones (Suárez-Díaz and Anaya-Munoz, 2008). More recently, Bayesian methods of statistical inference have been incorporated in the construction and election of phylogenetic trees; this movement has led to development of new software packages, like the very popular MRBAYES (Huelsenbeck and Ronquist, 2001). Moreover, the discovery of large amounts of horizontal genetic transfer, not only in bacteria, questioned the idea itself of the evolutionary tree (Doolittle, 1999; Sapp, 2007). As complete genome sequences have been produced in the last decade, new methods for genome comparison aim to surpass some of the limitations rising from one-to-one gene comparisons. Still, the contribution of molecular phylogenetics to our knowledge of the history of life has been enormous.

DNA sequencing was introduced into the study of mechanisms of evolutionary genetics by Kreitman (1983). As a Richard Lewontin's graduate student at Harvard, Kreitman used the sequencing techniques he learned in Walter Gilbert's laboratory to analyze the sequences of alcohol dehydrogenase (ADH) genes in *Drosophila melanogaster*. ADH had known a well-known polymorphism for fast and slow moving electrophoretic variants. Kreitman's investigation of the DNA sequences of the fast/slow ADH polymorphism revealed many differences between the DNA sequences of eleven different alleles, but only one DNA difference that corresponded to an amino acid difference. This nonsynonymous DNA substitution was at the site of the fast–slow protein polymorphism.

The striking difference between synonymous changes (which cause no change in amino acid sequence) and nonsynonymous changes (which cause a change in amino acid sequence) led Kreitman and his collaborators to devise new statistical tests for selection. Kreitman extended Kimura's 1983 idea of comparing synonymous and nonsynonymous substitutions by contrasting changes within and between species. The resulting McDonald–Kreitman test compares the ratio of nonsynonymous to synonymous changes within a species and between two species. If the sequences are neutral, the ratios should remain the same. If there is positive selection, then nonsynonymous changes should have accumulated over time, so there would be more nonsynonymous changes between species than within a species. The McDonald–Kreitman test and many other statistical tests that followed allowed evolutionary biologists to detect balancing selection, adaptive protein evolution, and population subdivision (McDonald and Kreitman, 1991; Kreitman, 2000). Where earlier statistical tests using electrophoretic data had been stalled by low power, these comparisons using DNA sequence data proved more effective in distinguishing the effects of drift and selection.

The success of tests of selection did not tip the balance of the neutralist–selectionist controversy in favor of the selectionists. On the one hand, tests of selection allowed the controversy between neutralists and selectionists to move beyond earlier either/or dynamics toward an acknowledgment that both selection and drift acted at the molecular level and were worthy of investigation. On the other hand, test of selection allowed biologists to recast the neutral theory that cast it as a null model, as the methodological starting place for molecular evolutionary analysis (Crow, 1987). When Kimura first championed the neutral theory in 1968, most evolutionary biologists understood natural selection to be the most important factor in biological evolution and as a result assumed

that searching for selection and its effects was the method of choice – they were panselectionists (Kimura, 1983; Gould and Lewontin, 1979). Indeed, in 1983, Ernst Mayr argued that biologists should continue to give selectionist explanations priority because random drift could not be demonstrated (Mayr, 1983). Mayr's confidence in selection was the result of earlier efforts that reinterpreted supposed cases of random drift governing the fate of morphological traits as actually the result of natural selection. As a result, for Mayr and many others drift became equated with an admission of ignorance of how selection was in fact operating (Beatty, 1987b). Mayr would have been hard pressed to hold such a stringent denial of drift only a few years later when statistical tests based on newly available DNA sequences became accepted tools in molecular evolution. By the late 1980s, both proponents and critics of the neutral theory recognized that neutrality, not selection, was a useful starting hypothesis when analyzing DNA sequences (Hudson *et al.*, 1987; Kreitman, 2000; Beatty, 1987b). In his review of methods to detect selection, Kreitman argues that "Kimura's theory of neutrally evolving mutations is the backbone for evolutionary analysis of DNA sequence variation and change" because a "substantial fraction" of the genome is best modeled as selectively neutral, because selective neutrality is a "useful null hypothesis," and because "statistical analysis of (potentially) neutral variation in a gene (or other region of the genome) can be informative about selection acting at linked sites" (Kreitman, 2000, pp. 541–542). Kreitman's view accepts both that there is a substantial amount of neutral variation and that the neutral theory is essential for detecting selection at the DNA level.

The acceptance of neutrality as a starting point for molecular evolutionary research demonstrates an important shift away from panselectionism in evolutionary biology. Over time, molecular techniques introduced information about a new level of biological organization: the molecular level, where drift plays a significant role. Molecular evolutionists helped create the divide between the molecular and morphological levels as a way of carving out space where their research could develop independently of the selectionist agenda of the architects of the Neo-Darwinian synthesis (Dietrich, 1998; Aronson, 2002; Hagen, 1999).

See also: Evolutionary Genetics, History of. Mutation and Genome Evolution

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Molecular Evolution, Models of

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Glossary

Covarion model A model proposed by Fitch and Markowitz in 1971 hypothesizing that the evolutionary process at a given site in a molecule is dependent on the state at other sites.

DNA transition A change between biochemically similar DNA states. Changes from one purine to another (A and G) or from one pyrimidine to another (C and T) are considered transitions.

DNA transversion A change between biochemically dissimilar DNA states. Any change from a purine to a pyrimidine, or from a pyrimidine to a purine, is considered a transversion.

Equilibrium distribution A steady-state probability distribution of possible states that does not depend on the initial states. The distribution represents a stable condition, and implies stable statistical behavior.

Instantaneous rate The rate of a change in state over an infinitesimally small unit of time.

Markov model A model for a stochastic process of change between states where the probability of an event depends only on the current state, and not on any of the events that may have preceded it.

Model bias The difference between the average performance of a model, often referred to as the expected value of the model, and the true value.

Model variance The difference between the individual results of a model and the long run average of the results when the modeling process is repeated many times.

Stationarity Pertains to a Markov process that has attained its equilibrium distribution and has no tendency to move away from that distribution.

Steady-state distribution An alternative term for the equilibrium distribution, which is defined above.

Stochastic Pertains to a process having a randomly determined sequence of events. A stochastic, or random, process can change over time according to any one of many different series of events.

Substitution rate matrix The matrix of instantaneous rates of character state change required to completely specify the evolutionary dynamics of a molecular sequence.

Time-homogeneity The property of a stochastic process where the transition probabilities for all changes among states are constant over time.

Time-reversibility The property of a stochastic process at equilibrium where the amount of change from state i to state j is exactly equal to the amount of change from state j to state i.

Introduction

Modern methods of phylogenetic analysis require a mathematical model to describe the process of sequence change over time. Often, the scientific goal will be to learn the evolutionary relationships from the sample of sequences. Although a model of the evolutionary process is an essential part of phylogenetic inference, it is sometimes referred to as a nuisance variable because it is not the objective of the study. Other times, the scientific purpose of the study will be to learn how molecules change over evolutionary time. Then, the phylogeny becomes the nuisance variable. In both settings, the evolution of the sequences underpins the endeavor, and success will depend on building an adequate representation of how the sequence changes over evolutionary time.

Sequence evolution is modeled by explicitly describing the probability of change between character states. Taking DNA sequence as an example, the model specifies the probability that a nucleotide will change from a given state (A, C, G, or T) to another given state (again A, C, G, or T) after some amount of time has passed. Because evolution of DNA proceeds by mutation of an existing nucleotide in a population, and its ultimate fixation in that population is by drift or by natural selection, it is reasonable to assume that the probability of a change depends only on the current nucleotide in the population. This assumption coincides with a special class of stochastic models that have very useful properties, called Markov models, where the probability of change does not depend on the history of past changes. Further restrictions are typically placed on Markov models because they provide computational benefits. The most common restrictions include the assumption that sites in an alignment evolve independently, the process does not change over time (time-homogeneity), the character states are at their equilibrium frequencies (stationarity), and the process is the same whether evolution was run forward or backward through time (reversibility). All these assumptions minimize the complexity of the model, which can aid the interpretability of the results.

Markovian models can be used to compute distances between pairs of sequences. These are the basis for the distance-based phylogenetic methods. The probability calculations of the model are critical to this approach because the amount of dissimilarity observed between a pair of sequences is not a linear function of evolutionary time; the observed difference between sequences progressively underestimate the actual evolutionary distance as time increases. A Markovian model is employed to estimate the actual evolutionary difference between sequences. Note that these models also provide the

probability calculations that underpin both the likelihood and Bayesian methods of phylogenetic analysis.

Parameters are used to define how different biological aspects of evolution determine the probabilities of a state change. Again, taking DNA as an example, it is well known that DNA transitions (A↔G and T↔C) occur more frequently during evolution than DNA transversions (all the other types of DNA changes). Thus, a parameter that permits the probabilities to differ between these two types of changes is often considered important to include in a DNA model. Model realism is considered improved only when parameters are subsequently found to provide a benefit to real data analysis. Model development has generally followed one of two tracks. Mechanistic models employ parameters to describe how a specific biological process impacts probabilities of change. The actual values for these parameters are not specified, but rather they are estimated by fitting the model to a sample of data (e.g., by using maximum likelihood). Through these estimated values, the model can serve as a tool for learning about the process of sequence evolution. Alternatively, the empirical models employ parameter values that have been previously estimated from a large reference dataset that is presumed to be broadly representative of sequence evolution. By leveraging the information in a large dataset, an empirical model can apply information about very complex biological processes to a relatively small dataset with little computational burden. The limitation is that any parameters that are fixed to database-derived values cannot be used to gain additional insight about the process of sequence evolution. For this reason, empirical models are more often used when the primary objective is phylogeny reconstruction, and a relatively complex model is warranted.

Mathematical Modeling of Substitution Dynamics

The task of constructing a model for sequence evolution begins with the assumption that the process is Markovian. Although this is a reasonable assumption, some conditions will be imposed on the process to facilitate otherwise difficult computation. In this section, changes among the states of a DNA sequence are used to illustrate the basis of model construction. As the purpose is to introduce the major properties of Markov models, readers can skip ahead to the different types of sequence models presented in Section 'Modeling the Evolution of Molecular Sequences' without loss of continuity.

Continuous Time Substitution Rates

The evolutionary dynamics implied by a Markov model are fully determined by the current states of the system, and the substitution probabilities from each state to all other states. Taking just adenine (A) as an example, let the current state of the system be the frequency of A at time t, $f_A(t)$. The frequency of A after some amount of evolutionary time has elapsed $(t+\mathrm{d}t)$ is determined by taking the current frequency of A, and subtracting the frequency of A lost by substitution of an alternative nucleotide (C, G, or T) and adding the frequency of new A's fixed by substitution

$$f_A(t+\mathrm{d}t) = f_A(t) - \sum_{x \neq A} f_A(t)\mu_{Ax}\mathrm{d}t + \sum_{x \neq A} f_x(t)\mu_{xA}\mathrm{d}t$$

where μ_{Ax} is the rate of substitution from A to any alternative state (C, G, or T), and μ_{xA} is the rate of substitution from an alternative state x to A. The substitution rate indicated by μ is called an 'instantaneous rate' because it gives the rate of a nucleotide substitution over an infinitesimally small interval of time, i.e., as $\mathrm{d}t$ tends to zero. A full description of the system requires three more equations:

$$f_C(t+\mathrm{d}t) = f_C(t) - \sum_{x \neq C} f_C(t)\mu_{Cx}\mathrm{d}t + \sum_{x \neq C} f_x(t)\mu_{xC}\mathrm{d}t$$

$$f_G(t+\mathrm{d}t) = f_G(t) - \sum_{x \neq G} f_G(t)\mu_{Gx}\mathrm{d}t + \sum_{x \neq G} f_x(t)\mu_{xG}\mathrm{d}t$$

$$f_T(t+\mathrm{d}t) = f_T(t) - \sum_{x \neq T} f_T(t)\mu_{Tx}\mathrm{d}t + \sum_{x \neq T} f_x(t)\mu_{xT}\mathrm{d}t$$

The total process (for all the nucleotide states) can be summarized by using a single equation, and a 4×4 substitution rate matrix, Q, that specifies the full set of substitution probabilities

$$F(t+\mathrm{d}t) = F(t) + QF(t)\mathrm{d}t$$

where

$$Q = \begin{bmatrix} -\mu_A & \mu_{AC} & \mu_{AG} & \mu_{AT} \\ \mu_{CA} & -\mu_C & \mu_{CG} & \mu_{CT} \\ \mu_{GA} & \mu_{GC} & -\mu_G & \mu_{GT} \\ \mu_{TA} & \mu_{TC} & \mu_{TG} & -\mu_T \end{bmatrix}$$

Because the values of Q fully determine the dynamics of the substitution process, the different DNA models are defined according to their unique 'substitution rate matrix,' Q. The off-diagonal elements of Q are used to determine the probability that a given state will change to another state over some fixed amount of time. The diagonal values of Q are set so that each row of the matrix sums to zero. Each diagonal value gives the total substitution rate of the nucleotide, for example, $\mu_A = \mu_{AC} + \mu_{AG} + \mu_{AT}$.

To construct a Markov model to describe the substitution dynamics of amino acids, or sense codons, the substitution rate matrix is simply expanded to accommodate the larger number of states. As there are 20 amino acids, a model for protein sequences will have a 20×20 matrix of instantaneous rates. A model for evolution under the universal genetic code will be even bigger, having a matrix of 61×61 instantaneous rates. The use of Markov chain theory is the same in these cases, so the DNA model serves as a good example.

Calculating Substitution Probabilities

Whether the states are nucleotides, codons, or amino acids, the instantaneous rate from any initial state i to another state j is given by μ_{ij}. This is the rate of a substitution over an infinitesimally small amount of time, but we want the substitution probability for a finite amount of evolutionary time, $p_{ij}(t)$ where $t > 0$. This quantity is known as a 'substitution probability,' and the matrix that gives the full set of these, $P(t)$, is called the 'substitution probability matrix.' This matrix is

given as

$$P(t) = \begin{bmatrix} p_{AA}(t) & p_{AC}(t) & p_{AG}(t) & p_{AT}(t) \\ p_{CA}(t) & p_{CC}(t) & p_{CG}(t) & p_{CT}(t) \\ p_{GA}(t) & p_{GC}(t) & p_{GG}(t) & p_{GT}(t) \\ p_{TA}(t) & p_{TC}(t) & p_{TG}(t) & p_{TT}(t) \end{bmatrix}$$

The diagonal elements of this matrix are set, so the rows sum to 1. In addition to being necessary to correct for multiple substitutions between states at the same site, the resulting values of $P(t)$ can be used to provide insights into the process of molecular evolution.

Given the instantaneous dynamics determined by Q, the behavior over a fixed amount of time t can be obtained by solving the differential equation

$$\frac{dP(t)}{dt} = P(t)Q$$

The solution gives $P(t)$ as the exponential of Qt

$$P(t) = e^{Qt}$$

There are several ways to obtain the exponential of the Q matrix, but this task is most easily handled by diagonalizing Q. If an invertible matrix U exists such that $Q = U^{-1}DU$, where D is a diagonal matrix, then:

$$P(t) = e^{Qt} = e^{(U^{-1}DtU)}$$

$$P(t) = U^{-1}e^{Dt}U$$

The matrix U is comprised of the eigenvectors of Q, and U^{-1} is the matrix inverse of U. The diagonal matrix is comprised of the eigenvalues of Q. Taking a 4×4 DNA matrix as an example for Q, the matrix $D = \text{diag}(\lambda_1, \lambda_2, \lambda_3, \lambda_4)$. The exponential of D, required to compute $P(t)$, is simply

$$P(t) = U^{-1}\text{diag}\{e^{\lambda_1 t}, e^{\lambda_2 t}, e^{\lambda_3 t}, e^{\lambda_4 t}\}U$$

A very common assumption of models used for inferring a phylogeny is that the substitution dynamics specified by Q are valid over the entire interval of time under study (e.g., over the entire evolutionary tree). This assumption is called 'time-homogeneity.'

Steady-State Distribution

The above Markov process has an interesting property worth noting. When the process has been running for a very long time, i.e., when time (t) tends toward infinity, the state frequencies will approach a distinctive distribution. This distribution is called the 'equilibrium' or 'steady-state distribution,' because this distribution does not depend on the initial state of the process. As long as the process is run long enough it will attain the 'steady-state distribution' and remain there.

Continuing with the DNA model as an example, let π^0 be an initial distribution of nucleotide frequencies and π^t be their distribution after some time (t) has elapsed, where

$$\pi^0 = (\pi^0_A, \pi^0_C, \pi^0_G, \pi^0_T), \text{ and}$$

$$\pi^t = (\pi^t_A, \pi^t_C, \pi^t_G, \pi^t_T)$$

The nucleotide frequencies at a point in time (t) are given by

$$\pi^t = \pi^0 P(t)$$

When the current distribution is the steady-state distribution (i.e., $\pi^t = \pi^{t+1}$), the Markov process is said to be 'stationary.' In the DNA case, if the process is stationary, the empirical proportions of the nucleotides (A, C, G, and T) in the evolving sequence will correspond to the steady-state distribution. This means that the frequency of, say, nucleotide A in those sequences gives an estimate of the expected proportion of time the Markov process at a given site was in the A state (π_A).

Time-Reversibility

Under the assumption of stationarity, we can be even more precise about what we expect for this process: the amount of 'movement' of the process from some state i into another state j will be given by $\pi_i q_{ij}$, and the movement out of that state j will be given by $\pi_j q_{ji}$.

'Time-reversibility' is just the special case where the steady-state amount of change from state i into j is exactly equal to the amount of change from state j into i for all possible pairs (i, j) of states:

$$\pi_i \mu_{ij} = \pi_j \mu_{ji}$$

Note that reversibility can be imposed on a Markov process when it is thought to be correct, or simply for mathematical convenience. Mathematical convenience is usually the reason for specifying a time-reversible evolutionary model, as there are few biological conditions that justify such a restriction on the process. Indeed, time-reversibility imposes an asymmetry in the substitution process between the two strands of DNA. Strand asymmetry can be relaxed by specifying a certain type of 'nonreversible model' (see Section 'Models for DNA Sequences' for further details), and such nonreversible models can have a stationary steady-state distribution.

Another convenience of a time-reversible model is that the off-diagonal substitution rates of Q can be expressed as the product of an 'exchangeability parameter' (s_{ij}) and the steady-state frequencies

$$\mu_{ij} = s_{ij}\pi_j$$

with time-reversibility imposed by the restriction

$$\pi_i s_{ij} \pi_j = \pi_j s_{ji} \pi_i$$

Thus, states i and j need not be equally frequent to have time-reversibility, making it possible to specify a time-reversible DNA model that allows biased nucleotide frequencies when at equilibrium.

Modeling the Evolution of Molecular Sequences

Evolutionary processes differ among genes, genomes, and organisms. Coding sequences are constrained by the function of the encoded proteins, whereas noncoding sequences are not.

Mitochondrial genomes are subject to very different evolutionary dynamics than the nuclear genes of the same species. The genome of a virus experiences different rates and patterns of spontaneous mutation than those of its hosts. Given that these examples represent just a small fraction of the natural diversity in evolutionary processes, it is not surprising that many different types of models have been developed for sequence evolution.

Different models are defined by different parameterizations of the rate matrix. The three most popular types of models differ according to the number of character states (DNA, amino acid, and codon). DNA models, having just four states (A, C, G, and T), have a relatively small rate matrix with $4 \times 4 = 16$ elements. Amino acid models allow up to 20 distinct states, so they employ a much larger rate matrix having 400 elements. Codons are triplets of nucleotides that encode an amino acid, and the 61 sense codons of the universal genetic code yields an even larger rate matrix. A very large number of alternative evolutionary models have been derived from these three types of rate matrix. While it is not possible to review them all, descriptions of selected models are provided in the following sections.

Models for DNA Sequences

The first Markov model for sequence evolution, proposed by Jukes and Cantor (1969), is also the most simple. This model assumes that every nucleotide substitution has the same probability of occurring. If the rate parameter is denoted by α, then the substitution rate matrix for this model is

$$Q = \begin{bmatrix} \cdot & \alpha & \alpha & \alpha \\ \alpha & \cdot & \alpha & \alpha \\ \alpha & \alpha & \cdot & \alpha \\ \alpha & \alpha & \alpha & \cdot \end{bmatrix}$$

Recall that the diagonal elements of a rate matrix must be set so that each row will sum to zero. For this model, the diagonal elements would be -3α, and are excluded for simplicity. A consequence of this model is that if a sequence had evolved for a sufficiently long period of time (i.e., the substitution process is at equilibrium), the resulting gene sequences would exhibit nearly equal nucleotide frequencies regardless of their frequencies at the start. This is a strong expectation that is clearly violated in the majority of real sequences.

Recognizing that different genes have different nucleotide frequencies, Felsenstein (1981) proposed a model whereby the substitution rate depends on the equilibrium frequencies:

$$Q = \begin{bmatrix} \cdot & \alpha\pi_C & \alpha\pi_G & \alpha\pi_T \\ \alpha\pi_A & \cdot & \alpha\pi_G & \alpha\pi_T \\ \alpha\pi_A & \alpha\pi_C & \cdot & \alpha\pi_T \\ \alpha\pi_A & \alpha\pi_C & \alpha\pi_G & \cdot \end{bmatrix}$$

The frequencies (π_x) can be estimated by simply measuring the nucleotide composition of the sequences under study. This represents an increase in complexity, as this model has three more parameters than the Jukes and Cantor (1969) model. It is only an increase of three because given any three frequency parameters, the fourth can be obtained by subtraction.

Due to greater biochemical similarity between purines (A and G) and between pyrimidines (C and T), DNA sequences more frequently experience changes from purine to purine and from pyrimidine to pyrimidine. These are called DNA transitions. Changes from a purine to a pyrimidine, or vice versa, occur less frequently over evolutionary time and are referred to as DNA transversions. This difference is a clear example of a biologically relevant aspect of sequence evolution that can be easily built into a model. In fact, it is so critical that it has been built into DNA models on three different occasions (Kimura, 1980; Felsenstein, 2004; Hasegawa *et al.*, 1985). The model of Hasegawa *et al.* (1985) is

$$Q = \begin{bmatrix} \cdot & \beta\pi_C & \alpha\pi_G & \beta\pi_T \\ \beta\pi_A & \cdot & \beta\pi_G & \alpha\pi_T \\ \alpha\pi_A & \beta\pi_C & \cdot & \beta\pi_T \\ \beta\pi_A & \alpha\pi_C & \beta\pi_G & \cdot \end{bmatrix}$$

In this model, there are separate rates for the DNA transitions (α) and DNA transversions (β) in addition to the frequency parameters, π_x. Collectively, all these parameters determine the subsitution rates of this model. This is an increase in the complexity of the model (five parameters) relative to Felsentein's 1981 model (four parameters).

All the models presented so far make the time-reversibility assumption described in the previous section. For DNA, the substitution rate matrix of a reversible model can have at most eight free parameters, three frequency parameters, and five exchangeabilities:

$$Q = \begin{bmatrix} \cdot & a\pi_C & \beta\pi_G & \gamma\pi_T \\ \alpha\pi_A & \cdot & \delta\pi_G & \varepsilon\pi_T \\ \beta\pi_A & \delta\pi_C & \cdot & \varsigma\pi_T \\ \gamma\pi_A & \varepsilon\pi_C & \varsigma\pi_G & \cdot \end{bmatrix}$$

There are only five free exchangeabilities in the above matrix because one is set to a value of 1. This is called a general time-reversible (GTR) model, and several studies have explored this for DNA sequences (Lanave *et al.*, 1984; Tavaré, 1986; Yang, 1994a). Recall that reversible models are largely motivated by mathematical convenience. Although the parameterization of the GTR model offers considerable flexibility, it forces the evolutionary process to be strand-asymmetric; i.e., it requires that the evolutionary process differs between the two strands of DNA.

Strand-symmetry, or strand-asymmetry, are examples of biologically relevant aspects of the evolutionary process that impact the probability of a change in state. Several authors (Takahata and Kimura, 1981; Gojobori *et al.*, 1982; Yang, 1994a; Bielawski and Gold, 2002) have explored alternative parameterizations of DNA models that are not time-reversible; i.e., within the substitution matrix the rate of $i \rightarrow j$ differs from the rate of $j \rightarrow i$. Bielawski and Gold (2002) proposed a model with five free parameters that describes a fully symmetric process for both strands of DNA. Specifically, this model ensures that complimentary substitutions on the two DNA

strands have the same rate:

$$Q = \begin{bmatrix} \cdot & \alpha & \beta & \eta \\ \delta & \cdot & \varepsilon & \gamma \\ \gamma & \varepsilon & \cdot & \delta \\ \eta & \beta & \alpha & \cdot \end{bmatrix}$$

Because this model is not reversible, likelihood calculations are conducted on a rooted tree (note that reversible models can employ unrooted trees for likelihood calculations). This model was not intended for use in phylogeny reconstruction. Rather, given a tree topology, the model served as the basis for explicitly testing certain types of evolutionary strand asymmetry.

The model of Bielawski and Gold (2002) is a simplified case of the most general form of a DNA substitution model without the restriction of reversibility. This model has 11 free parameters in the substitution rate matrix, and was introduced by Yang (1994a). Yang (1994a) referred to this model simply as the unrestricted model:

$$Q = \begin{bmatrix} \cdot & \mu_{AC} & \mu_{AG} & \mu_{AT} \\ \mu_{CA} & \cdot & \mu_{CG} & \mu_{CT} \\ \mu_{GA} & \mu_{GC} & \cdot & \mu_{GT} \\ \mu_{TA} & \mu_{TC} & \mu_{TG} & \cdot \end{bmatrix}$$

As it is not reversible, likelihood calculations must be carried out on a rooted tree. This is because likelihood scores can be different for the different rooted trees that correspond to a single unrooted tree. The unrestricted model is itself a special case of the model of Barry and Hartigan (1987) that employs a general substitution rate matrix for every branch within a phylogenetic tree.

In the majority of cases, the parameter values for the above mechanistic models are derived from the sequence data under study. There has been some exploration of empirical DNA models, where values for parameters are derived from reference datasets (Lanave *et al.*, 1984; Zharkikh, 1994; Arvestad and Bruno, 1997). Such models are not widely used in phylogenetic analyses due to concerns about finding suitably informative reference datasets. Furthermore, for the purpose of estimating a phylogenetic tree, the biological reality that is sacrificed by the reversible forms of DNA models does not seem to have much of a negative impact. However, the nice mathematical properties of reversible models (Keilson, 1979) greatly aid the task of phylogeny reconstruction. For this reason, the vast majority of phylogenetic analyses employ mechanistic models for a reversible DNA process.

Models for Double-Strand RNA

There are many types of biologically active RNA molecules (e.g., mRNA, tRNA, and snRNA) which are transcribed according to a DNA sequence template. Phylogenetic trees can be inferred from these molecules for the purpose of inferring the evolution of the organisms that carry them, or the evolution of the different gene families that encode them. Although the DNA models described above could be applied to RNA-encoding genes, the results would be biased. The problem is that the RNA molecules are biologically active as a single-stranded molecule that folds into secondary and tertiary structures. Hydrogen bonding between complementary nucleotides stabilizes secondary structures known as stems, and the evolutionary process is biased in favor of substitutions that preserve this structure. Because DNA models treat character state changes independently, paired substitutions that preserve stability are inferred to occur much less frequently than they should for RNA-encoding DNA.

To avoid these biases, Markov models have been formulated for evolution between nucleotides at different sites that pair within stem structures. Rather than the four individual DNA nucleotides (A, C, G, and T), these models specify probabilities of change between Watson-Crick RNA pairs (A:U, U:A, C:G, and G:C) and the 'wobble' pairing G:U and U:G. Because pairing occurs between sites within an RNA molecule, uracil (U) is used instead of thymine (T). There are 16 unique pairwise configurations, but only the six shown above are stable enough to form secure interactions. For this reason, the models are often restricted to either six or seven states (Tillier, 1994; Tillier and Collins, 1995, 1998; Higgs, 2000). The seventh state, M:M, is introduced to accommodate the 10 remaining pairs of nucleotides that do not form stable interactions. The most general form of this seven-state model was introduced by Higgs (2000):

$$Q = \begin{bmatrix} & AU & GU & GC & UA & UG & CG & MM \\ AU & \cdot & \alpha_1\pi_{GU} & \alpha_2\pi_{GC} & \alpha_3\pi_{UA} & \alpha_4\pi_{UG} & \alpha_5\pi_{CG} & \alpha_6\pi_{MM} \\ GU & \alpha_1\pi_{AU} & \cdot & \alpha_7\pi_{GC} & \alpha_8\pi_{UA} & \alpha_9\pi_{UG} & \alpha_{10}\pi_{CG} & \alpha_{11}\pi_{MM} \\ GC & \alpha_2\pi_{AU} & \alpha_7\pi_{GU} & \cdot & \alpha_{12}\pi_{UA} & \alpha_{13}\pi_{UG} & \alpha_{14}\pi_{CG} & \alpha_{15}\pi_{MM} \\ UA & \alpha_3\pi_{AU} & \alpha_8\pi_{GU} & \alpha_{12}\pi_{GC} & \cdot & \alpha_{16}\pi_{UG} & \alpha_{17}\pi_{CG} & \alpha_{18}\pi_{MM} \\ UG & \alpha_4\pi_{AU} & \alpha_9\pi_{GU} & \alpha_{13}\pi_{GC} & \alpha_{16}\pi_{UA} & \cdot & \alpha_{19}\pi_{CG} & \alpha_{20}\pi_{MM} \\ GC & \alpha_5\pi_{AU} & \alpha_{10}\pi_{GU} & \alpha_{14}\pi_{GC} & \alpha_{17}\pi_{UA} & \alpha_{19}\pi_{UG} & \cdot & \alpha_{21}\pi_{MM} \\ MM & \alpha_6\pi_{AU} & \alpha_{11}\pi_{GU} & \alpha_{15}\pi_{GC} & \alpha_{18}\pi_{UA} & \alpha_{20}\pi_{UG} & \alpha_{21}\pi_{CG} & \cdot \end{bmatrix}$$

The above matrix has been simplified according to various restrictions, such as base-pair reversal symmetry (e.g., Tillier and Collins, 1998) or restricting the number of exchangeability parameters (e.g., Tillier, 1994).

With 16 possible pairwise configurations, it is possible to construct a 16×16 rate matrix. In such a model, changes in state can proceed by all possible single- and double-nucleotide changes, regardless of stability. However, the most general form of this model would have 134 free parameters, making it impractical for maximum-likelihood-based inference. As with the six- and seven-state models, restrictions are used to simplify the rate matrix (Schöniger and Von Haeseler, 1994; Muse, 1995; Rzhetsky, 1995; Savill *et al.*, 2001).

Models for Protein Coding Sequences

Amino acid models are useful when phylogenetic signal is lost due to saturation of nucleotide substitutions. This modeling task represents a straightforward extension of Markov chain techniques to describe substitutions between the 20 different amino acids. The most simple Markov model for amino acids is reminiscent of the Jukes and Cantor (1969) model, as it is a symmetrical model having a single rate for all changes among the 20 amino acids (i.e., $\mu_{ij} = \alpha$). This model was first applied to phylogenetics of three species by Neyman (1971), and extended by Adachi and Hasegawa (1996) such that the substitution rate was proportional to the frequency of the target

amino acid (i.e., $\mu_{ij}=\alpha\pi_j$, where π_j is the frequency of amino acid j). These models are not often used in modern phylogenetic analyses, as they are considered too simplistic.

There is wide agreement that modeling replacement rates between different amino acids is critical to phylogenetic inference from amino acid data. Recall that these rates are determined by the off-diagonal values of a Q matrix, and they can be expressed as the product of an exchangeability parameter and a steady-state frequency ($\mu_{ij}=s_{ij}\pi_j$). Thus a 20×20 amino acid Q matrix will have 189 exchangeabilities (s_{ij}), and these will be difficult to reliably estimate from a single gene dataset. For this reason, amino acid models in phylogenetics typically employ a matrix of 189 s_{ij} values previously estimated from a large database of proteins. Such a model can be thought of as an extension of the nucleotide GTR model to amino acid data, where each of the s_{ij} parameter values is estimated from the database via maximum likelihood.

Two empirical exchangeability matrices (WAG: Whelan and Goldman, 2001, and LG: Le and Gascuel, 2008) have been very widely used in phylogenetics because they are intended for use with 'generalized' globular proteins. However, these matrices might not be suitable for other types of proteins (e.g., transmembrane or mitochondrial proteins) or domains of life (e.g., viruses). As mitochondrial encoded proteins have long been used for phylogenetic inference, the greatest choice of substitution matrices is available for these types of proteins. In addition to a very generalized matrix (mtZoa: Rota-Stabelli *et al.*, 2009), there are matrices tailored to specific lineages (e.g., mtREV20: Adachi and Hasegawa, 1996; mtMam: Yang *et al.*, 1998; mtArt: Abascal *et al.*, 2007; mtPAN: Carapelli *et al.*, 2007). Substitution matrices are also specifically available for viral proteins (e.g., HIV-B and HIV-W: Nickle *et al.*, 2007; FLU: Dang *et al.*, 2010). **Figure 1** illustrates differences among selected matrices.

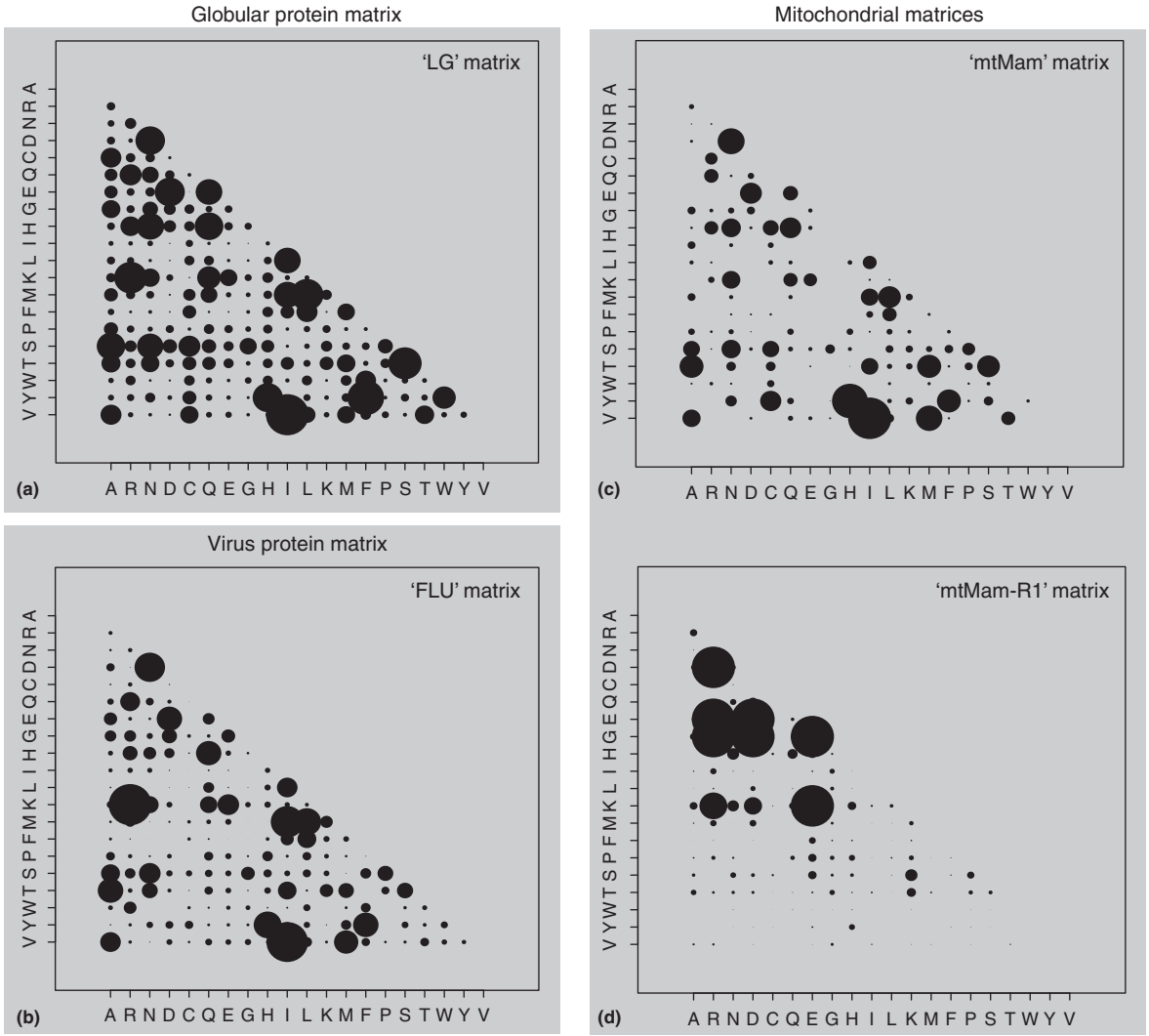

Figure 1 Examples of empirically estimated amino acid exchangeability matrices. The exchangeabilties are plotted as circles within a 20×20 matrix, where the diameter of the circle is proportional to parameter value. (a) The LG matrix for globular proteins. (b) The FLU matrix for viral proteins. (c) The generalized mtMam matrix for mitochondrially encoded proteins of mammals. (d) The mtMamR1 matrix for a specified subset of amino acid sites (1750 sites) with distinct physiochemical properties (bulky, hydrophobic, and amenable to alpha helices and beta structures).

In addition to exchangeability parameters, protein databases have been used to obtain a generalized set of steady-state amino acid frequencies (π_j). However, most researchers do not use them, preferring to set the π_j values in their model to the amino acid frequencies observed within their own data. This preference is likely because it is not computationally costly to obtain them from a single dataset.

It is possible to combine information about both the evolution of the DNA and the encoded protein in a model describing substitutions between triplets of nucleotides (codons). These codon models have the added benefit of accommodating the dependancies between adjacent DNA sites that are ignored by DNA models. The drawback is that the Q matrix grows to 61×61 (universal genetic code with stop codons removed), making these models much more computationally expensive to employ even though no new theory is required to carry out the probability calculations. The basic codon models were independently developed by Goldman and Yang (1994) and Muse and Gaut (1994). Although the rate matrix is very large (3721 elements), each substitution rate can be determined from the knowledge of just a few parameter values. For example, a simplified version of the Goldman and Yang (1994) codon model can be completely described by

$$q_{ij} = \begin{cases} \pi_j & \text{if synonymous transversion,} \\ \kappa\pi_j & \text{if synonymous transition,} \\ \omega\pi_j & \text{if nonsynonymous transversion,} \\ \omega\kappa\pi_j & \text{if nonsynonymous transition,} \\ 0 & \text{if codons differ by } >1 \text{ nucleotide,} \end{cases}$$

where κ is the DNA transition to transversion rate ratio, ω is the nonsynonymous to synonymous rate ratio and π_j is the steady-state frequency of the j^{th} codon. Many variants of the basic codon models have been proposed, and nearly all are used to study the process of codon evolution rather than infer a phylogenetic tree (reviewed in Anisimova and Kosiol, 2009). Because of the added benefits over amino acid models, they should also be desirable for phylogenetic analyses (e.g., Ren *et al.*, 2005; Miyazawa, 2013; Gil *et al.*, 2013). However, they have not yet been widely adopted because of their additional computational costs. Their use is likely to increase over time now that highly efficient implementations tailored for phylogenetic analyses are available in programs such as Garli (Zwickl, 2006) and CodonPhyML (Gil *et al.*, 2013).

Heterogeneous Evolutionary Dynamics and More Complex Models

The evolutionary models considered so far are defined in terms of a single substitution rate matrix. The problem is that real gene sequences typically evolve according to a process that differs among sites (e.g., membrane-bound versus non-membrane-bound sites of a transmembrane protein), and possibly over time (e.g., via lateral gene transfer events). Naive application of a constant rate matrix, no matter how sophisticated, to a heterogeneous substitution process can negatively impact biological inferences (Whelan, 2008). There are two broad approaches to modeling spatial and temporal heterogeneity. In the first approach, a priori biological knowledge is used to assign sites within a gene, or branches within a tree, to one of several discrete classes having different substitution parameter values. The heterogeneity among classes is referred to as a fixed effect because the relationship to the data (sites or branches) is fixed within the model. The second approach is used when suitable biological information is unavailable. Sequence heterogeneity is then modeled by using a statistical distribution, and this is referred to as a random effect within the model. Because biological knowledge is typically imperfect, the latter strategy is more common.

Site-wise heterogeneity in the substitution rate can be dramatic, and nearly all modern approaches to sequence analysis employ the gamma distribution to model rate variation. Although it is possible to use the continuous form of this distribution (Yang, 1993), it is computationally costly. A discretlized approximation of the gamma distribution works nearly as well, and is far more practical (Yang, 1994b; Waddell *et al.*, 1997). Note that the gamma distribution is used because it is convenient, and other strategies are possible. For example, Gu *et al.* (1995) proposed adding a simple parameter for the proportion of invariant sites, and Waddell *et al.* (1997) considered several alternative distributions. The underlying concept is that the rate at a site is modeled as a random draw from a statistical distribution so that rates do not have to be specified a priori on a site-by-site basis. Variation in other kinds of parameters can be modeled via this strategy (e.g., ω in codon models (Yang *et al.*, 2000)). Even the whole rate matrix, Q, can vary among sites as a random effect (e.g., Le *et al.*, 2008, 2012), or as a fixed effect (e.g., Dunn *et al.*, 2013), within a model.

The above models for spatial heterogeneity assume that the process is temporally homogeneous. Temporal heterogeneity can be accommodated by adding a second rate matrix to model the process of 'switching' between different evolutionary regimes over time (Wang *et al.*, 2007). The regime can represent binary states for the evolutionary process such as 'on' and 'off' (Tuffley and Steel, 1998), or alternative substitution rates (Galtier, 2001), or even alternative selective regimes modeled via the ω parameter of a codon model (Guindon *et al.*, 2004). These models are often referred to as 'covarion-like' because the Markovian 'switching' process is a computationally tractable approximation of Fitch and Markowitz's (1970) covarion model for coevolving sites. A very important difference is that the Markovian process of regime switching treats the evolution of the data at each site as independent of evolution at the other sites.

Model Selection

In phylogenetics it is often the case that some aspect of the results (topology, branch lengths, or measures of support) are sensitive to the assumed model. Thus, model selection is just as fundamental to modern phylogenetics as it is to other scientific disciplines (Posada and Crandall, 2001; Posada and Buckley, 2004). The task of model selection for phylogenetics is inherently statistical; the goal being to minimize different sources of statistical error by balancing the impact of using too complex a model against the impact of using too simple a model.

Bias and Variance

The process of model selection seeks to find a good balance between two different types of errors: bias and variance.

Variance is perhaps the more familiar type of error. In phylogenetics the goal is to predict the tree topology and branch lengths. Because there will be randomness in finite datasets, we expect a model to make somewhat different predictions for different samples of data. If we were to sample a gene, say, twice, and each time we sampled that gene from different species to represent the same lineages of interest (say, families), we would not be surprised to sometimes observe differences among tree topologies estimated under the same model. This is an example of variance, and it refers to the difference between results obtained under the same model when the modeling process is repeated multiple times.

Bias is less familiar because this type of error concerns how a model's mean result differs from the true values of the variables (which are typically unknown). When some aspect of the model is mis-specified, it will make some fundamental mistakes with the variability in the data. This leads to differences between the average of the predictions made by the model and the true value we want to predict; this is the error due to bias. Model bias is measured relative to the average prediction because model variance yields different predictions for different samples of data.

Figure 2 illustrates how both bias and variance contribute to the total error under a model. In this representation, the true value that we want to estimate is the center of the target, and each spot on the target is a result obtained under the same model when the modeling process is repeated multiple times. Minimizing model variance entails minimizing the spread of the results about the average result. If the model happens to be biased, this leads to estimates that consistently miss the target (Figure 2). Attempting to minimize bias, regardless of variance, might seem appealing because such a model could be thought of as being 'on average' correct. But this is also undesirable because the individual results can be as far, or farther, from the true value as they are under the biased model (Figure 2). Furthermore, in phylogenetics you need the model to be as good as it can be for the data in hand; you do not typically have data suitable for measuring a long run average. Thus both bias and variance must be managed when choosing a model for phylogenetics.

In general, increasing model complexity increases variance and decreases bias. Statistical methods of model selection attempt to manage both sources of error by trading off the negative effects of overly complex models (too high variance) against the negative effects of overly simple models (too high bias). Figure 2 illustrates how the contribution of variance and bias to the total error changes according to the complexity of the model. From Figure 2 we see that there is a level of model complexity that minimizes the total model error through a particular balance of variance and bias. A more complex model than this one is said to be 'over-fit' and a less complex model is said to be 'under-fit.'

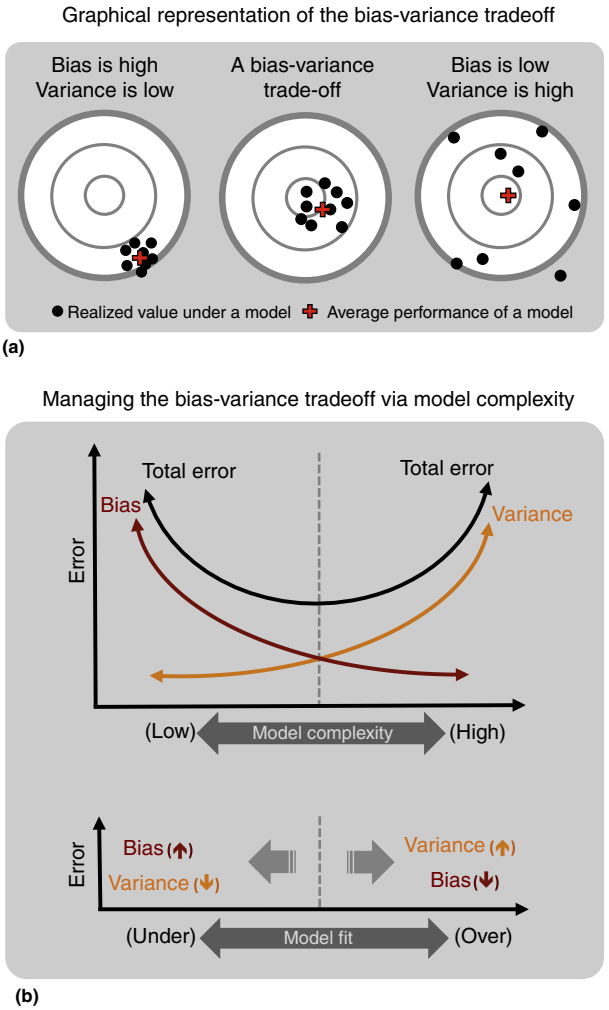

Figure 2 Model fitting as a means to manage the error due to bias and the error due to variance. (a) A graphical representation of the bias-variance trade-off. Here there are three 'targets,' each representing the predictions of a different model. A perfect model would make predictions that 'hit' the center of its target. Assuming that the modeling process can be repeated, the black dots represent individual realizations of the model, and the red plus sign represents the average performance of the model. The further the black dots are from the bulls-eye, the worse the predictions. (b) Managing the bias-variance trade-off via model complexity. Total error can be decomposed into bias and variance. Bias is the difference between the average predictions of the model and the 'target' value. Variance is the difference among the model predictions when the modeling process is repeated multiple times. Bias and variance depends on the complexity of the model. By managing the relationship between bias and variance, the total error associated with the model can be minimized. Relative to the point of minimal error, models that increase the total error by increasing variance are said to be over-fit, and models that increase the total error by increasing bias are said to be under-fit.

Testing Model Fit

In practice, there is no analytical solution for the level of model complexity that minimizes total model error. Thus, decisions about model complexity must be made according to how competing models fit the data in hand. The challenge is to use this information to choose a model that neither over-fits nor under-fits the data. This section presents three of the more widely used approaches (LRT, AIC, and BIC). Each compares competing models to determine if additional complexity yields a significant improvement in model fit. Lacking such an improvement, the simpler model is preferred. In this way, a

model can be selected that provides the best explanation of the data without over-fitting it.

The likelihood ratio test (LRT) is used to compare the fit of two models where one model (the null model, H_0) is a parametric simplification of the other model (the alternative model, H_1). As the pair of models are nested, this test focuses on how adding complexity to the null model improves model fit. The alternative model will always fit at least as well as the null, so the ratio of likelihoods is used to measure how much more likely the data would be under the alternative model. When certain regularity conditions are satisfied, two times the likelihood ratio serves as the basis of a formal statistical test. The test is typically applied to the logarithm of the ratio, defined as

$$2\delta l = 2\ln\left(\frac{\text{likelihood for } H_1}{\text{likelihood for } H_0}\right)$$

This statistic is approximately χ^2 distributed when the null is true, with degrees of freedom equal to the difference in the number parameters between the two models. Thus the significance of the improvement can be assessed according to the p-value of the observed likelihood ratio.

Several information theoretic approaches can be used to assess model error via the estimated loss of information associated with a candidate model. The most popular of these is the Akaike information criterion (AIC). The AIC score is defined as a function of the likelihood of the fitted model and the number of parameters in the model (k):

$$\text{AIC} = -2\ln(\text{likelihood}) + 2k$$

The second part serves to penalize the model fit according to its complexity. A different information theoretic approach is the Bayesian information criterion (BIC). It works in a similar way to AIC, but employs a stronger penalization for model complexity that depends on the sample size n (sequence length):

$$\text{BIC} = -2\ln(\text{likelihood}) + k \cdot \ln(n)$$

Under both AIC and BIC, models with a small score are preferred. A general guideline for avoiding over-fitting the data is to prefer simple models unless the difference in scores between competing models is greater than 2. Unlike the LRT, this approach does not require that the candidate models are nested.

Bayesian methods are now widely used alongside more traditional methods for phylogenetic inference. In a fully Bayesian setting, the relative fit of competing models is measured by a statistic called the Bayes factor, which is the ratio of the posterior odds of the two models to their prior odds. The Bayes factor is attractive to Bayesians because it permits use of the preferred prior for each model. When the two models (denoted M_1 and M_2) are believed to be equally probable a priori, the Bayes factor is approximated as the ratio of the marginal likelihoods under the competing models:

$$B_{12} = \frac{pr(D|M_1)}{pr(D|M_2)}$$

There are several interpretations of Bayes factors for model selection, but a widely used approach is to accept model M_1 when the Bayes factor is >1. Note that BIC is often viewed as a conservative approximation of the Bayes factor, and is thought to be reasonable for cases where there is little prior information. Model selection according to Bayes factors has advantages over the LRT in that it can be used for non-nested model comparisons and it can account for model uncertainty. Bayes factors can be challenging to compute and are only defined when the marginal probabilities densities under both models are proper.

Robustness

By trading off the negative effects of overly complex and overly simple models, the one that is chosen will not match reality in every detail. The model is necessarily simplified, and the researcher hopes that it has captured the features of the data that are most relevant to the inferences to be made. Implicit in this activity is the desire for a robust model; i.e., a model that has good performance under the small, and inevitable, departures from the model's assumptions. Although the robustness of the commonly used models for phylogenetic inference has been widely explored in the literature (e.g., Ripplinger and Sullivan, 2008, 2010), there will be many situations where it is prudent, and informative, for the researcher to further explore model robustness.

See also: Bayesian Phylogenetic Methods. Maximum Likelihood Phylogenetic Inference

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Multivariate Quantitative Genetics

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Glossary

Additive genetic correlation (r_A) A standardized measure of the additive genetic covariance (Cov_A) between a pair of traits in the population. r_A is calculated as $Cov_A/\sqrt{V_{A(X)} \cdot V_{A(Y)}}$ and is constrained to range between -1 and 1.

Additive genetic covariance (Cov_A) A measures of the degree to which two traits are influenced by the same genes (i.e., pleiotropy) or pairs of genes (linkage disequilibrium).

Additive genetic variance (V_A) Trait variation that is due to the additive effects of genes.

Artificial selection The process of selective breeding used to produce organisms (typically domesticated animals) with more desirable traits. It is also used as a tool by evolutionary genetics to understand the dynamics of trait evolution, such as demonstrating that a trait has genetic variation and determining if it is genetically correlated to other traits.

Correlated response (to selection) The evolutionary change in a given phenotypic trait that is not due to direct selection acting on that trait but due to selection acting on other genetically correlated traits.

Diagonalization It refers to the process of extracting the eigenvalues and eigenvectors of a symmetric matrix. Principal Component analysis is one of the most common methods of matrix diagonalization.

Principal component analysis It is one of the most common methods of matrix diagonalization.

Direct response (to selection) The evolutionary change in a given phenotypic trait that is due to selection directly targeting that trait. The direct response to selection depends on the strength of selection targeting that trait and its additive genetic variance.

Genetic architecture It refers to the pattern of genetic effects that underlie variation in a given phenotypic trait.

Heritability It refers to the proportion of the total phenotypic variance (V_P) that is due to genetic causes. Heritability can be estimated in either the broad sense or narrow sense. Narrow-sense heritability (denoted h^2) refers to the proportion of total V_P that is specifically due to additive genetic variance (V_A) and broad-sense heritability (denoted H^2) refers to proportion of total V_P that is due to all forms of genetic effect (V_G). Narrow-sense heritability is therefore estimated as V_A/V_P, whereas broad-sense heritability is estimated as V_G/V_P.

Linear selection gradient (β) A measure of the direct linear selection acting on a trait after removing the effects of indirect selection acting on other correlated traits being examined. β for a given trait is typically derived from a multiple regression analysis of the standardized measures of the traits being examined (where the standardize trait values (z) are determined as $z=x-\mu/\sigma$) against relative fitness. Depending on the sign, a significant β is predicted to either increase or decrease mean trait size.

Phenotypic variance (V_P) A measure of the variation present in a population for a given phenotypic trait.

Relative fitness (w) The fitness of an individual relative to the mean fitness of the population. w is typically calculated by dividing absolute fitness of an individual by the mean absolute fitness of the population.

Selection differential (S) A measure of the total phenotypic selection (both direct and indirect) acting on a trait. Under a regime of truncation selection, S can be estimated as the difference in the mean trait size of the selected individuals and the mean trait size of the entire population before selection.

Truncation selection The most common form of artificial selection where individuals subject to selection are ordered by the trait being selected and a proportion (p or $1-p$) is selected to reproduce and derive the next generation.

Vector A quantity or phenomenon that has both a magnitude and direction. In the case of $\Delta\bar{z}$ and β used in the multivariate breeder's equation, these vectors represent a single column with the number of rows equaling the number of traits being examined.

Evolution Is a Multivariate Process

Most traits of interest to evolutionary biologists, including behavior, morphology, life history, and physiology, are targeted by natural and/or sexual selection and their expression is controlled by the action of many genes (i.e., polygenic) (Falconer and Mackay, 1996; Lynch and Walsh, 1998). In the simplest sense, the evolutionary change in a trait can be predicted by the breeder's equation (Lush, 1937):

$$R = h^2 S$$

where R is the per generation *response to selection* of the trait being examined, h^2 represents the narrow-sense *heritability* of the trait, which encompasses the pattern of polygenic inheritance, and S represents the *selection differential*, which represents the covariance between the trait and *relative fitness*. Consequently, the breeder's equation measures the *direct response* of a given trait to selection and clearly illustrates the crucial point that R increases as either h^2 or S (or both) increases (Lush, 1937). While this fundamental equation has a long and successful history in predicting short-term evolution in a variety of agricultural and laboratory systems (e.g., Roff, 1997), this same

level of success does not appear to extend to natural populations (Morrissey *et al.*, 2010). It is therefore important to consider the underlying assumptions of this equation.

The breeder's equation assumes (1) that the trait being studied is not correlated with other traits that have a causal effect on *relative fitness* and (2) that the genes controlling variation in this trait are not associated with the genes that control variation in other traits. However, decades of quantitative genetic research has shown that selection rarely targets single traits but rather combinations of traits (Lande and Arnold, 1983) and that few traits are likely to be genetically independent (Falconer and Mackay, 1996; Lynch and Walsh, 1998). In quantitative genetics, the degree to which the genes for two traits are associated can be quantified by the sign and strength of the *additive genetic correlation* (r_A) between these traits. Values of r_A vary between -1 and 1 and the strength of the genetic association between traits increases (and therefore the degree of genetic independence decreases) with the absolute magnitude of r_A. Whenever traits are genetically correlated, the evolutionary response of a given trait will not only depend on the direct selection targeting this trait but also on the selection that targets any correlated traits. Therefore, just as the h^2 determines the magnitude of the direct response of a trait to a given strength of selection, r_A determines the magnitude and direction of an evolutionary response to a given level of selection on a correlated trait; a process referred to as a *correlated response to selection*. Genetic correlations between traits can therefore have dramatic effects on trait evolution, including altering the rate (e.g., Marchini *et al.*, 2014) and direction (e.g., Norry *et al.*, 2000) of evolution of a given trait and can even facilitate the evolution of this trait when it is not the direct target of selection (e.g., Hall *et al.*, 2004). Moreover, countless breeding experiments and analyses of natural pedigrees have shown that genetic correlations between traits appear commonplace (Roff, 1996) and *artificial selection* has also been used to demonstrate that these genetic correlations do indeed generate correlated responses to selection (Falconer and Mackay, 1996; Roff, 1997; Lynch and Walsh, 1998).

When selection operates on multiple, genetically correlated traits, a multivariate extension to the simple breeder's equation is needed to incorporate how these genetic associations between traits influences the response of a given trait to selection. This extension is known as the multivariate breeder's equation (Lande, 1979):

$$\Delta\bar{\mathbf{z}} = \boldsymbol{\beta}\mathbf{G}$$

where $\Delta\bar{\mathbf{z}}$ is the *vector* of mean trait responses over a single generation and $\boldsymbol{\beta}$ is vector of standardized *linear selection gradients* that directly targets each trait. **G** represents the additive genetic variance–covariance matrix that describes the *genetic architecture* of the traits being examined:

$$\mathbf{G} = \begin{bmatrix} Var_{1,1} & Cov_{1,2} & Cov_{1,3} \\ Cov_{1,2} & Var_{2,2} & Cov_{2,3} \\ Cov_{1,3} & Cov_{2,3} & Var_{3,3} \end{bmatrix}$$

G is described as a square matrix because it has an equal number of columns and rows, with one row and column for each trait being examined: the above example provides an illustration of **G** for three traits, represented by the numbers in subscript. The diagonal elements of **G**, in which both subscripts are the same, represent the *additive genetic variances* (V_A) of the traits (e.g., $Var_{1,1}$ equals the additive genetic variance in trait 1). The off-diagonal elements of **G**, with different subscripts, represent the *additive genetic covariances* (Cov_A) between pairs of traits (e.g., $Cov_{1,2}$ equals the additive genetic covariance between trait 1 and 2). **G** is also referred to as a symmetrical matrix because each genetic covariance occurs twice in the matrix: once above and once below the diagonal (since $Cov_{1,2} = Cov_{2,1}$, and therefore the same subscripts are used for both entries in the matrix). It is important to note that V_A and Cov_A are used in **G** and not estimates of h^2 and r_A. The reason for this is because scale matters when considering units of evolutionary change and estimates of h^2 and r_A are scaled in different units: h^2 is scaled by the phenotypic variance of the trait, whereas r_A is scaled by the square root of the sum of the additive genetic variances for the two traits (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

The easiest way to understand how **G** influences both the direct and correlated responses to selection is to write out the individual equations for evolutionary change for the set of traits derived from the multivariate breeder's equation (i.e., the $\Delta\bar{z}$):

$$\begin{bmatrix} \Delta\bar{z}_1 \\ \Delta\bar{z}_2 \\ \Delta\bar{z}_3 \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} \begin{bmatrix} Var_{1,1} & Cov_{1,2} & Cov_{1,3} \\ Cov_{1,2} & Var_{2,2} & Cov_{2,3} \\ Cov_{1,3} & Cov_{2,3} & Var_{3,3} \end{bmatrix}$$

$$\Delta\bar{z}_1 = \beta_1 Var_{1,1} + \beta_2 Cov_{1,2} + \beta_3 Cov_{1,3}$$
$$\Delta\bar{z}_2 = \beta_1 Cov_{1,2} + \beta_2 Var_{2,2} + \beta_3 Cov_{2,3}$$
$$\Delta\bar{z}_3 = \beta_1 Cov_{1,3} + \beta_2 Cov_{2,3} + \beta_3 Var_{3,3}$$

where the terms in red capture the direct response to selection for each trait, whereas the terms in black capture the correlated response to selection. For example, the evolutionary response of trait 1 ($\Delta\bar{z}$) is the sum of direct selection (β_1) acting on the acting on the additive genetic variance in the trait ($Var_{1,1}$), noting that $\beta_1 Var_{1,1}$ (or $\beta_1 V_A$) is just an alternate form of the 'univariate' breeder's equation outlined above ($R = h^2 S$), and the correlated response to selection caused by the fact that trait 1 is genetically correlated with traits 2 and 3 (i.e., $Cov_{1,2}$ and $Cov_{1,3}$) that are also targeted by linear selection (β_2 and β_3).

Here we provide an overview of multivariate concepts in quantitative genetics, paying specific attention to the importance of **G** in directing phenotypic evolution. Although we appreciate the fundamental contribution of multivariate selection to the evolutionary process, we direct the reader to other articles in this encyclopedia for information on the topic and how linear and nonlinear selection gradients can be quantified in natural populations.

What Does G Tell Us about Evolution?

As **G** contains the raw ingredients for evolution (V_A and Cov_A), this matrix can be used to describe the potential of a population to evolve (i.e., evolvability) and the extent to which

genetic architecture restricts or limits the course of phenotypic evolution (i.e., genetic constraint, Arnold, 1992). However, as the number of phenotypic traits contained in **G** increases it becomes progressively more difficult to understand these processes by interpreting the individual elements of **G**: for n traits, there will be $n(n+1)/2$ individual elements contained in **G** to interpret. Consequently, matrix algebra and trigonometry is often useful in describing the geometry of **G** (Blows, 2007) and therefore in determining how **G** influences evolvability and genetic constraint in a population. A useful mathematical property of all symmetrical matrices is that they can be *diagonalized* to find a set of real eigenvalues and orthonormal eigenvectors. Each eigenvector of **G** represents a linear combination of the original traits (known as a dimension) and the set of eigenvectors collectively describes the genetic space encompassed by the original traits. Each eigenvalue represents the scale factor that describes the length of the associated eigenvector and in the case of **G** this represents the genetic variance in the original traits. These two properties of **G** form the basis of a diverse array of metrics that have been used to characterize evolvability and genetic constraint in a population (Blows and Hoffman, 2005; McGuigan, 2006; Hansen and Houle, 2008; Agrawal and Stinchcombe, 2009; Kirkpatrick, 2009; Walsh and Blows, 2009). Here we provide a brief overview of some of these commonly used metrics and use an empirical data set on three important life-history traits in male Indian meal moths to help illustrate the utility of these metrics. Our list is not exhaustive and we classify these metrics in two broad categories: metrics of evolvability and genetic constraint that are derived exclusively from **G** and those that incorporate information on both selection and **G**.

An Empirical Example

The Indian meal moth (*Plodia interpunctella*) is a cosmopolitan pest of stored food products. In a study examining the evolution of sexual dimorphism in life-history traits in *P. interpunctella*, Lewis *et al.* (2011) quantified multivariate selection acting on three important life-history traits (development time, lifespan, and body size) in the sexes, as well as **G** for these traits within and between the sexes using a half-sib breeding design. Here, we use the quantitative genetic data on males as an example and the vector of standardized linear selection gradients, $\boldsymbol{\beta}$ and $\Delta\bar{z}$ for these traits is presented in Table 1. Table 2 presents the eigenvectors and eigenvalues of **G** derived by matrix diagonalization. The dominant eigenvector (referred to as $\boldsymbol{g}_{\max}$, Schluter, 1996) explains 95% of the genetic variation in **G** and is positively loaded to development time and to a lesser degree body size. $\boldsymbol{g}_2$ explains the remaining 5% of the genetic variance in **G** and is negatively weighted to lifespan and body size and positively weighted to development time. The final eigenvector ($\boldsymbol{g}_3$) is positively loaded to body size and negatively loaded to lifespan and development time but does not explain any of the genetic variance in **G**.

Table 1 The standardized linear selection gradients ($\boldsymbol{\beta}$), the additive genetic variance–covariance matrix (**G**), and the predicted response to selection ($\Delta\bar{z}$) for development time (DT), lifespan (LS), and body size (BS) in male *Plodia interpunctella*

Traits	β	**G**			$\Delta\bar{z}$
		DT	*LS*	*BS*	
DT	−0.07	4.74	0.03	1.52	−0.27
LS	0.11	0.03	0.12	0.16	0.02
BS	0.05	1.52	0.16	0.66	−0.06

Source: Data are taken from Lewis, Z., Wedell, N., Hunt, J., 2011. Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. Evolution 65, 2085–2097.

Evolvability and Genetic Constraint Based on Estimates of G

G will always contain as many dimensions as original traits and if each of these dimensions contains additive genetic variance, phenotypic traits will in theory be able to evolve in all regions of genetic space. However, if one or more of these dimensions have zero eigenvalues (as occurs if **G** is singular), then certain trait combinations will not contain additive genetic variance and therefore will not be evolutionary accessible in the population. Consequently, the rank of **G** (i.e., the number of nonzero eigenvalues) provides a useful measure of absolute genetic constraint (c) in the population (Table 3). The difficulty with this metric, however, lies in determining exactly how many dimensions of **G** have eigenvalues that differ significantly from zero, although bootstrapping (Mezey and Houle, 2005) or factor analytic modeling approaches (Kirkpatrick and Meyer, 2004) exist to test this directly. This latter approach provides statistical support for $\boldsymbol{g}_{\max}$ having an eigenvalue that is significantly greater than zero but not $\boldsymbol{g}_2$ or $\boldsymbol{g}_3$ for *P. interpunctella* (Table 2). This suggests that these lower two dimensions of **G** are unlikely to support evolution and therefore pose an absolute constraint.

The size of **G** can be quantified as the sum of the eigenvalues (known as the trace of **G**) and this value must equal the sum of the additive genetic variances of the original traits. Consequently, the trace of **G** provides a measure of the total amount of additive genetic variance (v_T) present in **G** and therefore an useful overall measure of the evolvability of a population: in theory, higher v_T will provide greater potential for the population to evolve (Table 3). The high V_A for development time and body size in male *P. interpunctella* (Table 1), which is substantially larger than in females due to sex linkage (Lewis *et al.*, 2011), results in a large v_T of 5.52

Table 2 Eigendecomposition (diagonalization) to extract the eigenvectors ($\boldsymbol{g}_{\max}$, $\boldsymbol{g}_2$, and $\boldsymbol{g}_3$) and eigenvalues of **G** provided in Table 1. The percentage of genetic variance explained by each eigenvector is also provided, as well as how each life-history trait contributes to these eigenvectors

Dimension	g_{max}	g_2	g_3
Eigenvalues	5.24	0.28	0.00
Variance explained (%)	95.00	5.00	0.00
Eigenvectors			
DT	0.95	0.25	−0.20
LS	0.02	−0.66	−0.75
BS	0.32	−0.71	0.63

Table 3 Some simple metrics measuring the evolvability and genetic constraint operating in a population that are based (A) exclusively on estimates of **G** and (B) on estimates of **G** and selection. We also provide values for each of these metrics based on the Plodia *interpunctella* data set provided in **Tables 1** and **2**

Metric	*Symbol*	*Formula*	*References*	*Value*
A. Based on G				
Absolute genetic constraint	c	$c=\lambda_i>0$	McGuigan (2006)	1
Total genetic variance	v_T	$v_T=\sum_{i=1}^{n}\lambda_i$	Kirkpatrick (2009)	5.52
Maximum evolvability	e_{max}	$e_{max}=\sqrt{\lambda_1}$	Kirkpatrick (2009)	2.29
Average evolvability	$\bar{e}$	$\bar{e}=\sum_{i=1}^{n}\lambda_i/n$	Hansen and Houle (2008)	1.84
Effective number of dimensions	n_D	$n_D=\sum_{i=1}^{n}\lambda_i/\lambda_1$	Kirkpatrick (2009)	1.05
B. Based on G and $\boldsymbol{\beta}$				
Angle between $\boldsymbol{\beta}$ and $\boldsymbol{g}_{max}$[a]	θ	$\theta=\cos^{-1}\left(\frac{\boldsymbol{\beta}\cdot\boldsymbol{g}_{max}}{\|\boldsymbol{\beta}\|\cdot\|\boldsymbol{g}_{max}\|}\right)$	Schluter (2009)	110.99°
Angle between $\boldsymbol{\beta}$ and $\Delta\bar{z}$[a]	θ	$\theta=\cos^{-1}\left(\frac{\boldsymbol{\beta}\cdot\Delta\bar{z}}{\|\boldsymbol{\beta}\|\cdot\|\Delta\bar{z}\|}\right)$	Walsh and Blows (2009)	61.54°
Rate of adaptation[b]	$\boldsymbol{R}$	$\boldsymbol{R}=\frac{\Delta W_c(\bar{z})}{\Delta W_i(\bar{z})}$	Agrawal and Stinchcombe (2009)	DT = 0.77 LS = 1.38 BS = −1.86
Respondability[b]	$r(\beta)$	$r(\boldsymbol{\beta})=\frac{\|\Delta\bar{z}\|}{\|\boldsymbol{\beta}\|}$	Hansen and Houle (2008)	DT = 3.86 LS = 0.18 BS = 1.20

[a] $\boldsymbol{\theta}$ is calculated in radians using the equations provided but can be converted to degrees by multiplying by $180/\Pi$.
[b] $\boldsymbol{R}$ and $r(\beta)$ are both measured on the specific life-history traits being examined: development time (DT), lifespan (LS), and body size (BS).
Note: In all formulas, λ refer to eigenvalues and n to the number of traits (or dimensions) in **G**.

(Table 3) and suggests that there is ample additive genetic variance for these life-history traits to evolve.

There are also a number of alternate ways of expressing evolvability and genetic constraint in a population that are based on how the additive genetic variation is distributed across the eigenvectors of **G**: the maximum evolvability (e_{max}), average evolvability ($\bar{e}$), and the effective number of dimensions (n_D) of **G**. In short, all three of these metrics describe how additive genetic variation is distributed across the eigenvectors of **G**. e_{max} is the square root of the eigenvalue of $\boldsymbol{g}_{max}$ and therefore describes the combination of traits in genetic space in which there is the maximum additive genetic variation for proportional change (Table 3). A value of 2.29 in *P. interpunctella* indicates substantial genetic variance is available in $\boldsymbol{g}_{max}$ to support phenotypic evolution (Table 3). $\bar{e}$ is the trace of **G** divided by the number of traits being examined and is therefore equivalent to the average trait additive genetic variance (Table 3). A value of 1.84 in *P. interpunctella* suggests a high average evolvability across dimensions of **G** but this finding should be interpreted with caution because $\bar{e}$ provides a weaker approximation of the average evolvability of the population when the number of dimensions is small and the eigenvalues are not of similar magnitude (Hansen and Houle, 2008). n_D is the sum of the eigenvalues divided by $\boldsymbol{g}_{max}$ and therefore a value close to one suggest that all genetic variation lies in a single dimension, whereas a value close to the total number of eigevectors suggests that genetic variation is distributed equally across dimensions (Table 3). A value of 1.05 indicates that most of the variation in **G** for male *P. interpunctella* is contained in $\boldsymbol{g}_{max}$: a pattern that is confirmed in Table 2.

Interestingly, a recent meta-analysis across plant and animal species found evidence that sexually selected traits evolved faster than life-history and morphological traits (Pitchers *et al.*, 2014). This pattern, however, was not related to differences in the strength of selection across these trait types or any of the above metrics evolvability and genetic constraint derived from published estimates of **G**. One interpretation of this finding is that these metrics of evolvability and genetic constraint do not, on average, limit phenotypic evolution. This should be viewed with caution; however, as few individual studies have information on the rate of evolution, selection, and **G** and therefore the ability to examine the interplay between parameters is limited.

Evolvability and Genetic Constraint Based on Estimates of G and Selection

As evolutionary change is the product of both selection and the genetic architecture of the traits subject to selection, arguably more information about evolvability and genetic constraint acting in a population will come from metrics that combine **G** with selection. This can be illustrated with a simple example. Consider the case in male *P. interpunctella* where only $\boldsymbol{g}_{max}$ has statistical support for an eigenvalue greater than zero. As we argue above, this provides evidence for an absolute genetic constraint on phenotypic evolution as there are regions in genetic space that cannot support evolution. But will this actually reduce evolvability and impose a genetic constraint on phenotypic evolution if selection is not targeting these particular trait combinations? This raises the more general issue of how well the direction of selection is aligned with the additive genetic (co)variance contained in **G** and whether this constrains phenotypic evolution. In theory, if the direction of selection and additive genetic (co)variance in **G** is poorly aligned, this could limit evolvability and represent a significant directional genetic constraint in the population (McGuigan, 2006). This issue was first raised by Schluter (1996) who showed that across a range of vertebrate species, the major

direction of divergence in phenotype was aligned with $\boldsymbol{g}_{\max}$ over an evolutionary timescale of at least 4 million years. Although this approach does not distinguish the observed phenotypic divergence to different evolutionary processes (i.e., drift and selection), it has stimulated the development of numerous methods to understand the importance of the alignment of **G** and selection to phenotypic evolution.

The simpler of these approaches, and the one used by Schluter (1996) to examine the alignment between the dominant eigenvector of the divergence matrix and $\boldsymbol{g}_{\max}$, is to use trigonometry to calculate the angle ($\boldsymbol{\theta}$) between two eigenvectors. Two different angles are useful when examining the interplay between **G** and selection: the angles between $\boldsymbol{\beta}$ and $\boldsymbol{g}_{\max}$ and between $\boldsymbol{\beta}$ and $\Delta\bar{z}$ (Table 3). The angle between $\boldsymbol{g}_{\max}$ and $\boldsymbol{\beta}$ measures the degree to which linear selection is aligned with the greatest amount of additive genetic variance in **G**. Therefore, an $\boldsymbol{\theta}$ approaching 0° indicates a strong alignment between these eigenvectors meaning that genetic constraint should be minimal, whereas an $\boldsymbol{\theta}$ approaching 180° indicates that these eigenvectors are poorly aligned and genetic constraint should be maximal. In the case of *P. interpunctella*, the large $\boldsymbol{\theta}$ of 110.99° suggests genetic constraint is likely to be important (Table 3). One limitation of this approach, however, is that it ignores the genetic variation present in the lower dimensions of **G** (Blows and Hoffman, 2005). While this is unlikely to be an issue in *P. interpunctella* because 95% of the additive genetic variance in **G** exists in $\boldsymbol{g}_{\max}$, this may not be the case in all species. In this regard, the angle between selection ($\boldsymbol{\beta}$) and the predicted evolutionary response to selection ($\Delta\bar{z}$) may provide a better estimate of genetic constraint in a population. While the interpretation of the magnitude of $\boldsymbol{\theta}$ between these eigenvectors is identical, the meaning is slightly different. As the estimation of $\Delta\bar{z}$ has been filtered through **G**, this angle measures the extent to which **G** biases predicted phenotypic evolution away from the direction favored by $\boldsymbol{\beta}$. Thus, a large $\boldsymbol{\theta}$ approaching 90° means that **G** is biasing the direction of phenotypic evolution, whereas as value of $\boldsymbol{\theta}$ that approaches 0° means that **G** is having little influence on the direction of phenotypic evolution. In *P. interpunctella*, $\boldsymbol{\theta}$ is 61.54° indicating that **G** is biasing the predicted evolution of life-history traits in this species (Table 3).

Another useful approach for examining the potential biasing effects of **G** on $\Delta\bar{z}$ is a metric known as the – rate of adaptation (***R***) (Table 3). ***R*** essentially compares $\Delta\bar{z}$ for a suite of traits when the genetic covariances in **G** have been set to zero ($\Delta W_l(\bar{z})$) versus when they are unaltered ($\Delta W_c(\bar{z})$). ***R*** is expressed as a ratio of these two parameters (Table 3), whereby if $\boldsymbol{R}=0.5$ for a given trait then the additive genetic covariance structure causes the fitness of the mean trait to increase by only 50% as quickly as expected if the trait was genetically independent. Alternatively if $\boldsymbol{R}=2$, then the additive genetic covariance structure accelerates evolution, such that the rate of adaptation occurs twice as fast as expected under genetic independence of traits. In *P. interpunctella*, the additive genetic covariance structure constrains the predicted evolution of development time. In the case of body size, the negative value of ***R*** occurs because of the difference in sign of $\Delta W_c(\bar{z})$ and $\Delta W_l(\bar{z})$ which is -0.062 and 0.033, respectively.

A final metric, known as the respondability ($r(\boldsymbol{\beta})$), measures the predicted evolutionary response of the population per unit of selection (Table 3). While $r(\beta)$ does not specifically measure evolvability along a given selection gradient, it does examine the potential for **G** to bias how rapidly a trait will respond when subject to directional selection (Hansen and Houle, 2008). Thus, large values of $r(\boldsymbol{\beta})$ indicates that the structure of **G** promotes evolutionary change, whereas small values indicates that **G** constrains such evolution. In the case of *P. interpunctella*, both development time and body size (largely owing to the larger V_A in these traits and reduced linear selection gradients, Table 1), whereas the reverse pattern in seen for lifespan (Table 3).

The Limitations Associated with Using G to Understand Multivariate Evolution

While the above examples provide a clear overview of why **G** is important in predicting phenotypic evolution, it is important to consider the limitations associated with using **G** in this manner. First, as is the case when examining multivariate selection operating on a suite of correlated traits, the utility of **G** in predicting phenotypic evolution is sensitive to the original traits contained in this matrix. Given the ubiquity of genetic covariance between traits and the large effects this can have on biasing evolution, it is tempting to include as many traits as possible in **G** and use matrix diagonalization to define 'new' composite traits (expressed as eigenvectors). It is important, however, to consider the biology of the traits being examined as there is the potential for this to obscure biological interpretation (Hunt *et al.*, 2007). That is, the 'new' traits described by the eigenvectors of **G** may not always have biological meaning. Thus, biological interpretation should always take precedence over statistical inference when determining which traits to include in **G** (Hunt *et al.*, 2007).

Second, the ability to predict phenotypic evolution using the multivariate breeder's equation is based on the assumption that **G** remains constant over evolutionary time. While this may be the case over one or a few generations, it is now widely accepted that **G** is unlikely to be stable over longer evolutionary timescales (Steppan *et al.*, 2002). The structure of **G** is ultimately determined by the frequency of alleles in the population and therefore any evolutionary process that alters allele frequency can also drive the evolution of **G**. While the effects of selection, drift, and mutation on the evolution of **G** have been examined mathematically (e.g., Jones *et al.*, 2003; Revell, 2007), we still know relatively little about the evolution of **G**. Until this is better understood, using **G** to predict evolutionary change will always face limitations.

See also: Evolvability, Quantitative Genetics of. Natural Selection, Measuring. Quantitative Genetic Variation, Comparing Patterns of

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Mutation and Genome Evolution

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Glossary

Biased gene conversion Unequal rates of utilization of the two DNA strands as templates in heteroduplexes containing gaps or mismatches during homologous recombination and/or recombinational repair.
Copy number variation, CNV Variation reflecting chromosomal and segmental deletions and duplications.
CpG hotspot A 5′CG3′ dinucleotide often methylated (C→^{m}C) in many genomes. Because deamination, a common mechanism of transitions, results in a ^{m}C→T change, such mutations cannot, unlike deamination of Cs into Us, be repaired by base excision repair, resulting in an abnormally high rate of C→T transitions at CpG sites.
Ectopic or illegal crossing-over Crossing-over between short areas of homology located at nonhomologous chromosomal sites. A major source of chromosomal and segmental deletions, duplications, inversions, and translocations.
Indels Insertions and deletions collectively, in particular in situation when it is impossible to distinguish between a deletion in one lineage and insertion in the other.
Isochores More or less (often less) well defined regions of high or low CG content in vertebrate genomes.
Microsatellites or short tandem repeats Hypervariable sites in which a motif of 2–6 nucleotides is repeated 10–200 times.
Mutation A heritable change in genetic material.
Mutational bias Intrinsic systematic differences in frequencies of mutations of different types; cause by either chemistry of mutagenesis, or by biased gene conversion/asymmetries in DNA repair mechanisms.
Neutral mutations Mutations conveying no fitness differences to organisms that carry them. Invisible to selection; their frequencies change by genetic drift.
Segmental duplications Relatively long (1–100 kbp) tandem duplications observed in many genomes.
Transition Nucleotide substitution within a class of nucleotide (purin to purine/pyramidine to pyramidine); as opposed to transversion, nucleotide substitution across nucleotide classes (purin to pyramidine); typically transitions are more likely events than trnsversions.
Transposons or transposable elements Selfish genetic elements capable of copying themselves (and occasionally other genetic material) into new genomic locations. DNA transposons cut themselves from one location and paste into another; if such transposition occurs during DNA replication from an already replicated to a not yet replicated region it is accompanied by the increase in number of transposon's copies. RNA- or retrotransposons multiply by transcribing themselves into an RNA copy which is then reverse-transcribed and the resulting cDNA copy inserted into a new location.

Introduction

For much of the 100 years since the rediscovery of genetics and the introduction of the New Synthesis, the field developed under unique conditions of extreme shortage of data on chemical nature of heritable differences among organisms. It was clear from the theoretical standpoint that such heritable changes occur randomly, affect the germline, and are inherited in a discrete manner, but, perhaps with the exception of large-scale chromosomal rearrangements, no such changes could be directly observed. As the result, evolutionary genetics possessed a well-developed theory that predicted the fate of an individual heritable change in the population, but could not be made specific to describe particular types of changes or predict their abundance in the genomes. The amount of polymorphism present in the genomes of living organisms at any given moment of time was greatly underestimated and quantification of genomic differences among species was intractable. Everything changed with the discovery, largely in 1960s, of the mechanisms of spontaneous and induced mutagenesis and off, first, protein-level (allozyme) and, later, nucleotide sequence-level polymorphisms. DNA sequencing methodologies, developed in 1970s and perfected in 1990s, allowed a significant flow of sequence data to enter the field, greatly expanding our understanding of the nature of heritable variation in genes and genomes. These data were, in turn, dwarfed by the flood of sequences resulting from the next generation sequencing methods developed since the onset of the twenty-first century, with thousands of full genomes from hundreds of different species now available for the analysis. Today we can fully apply the classic population-genetics theory to explain genomes' nucleotide composition, variability, and architecture. This article reviews recent insights on how properties of mutagenesis shape the evolution of genomes.

Theoretical Considerations: Rate of Substitution and Stable Polymorphisms For Neutral and Harmful Mutations

A fundamental result of neutral theory of evolution (Kimura, 1983) is that mutations free of the effects of selection reach fixation at the same rate at which they are introduced into populations by mutagenesis. Indeed, if one compares a finite diploid population of size N at two sufficiently distant moments of time (sufficient for coalescence of all gene lineages), then any new allele created by a mutation at the earlier moment has the $1/(2N)$ probability to become fixed. There are

$2\mu N$ such alleles at this moment of time (where μ is the mutation rate per generation), thus that the rate at which new mutations become fixed is equal to μ. This is true for all mutations and for any mutation of a particular type, thus the rate at which neutral mutations of different molecular nature reach fixation reflects their mutational probabilities. For example, if deletions are more frequent than insertions of similar size and both are neutral, genomes will tend to become more compact; if substitutions to CG pairs are more frequent than substitutions from CG pairs, higher equilibrium CG content will be reached. While strictly speaking this is true only for neutral mutations, similar mutation biases exist also for mutations reaching fixation under positive selection, if selection is not too strong, and differences in mutation rates are large (Stoltzfus and Yampolsky, 2009). Therefore, properties of mutagenesis influence genome architecture in both neutrally evolving genomic regions and in regions under moderate positive selection. Conversely, any part of the genome in which either current state or inferred evolutionary dynamics of nucleotide composition or genome architecture drastically differ from what can be predicted from mutational pressures, can be hypothesized to experience strong positive selection.

The generation of new mutations affects not only rates of substitution but also the occurrence of polymorphic sites and allele frequency in them. There are at least two mechanisms through which mutagenesis can maintain stable polymorphisms: direct-reverse mutation balance and mutation-selection balance (Crow and Kimura, 1970). Direct-reverse mutation balance occurs between neutral mutations introduced by the mutagenesis process at roughly equal rates, with equilibrium allele frequency at a 2-allele locus being $q_{eq}=\mu/(\mu+\nu)$, where μ and ν are direct and reverse mutation rates (exactly equal rates resulting in the equilibrium frequency of 0.5). The rate approach to this equilibrium in large populations is equal to total mutation rate, i.e., is extremely slow. Mutation-selection balance occurs because stabilizing selection is inefficient in eliminating rare mutations, in particularly recessive ones. As the result deleterious alleles can persist in a population at a low frequency if constantly introduced by mutations. This equilibrium frequency of a harmful recessive allele is $q_{eq}=\sqrt{(\mu/s)}$, where μ is the mutation rate to a harmful allele and s is the selection coefficient against it. This frequency can be many orders of magnitude higher than mutation rate if selection is not too strong. For example, with $\mu=10^{-6}$ and $s=0.001$, the harmful allele will segregate at the easily detectable frequency of $q_{eq}=0.032$. For a harmful codominant allele with dominance coefficient h ($h=0.5$ corresponds to full codominance, $h=1$ to complete dominance) the equilibrium frequency at mutation-selection balance can be approximated as $q_{eq}\approx\mu/hs$. It is believed that many segregating sites in the human genome, both those with and without known clinical effects, are maintained in polymorphic state by mutation-selection balance (Olson, 2012). This is particularly true for sites with high mutation rates, such as microsatellite sites or CpG sites.

Genome Size Under Mutational Pressure

Genome size depends on differences in the rates at which deletions and insertions occur and on the efficiency of natural selection in promoting or eliminating such changes (Lynch, 2007). Small changes in genome size are probably of negligible significance in terms of energetic costs of replication, particularly in multicellular eukaryotes, in which genomes are large and metabolic costs of locomotion and development are many orders of magnitude higher than those of genome duplication. Similarly, while large genomes have higher mutational liability, even in noncoding areas, the disadvantage of a small further increase is likely to be insignificant. Thus, a large fraction of noncoding regions are likely to evolve neutrally with respect to insertions and deletions (**Figure 1**).

Three major types of mutations contribute to genome size: small indels (single nucleotides to few dozens of nucleotides), transposone activity, and segmental duplications. As these mechanisms can lead to opposing pressures on genome size, the size of the genome greatly depends on their relative occurrence rates. Small indels occur as the result of polymerase slippage during replication (in particular at short tandem repeat or microsatellite sites) and as the result of imprecise repair of double-strand breaks. It appears that in many genomes (*Drosophila melanogaster* and *Caenorhabditis elegans* genomes being well documented examples) deletions occur at a higher rate than insertions and are on average longer, resulting in compact genomes (**Table 1**; Petrov, 2002; Witherspoon and Robertson, 2003). Compact size of such genomes is often explained by the net losses of nucleotides in neutrally evolving genome segments. There are also documented cases of a significant mutational bias toward net gain of nucleotides, for example, in pseudogenes in rice (Noutsos *et al.*, 2005). Yet, it is difficult to imagine that mutational bias observed for small indels can overcome or significantly enhance much stronger bias toward insertions imposed on genomes by transposition and segmental duplications (see below).

Transposable elements, retrotransposons in particular, impose a significant mutational pressure toward potentially nonadaptive expansion of genome size (Cordaux and Batzer, 2009;

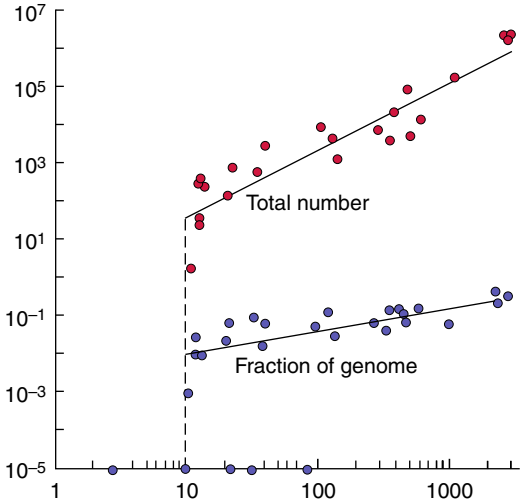

Figure 1 Number of transposons copies and fraction of genome made up by them vs. genome size (Mb) in a variety of organisms with fully sequenced genomes. Reproduced from Lynch, M., 2007. The Origins of Genome Architecture. Sunderland, MA: Sinauer Associates.

Table 1 Ratios between rates and average length of deletions and insertions in the worm, the fly, and mammalian (average of mouse, rat, and human) genomes

	Deletions: Insertions	
	Rates	*Average length*
Caenorhabditis	1.8	1.1
Drosophila	4.1	3.5
Mammals	1.9	0.8

Source: Modified after Lynch, M., 2007. The Origins of Genome Architecture. Sunderland, MA: Sinauer Associates (Table 2.1).

Lee and Kim, 2014), often demonstrating a linear relationship between the number of transposon copies and genome size. In addition to potential energetic cost of replicating and, possibly, transcribing extra genetic material, proliferation of transposons increases mutational liability in at least three different ways. Firstly, extra genetic material, even fully neutral, implies higher risks of deleterious gain-of-function mutations. Secondly, higher copy number of functional transposons leads to a higher genome-wide transposition rate, resulting in a higher rate of loss-of-function mutations due to transposition into coding or regulatory regions. Finally, high transposon copy number increases the likelihood of ectopic crossing-over by providing numerous areas of homology at non-homological sites, resulting in higher rate of chromosomal aberrations, including segmental duplications (see below). Thus, the enormous mutational bias toward increased copy number (transposition rates can be as high as 10^{-5} per transposable element per generation) is likely to be counterbalanced by selection favoring curbing transposon activity and deletion of existing copies. It has been argued that enormous genomes of some fish and amphibians may be a result of inefficiency of such selection in small populations, resulting in a runaway growth of genome size (Sun *et al.*, 2012).

Finally, genome size and variability may be affected by the mutational pressure of chromosomal and segmental duplications and deletions. Because each ectopic crossing-over event occurring between repeated areas of homology within the same chromosome generates both a deletion and a duplication of the region between these homologous sites, one should expect that the dynamics of the resulting copy number variation will be governed by direct-reverse mutation and mutation-selection biases. Indeed, CNVs in the human genome are numerous, result from mutations that occur at a high genomic rate, and their frequencies are well described by the mutation-selection model (Itsara *et al.*, 2010). Although much duplication also has detrimental effects, generally speaking deletions should be under stronger stabilizing selection than duplications. This may lead to a run-away process of proliferation of segmental duplications, as each additional duplication increases the opportunities for ectopic crossing-over. It is not currently clear in what situations segmental duplications drastically increase size and number of genes in a genome, but such genomes are known. For example, the genome of *Daphnia*, a plankton crustacean, contains numerous segmental duplications with about half of the genes represented by multiple paralogous copies residing in such duplications (Colbourne *et al.*, 2011).

Variation at Microsatellite Sites

Microsatellite sites are defined as sites at which a short (206 pb) sequence of nucleotides is repeated a large and variable number of times, typically 10–200. (Compare to a different type of hypervariable tandem repeats site, minisatellites, in which repeats are longer, 10–150 bp, and which mutate largely though the ectopic crossing over mechanism described above). While not contributing significantly to genome size, microsatellites are ubiquitous and harbor a significant amount of variability in the number of repeats. This variability is expensively used for identification purposes and sometimes has medical significance.

Microsatellites' abnormally high rates of insertions and deletions are caused by the phenomenon of polymerase, or replication slippage. During DNA replication the template and the newly synthesized strands of DNA sometimes become separated. It probably can happen in any area of the genome, but in the regions of high sequence complexity this has no consequences, as there is only one way the two complementary strands can pair up again. However, in the areas of low complexity, such as runs of single nucleotides or at microsatellite sites, the two strands may pair up with a short loop formed on either one or the other (**Figure 2**). Looping on the new strand results in an insertion, while looping of the template strand results in a deletion of a whole number of repeats. Generally speaking both outcomes are approximately equally frequent, so one may expect to observe the direct-reverse mutation balance. Moreover, each newly created allele with a larger number of repeats is now capable of generating even longer alleles, so microsatellite sites often contain numerous alleles, and therefore extremely high heterozygosity (**Figure 3**).

The majority of microsatellite sites are located in noncoding regions of the genome, such as intergene spacers and introns. Such microsatellites are likely to be neutral unless they disrupt a regulatory site or a splicing signal. However, a number of microsatellite sites located within coding portions of genes are known to cause severe hereditary diseases in humans. Clearly a 3-mer microsatellite within a coding portion causes a run of a particular amino acid in the polypeptide chain encoded by the gene, while microsatellites with repeat length of n nucleotides, where n is not a multiple of 3 cause repeats of n amino acids in the polypeptide chain; copy number mutations at such sites cause frameshifts. Probably for this reason, the majority of microsatellite sites located within coding regions are 3-mers. Diseases caused by microsatellites in coding portions of genes have several features in common. Firstly, they are more likely than single nucleotide substitutions to be gain-of-function and therefore dominant mutations. For example, the normal allele of huntingtin gene in humans contains a microsatellite site with 25 or less repeats of CAG triplet (i.e., a run of glutamines in the polypeptide sequence that is 25 residuals or less). Mutant alleles with over 40 repeats result in an abnormal protein that is characterized by a gain of a toxic function: it has the ability to aggregate and accumulate in neurons where huntingtin is normally expressed, disrupting the neurons' functions, resulting in Huntington's disease, a dominant hereditary condition. This also probably explains why there are many neurodegenerative

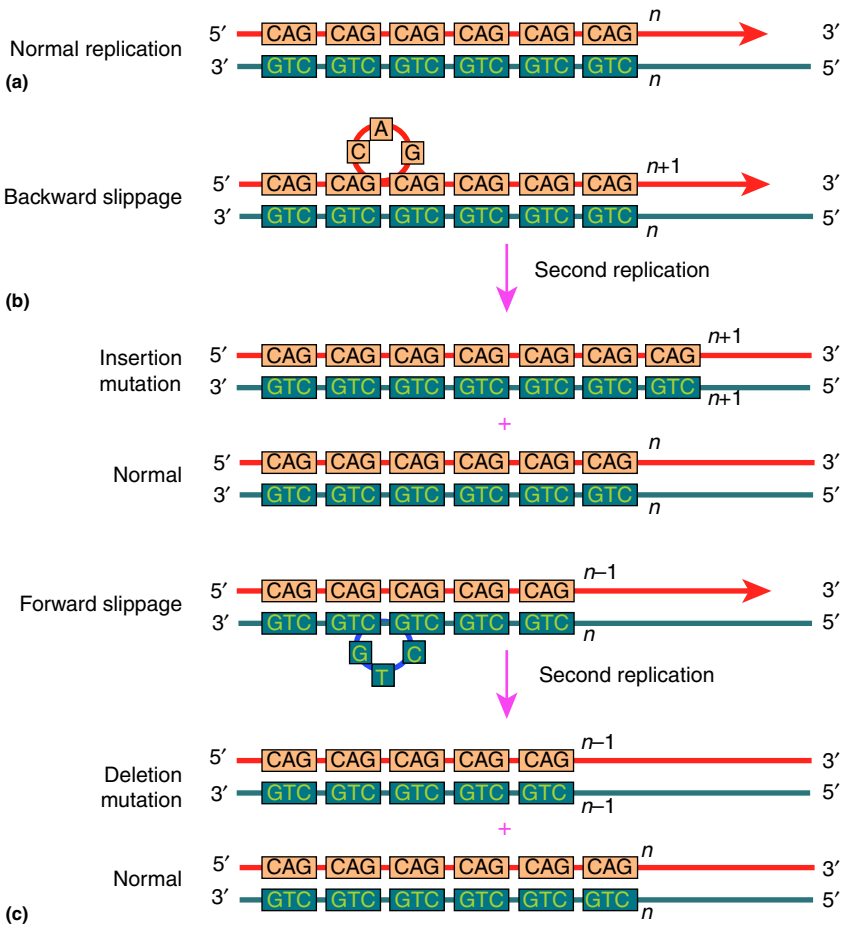

Figure 2 Polymerase slippage. Adapted from http://www.web-books.com/MoBio/.

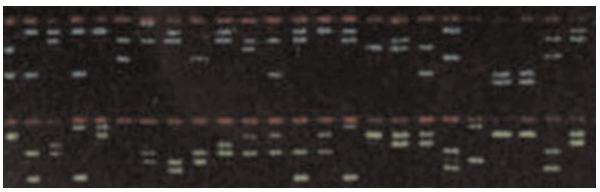

Figure 3 Multiplexed microsatellite gel showing variation at two microsatellite sites (amplified with different fluorescent dyes). Note that majority of individuals are heterozygotes for both loci. Adapted from http://125.218.212.107/fzswx/index.php?con=lesson&action=chapter&lcid=151.

conditions among microsatellite-caused diseases: neurons appear to be sensitive to gain-of-function mutations. Secondly, microsatellite-caused diseases often demonstrate different degrees of penetrance and the phenomenon of anticipation: the increase of penetrance and/or severity from one generation to the next. The reason for this is that (1) alleles with intermediate repeat number have a lower penetrance than alleles with high repeat number and (2) further mutations expanding already long alleles will generate alleles with increased severity or penetrance. This is typical for Huntington's disease as well as for fragile-X disease (a CGG repeat in gene responsible for normal functioning of synapses). Of course the bidirectional nature of microsatellite indel mutations will just as likely cause the decrease of the number of repeats from one generation to the next, but a decrease of penetrance and severity is much more difficult to detect (a mild allele becomes nearly normal and escapes clinical attention).

While the role of hypervariable microsatellite sites in shaping the spectrum of neurodegenerative hereditary diseases has been extensively studied, the degree to which they can serve as generators of adaptive variations is much less understood. Yet, some intriguing evidence exists that adaptive divergence among populations of grasses, behavioral changes in *Drosophila* and voles and skeletal changes in dogs during domestication are likely to have evolved by tapping into the endless pool of microsatellite mutations as the suppliers of raw material for adaptation (Kashi and King, 2006).

CG Content Across and Within Genomes

If all nucleotide substitutions occurred at the same rate, the equilibrium frequency of the 4 nucleotides in either DNA strand would have been 0.25 each, implying equal number of CG and AT pairs. This is almost never the case; CG content of whole genomes and parts of genomes deviate from this expectation widely. Recent findings in nucleotide composition

analysis indicate that CG content is determined by the interplay of mutational pressure, biased gene conversion, and, in some cases, selection. Mutational pressure typically favors CG→TA transitions over TA→CG ones (Hildebrand *et al.*, 2010; Hershberg and Petrov, 2010). This occurs because both C's and G's happen to be more liable to the two most common base-modifying mutagenesis processes than either A's or T's – deamination and oxidation. Deamination of cytosine results in a uracil, which will, in the next round of DNA replication, reliably pair up with an A instead of G. The product of oxidative conversion of guanines, 8-oxo-guanine (8oG) pairs up with T instead of a C. Thus, both processes lead to CG→AT transitions. Similar processes affecting A's and T's either are less likely to occur, or result in more promiscuous bases, meaning lower mutation probability per event, or result in bases that are easily removed by excision repair (such as hypoxanthine, the product of deamination of A's). Both U's and 8oG's present in DNA strands as the result of these processes can also be removed by base excision repair, eliminating the mutation. However, deamination of methylated cytosines results in a thymine base, which, as a base normally occurring in DNA, is invisible to excision repair mechanisms. As a result, methylation of cytosines in general, and in particular at CpG sites in animal genomes (see below), leads to even higher rate of CG→TA transitions (Krawczak *et al.*, 1998). The loss of CG pairs due to mutational pressure may also be a self-enhancing process. In particular, the deamination of C's is much more likely to occur in single-stranded DNA, and strand separation is more likely in AT-rich regions, because AT pairs are held together by two hydrogen bonds, not three, as CG pairs are (Fryxell and Zuckerkandl, 2000).

It is therefore not surprising that the rate of CG→TA transitions is universally 2–4 times higher than the rate of TA→CG transitions (Lynch, 2007, Table 6.1). Given these differences one could expect many genomes to be extremely CG-poor. This is not the case due to two other processes that oppose this mutational pressure: biased gene conversion and selection. Gene conversion occurs during crossing-over or double-strand break repair when a strand from one recombining homologous DNA is used as a template to build or repair the complementary strand from the other homologous DNA. If the choice of template strand in the recombinational heteroduplex was random, gene conversion would have been unbiased with respect to nucleotide composition. Yet, there is strong evidence that whenever G:T or C:A mismatches occur in a heteroduplex region, the strand containing G or C nucleotides is more frequently used as the template to repair the other strand, resulting in a bias favoring CG pairs over TA pairs. Relatively high CG content in animal and fungal genomes is almost certainly maintained by biased gene conversion opposing mutational pressure.

The role of selection in opposing the AT-favoring mutational pressure has been discussed in the context of CG-content differences among prokaryotes and CG-content heterogeneity within many eukaryotic genomes (isochores). In both cases selection in favor of DNA stability (i.e., higher CG content) in organisms inhabiting warmer environments or maintaining higher body temperature has been hypothesized. There is, however, little evidence for this effect either in prokaryotes (little if any correlation between temperature optimum and CG content) or for isochores in eukaryotic genomes (CG-heterogeneity is present in both homeotherm and poikiloterm vertebrates; it is difficult to see why some parts of genomes can be affected by thermostability more than others). Rather, it appears that the heterogeneity of CG content among prokaryotic genomes and within eukaryotic genomes is caused by the self-sustaining nature of mutational pressure: genomes or genomic regions with stochastically low CG content will tend to further lose CG pairs due to lower DNA stability.

Hypervariable CpG sites, which experience higher than average rate of transitions due to methylation of cytosines, are worse special attention. In animals, in particular in vertebrates CG→TA transitions are 10–20 times more likely at such sites than at non-CpG sites and constitute a disproportional share of variation within species (Krawczak *et al.*, 1998) and divergence among species (Nachman and Crowell, 2000). This results in a rapid loss of nonessential CpG dinucleotides in the genomes, suggesting that de-novo mutations at CpG sites are likely to be deleterious. Yet, recent evidence suggests that, at least in the context of adaptation to novel or extreme environments, CpG hypervariability may be a source of raw material for adaptive evolution (Galen *et al.*, 2015).

Replication and Mutation

We have already seen that transitions caused by base modification and mutations at hypervariable microsatellite sites occur during DNA replication. In fact, this is true about the majority of point mutations. Any mismatch of nucleotides caused by spontaneous or induced mutagenesis requires two rounds of DNA replication to become a permanent mutation affecting both DNA strands. Thus, non-replicating DNA (i.e., DNA in cells stalled in G1 or G2 phase of the cell cycle) will accumulate mismatches, which still retain the possibility of being repaired by DNA repair mechanisms, but few permanent mutations. There are several evolutionary consequences of this fact.

Firstly, because the two DNA strands spend different amount of time single-stranded during replication (lagging strand longer than the leading strand) and mutations frequently are initiated in the single-stranded state, the two strands should be expected to differ in the spectrum of mutations that occur in them, and thus in their nucleotide composition. Therefore, either strand on one side of the origin of replication should be expected to evolve the same nucleotide composition as the opposite strand on the other side of the origin. This phenomenon, known as strand asymmetry, is indeed observed, at least in prokaryotes (**Figure 2**). In contrast to CG-bias also mediated by single-strandedness (see above), strand asymmetry results, in most cases, not in excess of Cs and Gs over As and Ts, but in excess of Gs over Cs and Ts over As on the leading strand (Lobry and Sueoka, 2002; **Figure 4**).

Secondly, because most mutations occur during DNA replication, genomic mutation rate per generation strongly depends on the number of mitotic divisions from zygote to zygote. This has several consequences. Larger multicellular organisms have to deal with higher mutation rates, a problem exacerbated by the fact that larger organisms tend to have

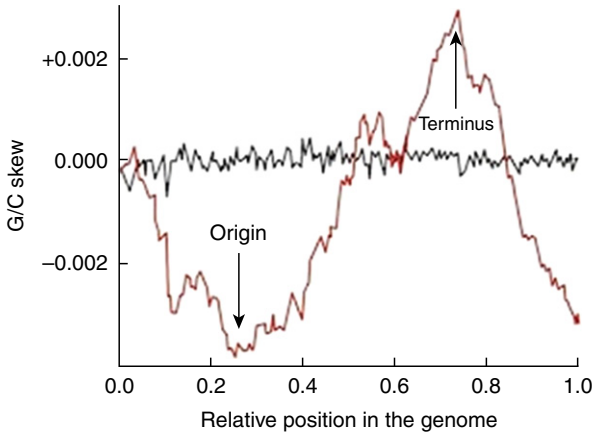

Figure 4 Local (black) and cumulative measure (red) of G/C skew on either side of the origin of replication in Mycoplasma pneumonia chromosome. Reproduced from Lynch, M., 2007. The Origins of Genome Architecture. Sunderland, MA: Sinauer Associates.

larger genomes (Lynch, 2006). Furthermore, in bisexual organisms with separate genders there is a universally higher number of mitotic divisions in males than in females, due to many orders of magnitude higher sperm production than egg production (Haldane, 1947; Miyata *et al.*, 1987). For example, in mammals there are about 15–30 mitoses in the female germ line, from an egg to an egg, but hundreds of mitoses in the male germ line. In a given species, this number is constant in the female germ line, but linearly increases with a male's age in the male germline, since spermatogenesis continues through a males' lifespan (Hurst and Ellegren, 1998). Thus, many more mutations initially occur in male than in female germ line and the number of new mutations increases with the paternal age (Kong *et al.* 2012; Campbell and Eichler, 2013).

Do Mutation Rates Evolve?

The entire above discussion assumes that mutation rates and biases are physical or chemical constraints that organisms have to deal with. However, much of the variation in these rates and biases is caused by details of complex enzymatic processes such as DNA polymerase fidelity, proofreading, and DNA repair rather than on extrinsic chemical constraints. Such processes can evolve and they do evolve. For example, one may hypothesize that the observed bias in rates of deletions versus insertions may be a result of selection favoring genotypes that are more efficient in streamlining genomes or that the degree of CG bias in gene conversion is the result of selection in favor of counterbalancing maladaptive effects of mutational pressure. Numerous theoretical and experimental studies show that mutator alleles that increase mutation rates (e.g., hypomorphs at genes responsible for DNA repair) are readily hitchhicked by positive selection in organisms with little or no recombination (Raynes and Sniegowski, 2014). However, it is difficult to demonstrate how hitchhiking of such mutator alleles with genome-wide effects can occur when recombination is frequent and when positive selection is moderate, because the cost of elevated mutation rates would be too high. Likewise, selection favoring extremely low mutation rate is both weak (and the lower the mutation rates are, the weaker this selection is) and limited by high costs of replication fidelity. On the other hand, gene-specific modulations of mutation rates and spectra have been described, at least in prokaryotes (Martincorena and Luscombe, 2012). Mechanisms of targeted hyper- and hypomutation include local changes in recombination rate and/or DNA repair activity, propensity of lagging strand to form secondary structures (thus reducing the mutation-prone singlestrandedness), and affinity of protective DNA-binding proteins. Targeted hypomutation can also be achieved by selection favoring removal of mutation-prone sequence elements such as microsatellites and CpG dinucleotides and of repeats facilitating ectopic crossing-over.

See also: Genome Organization, Evolution of. Molecular Evolution, History of. Mutation, Population Genetic Models of

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Mutation, Population Genetic Models of

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Models of mutation long predate the discovery of DNA as the hereditary material. Still, the development of new models of mutation have often followed advances in molecular genetics. The field of genetics was initiated by Gregor Mendel and he focused on dichotomous traits. In the 1860s and 1870s, Mendel collected data on seven characteristics of the common garden pea. For each trait, he recognized two alternative forms and hypothesized a specific underlying 'factor' associated with each alternative (what we now call alleles). When variation is described in this way, a natural way to model mutation is given below, where A and a indicate alternative alleles, u is the mutation rate from A to a, and v is the reverse mutation rate (Hartl and Clark, 1989)

$$A \underset{v}{\overset{u}{\rightleftharpoons}} a$$

This very simple model was developed extensively by population geneticists to explore the balance of evolutionary forces. If p is the population frequency of the A allele and q is the frequency of a ($q=1-p$) and mutation is the only process at work, then the frequency of A in the next generation is given by

$$p' = (1-u)p + vq \qquad [1]$$

and the population approaches a stable equilibrium,

$$\hat{p} = \frac{v}{u+v} \qquad [2]$$

Equation [2] illustrates the simple idea that if mutation to one allele is most probable, that allele will be most common in the absence of other forces.

This very simple two-allele model remains in use today, despite knowing, at the molecular level, that more than two types are possible even in the simplest situations. For example, there are four alternatives at any location in a DNA sequence (nucleotides A, G, C, and T). The two-allele model remains useful, because it is routinely possible to group all the possible molecular variants into two functional classes, such as wild-type (A) and detrimental (a). The detrimental allele may actually be constituted of many distinct DNA-level alleles, but to a first approximation, we treat them as equivalent. The basic model of 'mutation-selection balance' posits mutation recurrently introduces the detrimental allele into the population. It reduces fitness of heterozygotes by hs and of homozygotes by s, where h gives the degree of dominance and s the selection coefficient. If a is wholly recessive ($h=0$), the equilibrium frequency of the deleterious allele q is:

$$\hat{q} \approx \sqrt{\frac{u}{s}} \qquad [3]$$

while if a is not completely recessive:

$$\hat{q} \approx \frac{u}{hs} \qquad [4]$$

The mutation rate for a to A (v) does not contribute to eqns [3]–[4], because the a allele remains rare (unless mutation rates are unrealistically large or selection is very weak). Equations [3]–[4] illustrate the important effect of recessivity in allowing deleterious alleles to persist. An important practical implication of eqn [3]: For rare, recessive genetic diseases, for every affected individual, there are likely to be thousands of unaffected 'carriers' of the deleterious allele.

Wright (1931) extended mutation-selection balance to consider the effect of genetic drift. Now, a single value for $\hat{q}$ is replaced by a distribution:

$$f[q] = Ce^{-4Ns}\, q^{4Nu-1}(1-q)^{4Nv-1} \qquad [5]$$

where C is a constant insuring that the integral $f[q]$ from 0 to 1 equals 1. Here, $f[q]$ is the density function for q at the statistical equilibrium between mutation, selection and drift. Equation [5] assumes additive gene action ($h=½$) and that the adult population consists of N diploid adults in each generation. Equation [5] yields a number of important insights (Crow and Kimura, 1970, Chapter 9) about the likely frequency of an allele depending on how strong selection is relative to drift and mutation. Most basically, it illustrates that the relative values of different evolutionary parameters, and not their absolute values, often determine predictions. Both the selection coefficient (s) and the mutation rates (u and v) only appear as a product with the population size (N). If $N=1000$ and $s=u=v=0.01$, we get the same distribution for q when $N=100\,000$ and $s=u=v=0.000\,1$. This feature is not specific to eqn [5]. In eqns [3]–[4], u always appears in a ratio of u/s.

The fact that parameters only occur in specific combinations (in compound parameters) has both positive and negative implications. On the positive side, it simplifies exploration of the parameters space of a model. The parameter space of eqn [5] is basically three dimensional despite that four constants appear in the formula, N, u, v, and s. The fact that the population size N invariably occurs in a product with mutation rates and selection coefficients is also practically important. It suggest that one might be able simulate evolution using a relatively small N, say 1000, and obtain results valid for much larger population sizes if one adjusts the other parameters so that Ns, $4Nu$, and $4Nv$ remain unchanged. This will not always work, but it has proven an effective approximations in many population genetic studies. A negative feature of having parameters occur only in combinations is statistical identifiability. If one measures a predicted statistic, say the number of polymorphic sites in a sample of gene sequences (see below), one cannot estimate a particular value for u. Many different values of u will fit the data equally well because many different values for u produce the same product for $4Nu$.

Despite the utility of the two allele model for studying evolutionary process, many situations require an explicit consideration that a genetic locus can have more than two

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Figure 1 Motoo Kimura (1924–94) was a Japanese population geneticist who made many contributions to the theory of mutation.

alleles. From a modeling perspective, this is a straightforward generalization. We posit that there are K possible alleles at a locus and then specify the rate that each allele can mutate to every other allele. While simple in principle, this model can be cumbersome in that it is difficult to choose a reasonable value for K, and unless K is small, the number of mutation rates to be specified, $K(K-1)/2$, can be very large.

There are a large number of 'simplified' multi-allelic models of mutation in which the process can be specified with a tractable number of parameters. Perhaps the most elegant is the 'Infinite Alleles Model' of Kimura and Crow (1964). Motoo Kimura, pictured in **Figure 1**, made fundamental contributions to the theory of mutation throughout his career. The idea underpinning the Infinite Alleles Model is that mutation occurs at a constant rate u, but when it occurs, it invariably produces a novel allele. This is a sensible approach to many situations where the number of possible alleles is enormous (consider the number of unique permutations of A/G/C/T that are possible in a sequence of 100 nucleotides). The premise that all new alleles are different also makes some potentially difficult calculations quite simple. Consider heterozygosity in a population. For a selectively neutral locus in a closed population (no migration), heterozygosity is determined by a balance between mutation and genetic drift. Let F denote the probability that two randomly sampled alleles are the same in the current generation. In the next generation,

$$F' = (1-u)^2\left[\left(1-\frac{1}{2N}\right)F+\frac{1}{2N}\right] \qquad [6]$$

First term in brackets on the right hand side corresponds to event that distinct alleles are sampled from current generation (which were the same with probability F) while $1/2N$ is probability that same allele is sampled twice. Finally $(1-u)^2$ is the probability that neither allele mutates. After simplifying by assuming u and $1/2N$ are much less than 1, and noting that heterozygosity (H) is equal to $1-F$, we find the equilibrium heterozygosity:

$$\hat{H} = \frac{4Nu}{1+4Nu} \qquad [7]$$

The neutral theory of molecular evolution has been greatly elaborated over the last 45 years, but the Infinite Alleles Model provides a succinct route to one of its fundamental results. This is, the amount of genetic diversity within a population is determined by $4Nu$, a quantity often called θ in population genetics. This same product is also a primary ingredient in Wright's formula for the allele frequency distribution (eqn [5]), although the interpretation of u is a bit different between the two models.

Two basic difficulties emerge with the Infinite Alleles Model, at least when we are looking at DNA sequence data sampled from a population. The first is that we lose information by simply treating each sequence as a distinct allele. Some sequences are much more similar than others. In other words, we lose information about the haplotype or linkage disequilibrium structure among point mutations within a sequence. Also, if we treat the entire DNA sequence as the locus and each unique sequence as an allele, the process of recombination can generate new alleles. For a variety of reasons, we cannot easily absorb the recombination process into u.

The 'Infinite Sites Model' addresses these issues, while retaining simplicity. There is still a single mutation rate, but we are now thinking of this in terms of point mutations at a particular sequence location. The assumption (captured in the name) is that all new mutations occur at previously monomorphic sites. This model produces predictions for statistics more usually calculated for nucleotide sequence data, such as S (the number of polymorphic sites in a sample) and π (the average number of sites that differ between two sequences in the sample). At equilibrium of mutation/drift balance,

$$E[\pi] = 4Nu \qquad [8]$$

and

$$E[S] = 4Nu\sum_{i=1}^{n-1}\frac{1}{i} \qquad [9]$$

where n is the sample size (number of sequences), u is the overall mutation rate (product of per site mutation rate and the number of sites in the sequence), and E[*] is the expectation operator. Hudson (1993) reviews results employing the infinite sites model.

Equations [8]–[9] imply that different aspects of the data depend on the same quantity. This can be put to use. For example, if we take the observed average difference among sequence to estimate $4Nu$ from eqn [8], we can then predict how many sites should be polymorphic in the sample using eqn [9]. This prediction can be compared to the observed number of polymorphic sites as a way to evaluate the model, specifically the assumption that variation reflects mutation-drift balance. Of course, sampling variation must be properly accounted for (both evolutionary and experimental), but this is the basic idea behind the widely used neutrality test of Tajima (1989). Other neutrality tests share the same basic property: The mutation rate implies a relationship between different aspects of a dataset and the test asks whether the observed deviations from the predicted relationship are large enough to reject the model.

A number of other multi-allelic mutational models have been derived suitable to different data types. In some cases,

alternative alleles naturally form an ordered series. For example, microsatellite loci can vary in the number of times that a sequence motif (e.g., AAT) is repeated. Allozyme polymorphisms can be scored in sequential fashion based on how rapidly alleles migrate on a gel. The 'Stepwise Mutational Model' of Ohta and Kimura (1973) posits that mutations occur at a constant rate and involve the gain or loss of a single unit. This model has been extended in several ways, allowing gain and/or loss of multiple units in different ways. Regardless, analysis of the model reveals similarities with other mutational models, at least when variation reflects mutation/drift balance. In the Stepwise Mutation Model, the variance in allele length (number of repeats for a microsatellite locus) is proportional to the product of mutation rate and population size.

Multi-allelic mutational models are also used to study phenotypic evolution. The 'Continuum of Alleles Model' of Kimura (1965) is a good example. It shares features with the Infinite Alleles Model in that each new mutation is unique. However, the mutations are characterized by their effect on a quantitative trait. Quantitative traits are those where variation is due to both genetic and environmental effects, and the genetic component usually involves contributions from many loci. The Continuum of Alleles Model posits the effect of a new allele on the quantitative character is only slightly different from the parent allele from which it was derived. In this way, it is similar to the Stepwise Mutation Model. However, unlike the Stepwise Model, the amount by which new mutations differ from their progenitor is a continuous variable drawn from a probability distribution (or, more precisely, a density function). This revision owes to the fact that most quantitative traits exhibit a continuous distribution of values in the population.

The Continuum of Alleles Model has most usually been applied to a quantitative trait with a subsequent consideration of how that trait affects fitness. A final category of multi-allelic models makes this mapping more direct, attributing fitness effects directly to alleles. The Kondrashov (1985) 'mutational model' is a generalization of eqns [3]–[4], considering the mutation-selection dynamic distributed across the genome. At these many unlinked loci, deleterious mutations occur that reduce fitness by s in homozygotes and by hs in heterozygotes. The simplest version of the theory assumes that the population mates randomly and that the effects of different mutations combine multiplicatively to determine the fitness of an individual. However, the model has been extended to identify the important effects of non-multiplicative interactions between mutations ('epistasis') and of nonrandom mating ('inbreeding') on mutation-selection balance.

See also: Mutation and Genome Evolution. Neutral Models of Genetic Drift and Mutation

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Mutualism, the Evolutionary Ecology of

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Glossary

By-product mutualism An association among species in which the resources exchanged are cost free to at least one partner and are produced as by-products of other organismal functions such as metabolism.
Cheater An exploiter species that evolved from a mutualistic one and no longer reciprocates with its partner.
Coevolution Reciprocal evolutionary change caused by an interaction among species.
Coevolutionary hotspot Populations where all interacting species are evolving in response to one another.
Connectedness In a community of interacting mutualists, connectedness is the proportion of the total possible interactions among species that are observed within an interaction network.
Cooperation The act of individuals working together for a common benefit. Mainly applied to interactions among individuals within the same species.
Defensive mutualism An interaction among partner species in which one partner defends the other in exchange for a place to live or for nutrients needed for growth and reproduction.
Dispersive mutualism An interaction among species in which one partner moves the gametes, offspring, or individuals of the other species and in return receives nutrients or resources needed for growth and reproduction.
Exploiter A species from outside a mutualistic interaction that takes the resources or services provided by mutualistic species without providing anything in return.
Geographic mosaic of coevolution The concept that most of the coevolution among species occurs at different rates in different populations with some populations being hotspots of coevolutionary change.
Host sanctions A mechanism by which a mutualist can reduce the trade of resources or services in response to a partner that is of inferior quality.
Nutritional mutualism An interaction among species in which partners exchange nutrients needed for growth and reproduction.
Partner choice A mechanism by which mutualistic partners preferentially interact with high-quality partners.
Partner fidelity feedback Mutualisms where the increase in fitness of one mutualistic partner causes an automatic increase in the other resulting in a positive feedback loop. This is usually only possible between individuals of different mutualist species that associate long enough to experience changes in fitness.

Introduction

Species interact with one another in myriad ways with the ultimate goal of obtaining resources or services needed for survival and reproduction. Many of these interactions are antagonistic in which one species gains a benefit but the other suffers a cost. Predators, herbivores, and parasites either kill their hosts for energy and nutrients or take some resources directly from their hosts. In contrast, there are interactions in which the species involved all gain reciprocal benefits. Van Beneden (1873) was the first to call these interactions mutualisms. For example, bees visiting a flower gain energy from nectar rewards while providing pollen exchange for the plant. Since the term was coined, we have come to realize that mutualisms are the foundation of most ecosystems (**Figure 1**). For instance, in terrestrial systems, plants interact with a variety of species that help in the procurement of nutrients, the dispersal of pollen and seeds, and defense against herbivores. In aquatic systems, coral and their symbiotic algae build the extensive reef ecosystems that harbor much of the ocean's biodiversity. Furthermore, mutualisms involve a taxonomically diverse set of species, occur in every environment, and occur among highly mobile free-living species as well as those that live on or within other species. For this reason, many additional terms have been synonymized with mutualism such as cooperation, facilitation, and symbiosis. Each of these terms can apply to mutualistic interactions, but not all mutualisms include cooperation or facilitation, and not all symbioses are mutualisms.

Mutualistic interactions are traditionally divided into three different types based on the resources or services that are traded between species. Nutritional mutualism involves interactions among species that are trading resources that are needed for growth and reproduction. A classic example is the trade of carbon and minerals between plants and mycorrhizal fungi. Plant roots form intimate associations with the hyphae of fungi that are very fine and can forage efficiently for phosphorus, nitrogen, and micronutrients in the soil (Courty *et al.*, 2010). The roots exude rich carbon compounds that are used as food by the fungi which in return provide the plant with scavenged water and minerals. Dispersive mutualism involves one partner species distributing gametes, offspring, or individuals of another species in return for a resource. The diversification of angiosperm plants is partly attributed to their mutualism with pollinating insects that act as directed pollen dispersal agents (Grimaldi, 1999). Likewise, plants also often trade food rewards with a diversity of mammals, birds, and insects that inadvertently disperse plant seeds. Lastly, defensive mutualism involves one species defending another in exchange for resources and/or a place to live. For example, ants protect acacia plants from herbivores such as elephants in exchange for nesting locations within the tree and packets of

Figure 1 Examples of mutualisms. (a) Eastern deciduous forest in central New York, USA highlighting young maple seedlings that will need to establish mutualistic interactions with ectomycorrhizal fungi. (b) Coral reef in the British Virgin Islands. (c) Fruiting body of ectomycorrhizal fungus species at the Archbold Biological Station in Florida, USA. (d) A species of bombyliid fly nectaring on a flower in central Florida, USA.

proteins produced by specialized leaves (Goheen and Palmer, 2010).

Regardless of the type of mutualism, there are a variety of ecological and evolutionary factors that influence the formation and continued functioning of the interaction. As a consequence, research in mutualism is focused on answering at least four major questions (Bronstein *et al.*, 2006). The first is to understand how mutualism originates. Why do species in essence hitch their futures together by relying on one another for goods or services? The second major question is: How is mutualism maintained among species that are under constant selection to obtain resources from their partners while reducing the cost of reciprocating? Why don't the partners cheat and reduce their costs? The third major question is: Why are some mutualistic interactions very specialized and involve, in some cases, just a pair of species and in other cases involve several or even dozens of other species? Finally, given that mutualism is the foundation of many communities, how does the addition of other community members that interact with mutualist species influence the dynamics of mutualism?

The Origin of Mutualism

Many of the mutualisms we observe today are likely the product of a long-term process of adaptation among one or both mutualists (Frederickson, 2013). In considering the origin of mutualism, a poignant question to address is whether the interactions were mutualistic from the beginning or did they gradually transition into mutualism over time. If it is the former, what conditions favor this scenario? If it is the latter, how do some antagonistic interactions eventually change into mutualistic ones? Additionally, are traits important to mutualistic interactions co-opted from existing traits or must novel traits evolve *de novo*?

Determining the origin of mutualism is a difficult process, especially given that interaction outcomes can change depending on the environmental context. Addressing this question also requires combining information on the phylogenetic history of the species involved and the dynamics of the interaction and the traits important for the mutualism (Sachs *et al.*, 2014). Some of the best evidence for how mutualism originates comes from studies of yuccas (Asparagaceae: Agavoideae) and their pollinating moths. Moths in the family Prodoxidae are plant feeders in which the larvae feed internally on plant tissues, either in the leaves, flowering stalks, or within developing fruits (Davis, 1967). Phylogenetic analysis of the three closely related prodoxid genera that feed on yuccas, *Prodoxus*, *Parategeticula*, and *Tegeticula*, reveals an interesting progression of the overall interaction from antagonism to mutualism (**Figure 2**).

Prodoxus moths colonized yuccas as herbivores and diversified to feed within galls on the leaves, within the

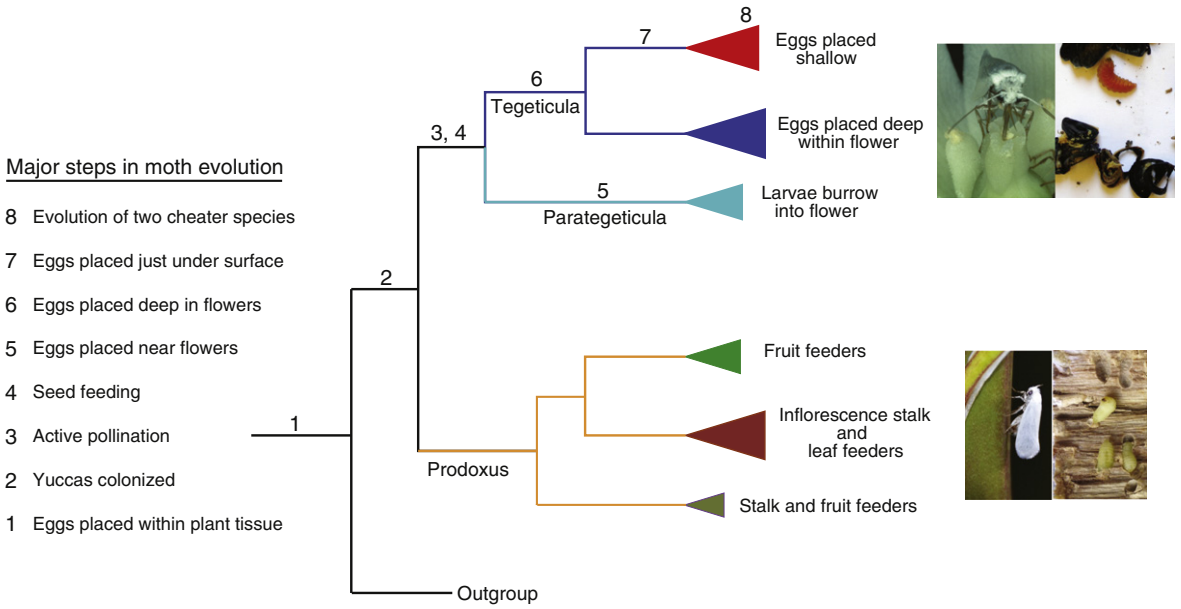

Figure 2 The evolutionary transitions between antagonism and mutualism in the yucca moth genera, *Prodoxus*, *Parategeticula*, and *Tegeticula*. Numbers along the branches correspond to major evolutionary changes in moth biology. Top right picture shows a female *Tegeticula* using the special 'tentacles' to actively deposit pollen into a yucca flower. Adjacent picture shows a larva and its feeding damage to the developing seeds. Bottom right picture shows a *Prodoxus* female laying eggs into a yucca flowering stalk. The larvae feed with the stalk and tunnel through the plant tissue.

flowering stalk, or within galls on developing fruit of yuccas (Pellmyr *et al.*, 2006). A female *Prodoxus* deposits her eggs into the plant tissue during the flowering season of her host plant species and the larvae develop internally, feeding on host plant tissue, but never on seeds. The interaction between *Prodoxus* and yuccas likely ranges from neutral to slightly antagonistic as larval feeding does not seem to impact plant reproductive success much (Althoff *et al.*, 2004). Like some *Prodoxus* species, *Parategeticula* moths also feed within galls on developing fruit, but unlike *Prodoxus*, the galls encompass a small portion of the developing yucca seeds that the larvae also consume (Powell, 1992). A *Parategeticula* female scrapes shallow pits into which she deposits her eggs in the flower pedicel or the small side branches holding the flowers. The larva then crawls to a flower and burrows into the base of the pistil to initiate a gall that replaces a few yucca seeds. Up to this point, the interaction would be considered antagonistic as moth larvae are killing potential plant offspring. However, the female *Parategeticula* also pollinates the flowers near where she deposits her eggs. This action increases the probability that the flowers will develop into fruit in which the larvae can feed and develop. To accomplish pollination, a female uses a completely novel trait – specialized mouthparts called tentacles – to collect yucca pollen into a ball that she carries beneath her head and to actively deposit some of the pollen into the stigmatic cavity of the yucca flower (Davis, 1967). Because a female moth transfers enough pollen to fertilize many more seeds than her offspring will eat, the net outcome for the plant is positive. The plant loses a small subset of its seeds to the moth larvae, but gains many more through active pollination by a highly specialized and efficient pollinator.

In *Parategeticula*, there has been a switch in the outcome of the interaction from antagonism, as in the case of *Prodoxus*, to mutualism in the case of *Parategeticula*. The only reason that the outcome of the interaction between yuccas and *Parategeticula* is considered a mutualism, however, is because the larvae only consume a small subset of the seeds in the yucca flower that their mother pollinated. The plant loses some seeds to the larvae, but experiences an overall positive fitness increase due to the pollination by the female moths. This overall interaction highlights a very important dynamic of mutualism – there is always some level of antagonism involved and it is the relative increases and decreases in fitness that determine whether we call an interaction an antagonism or a mutualism (Bronstein, 1994). If we just considered larval feeding, *Parategeticula* reduces yucca fitness much more than *Prodoxus* that do not feed on seeds. The evolution of active pollination, likely as a result of selection on female moths to increase their offsprings' survival, was the key event that shifted the outcome of the overall interaction. The effectiveness of active pollination by *Parategeticula* also likely selected for the loss of other pollinators of yuccas. Yucca flowers produce no nectar and there are no other known pollinators except for a single case of an introduced yucca in the United States, *Yucca aloifolia*, that is inadvertently pollinated by honeybees (Rentsch and Leebens-Mack, 2014). Thus, the interaction between moths and plants has become highly specialized and absolutely critical to the evolutionary success of both lineages.

Evolution within the moth genus *Tegeticula* further highlights the ongoing dynamic of antagonism within mutualism. This genus contains over 17 species of pollinator moths, but there has also been the evolution of at least two cheater species (Pellmyr, 1999). These cheater species no longer pollinate and accordingly have even lost the tentacles, yet the larvae still feed on yucca seeds. Like *Parategeticula* females, a female *Tegeticula* pollinates flowers but pierces through the locule wall and

deposits her eggs next to the developing plant ovules rather than in shallow pits outside of the flower. Egg and larval survivorship is likely increased because the eggs are protected inside of the flower. The tradeoff, however, is that the female's ovipositor damages some of the developing ovules. If there are many oviposition attempts on a single flower, the ovule damage triggers floral abscission and kills the eggs/larvae. Within *Tegeticula*, another lineage of moth species evolved probably in response to this floral abscission mechanism. In this second lineage, females lay their eggs superficially just under the style or pistil surface. This placement prevents ovule damage and potentially allows females to lay many eggs (Segraves, 2008). From within this superficially egg laying lineage arose the antagonistic cheater moths. There are two cheater species that lay eggs into early fruit or late fruit and do not pollinate. These species are capitalizing on the remaining seed resource that would not be eaten by the pollinator larvae. Thus, within the yucca moths, the interaction with yuccas has shifted from antagonism to mutualism and back to antagonism, even though all moths feed on yuccas.

The Maintenance of Mutualism

As evidenced by the evolution of cheater moth species in the yucca–yucca moth system, mutualism can be the target of exploitation, both from within mutualistic lineages and from without (Yu, 2001). Indeed, the prevention and mitigation of exploitation has played a significant role in directing empirical studies and in the development of mutualism theory. Two types of exploitation have been distinguished. The general term 'exploiter' refers to organisms that take the benefits of a mutualism without offering anything in return; thus, exploiters can be opportunists from the community or they can originate from the mutualism itself (Sachs and Simms, 2006). The term 'cheater' specifically refers to an exploiter that is evolutionarily derived from a mutualist lineage. Cheaters could be mutant mutualists that no longer provide a reward or, as in the case of yucca moths, they could be entirely different species that evolved from a mutualistic ancestor. The presence of exploitation in mutualism presents an evolutionary paradox. The reasoning is simple: because mutualisms generate resources that are often costly to make, individuals that can obtain these benefits without reciprocating will be favored by natural selection. Thus, exploiters of mutualism avoid the costs of participating in the interaction yet are able to obtain the benefits that enhance fitness. Natural selection, then, should favor exploitative individuals that maximize the benefits while reducing the costs, potentially leading to mutualism breakdown.

A long-standing question in the study of mutualism is to understand how mutualists prevent their partners or other organisms from overexploiting the benefits of the mutualism. Theoretical work has identified four general ways that mutualisms remain stable: by-product mutualisms, partner fidelity feedbacks, host sanctions, and partner choice. First, by-product mutualisms remain stable because one or both partners produce a cost-free by-product. The release of the by-product directly benefits the individual producing it, and as a consequence, there is no incentive for exploitation. For example, a bacterium might produce a waste product that is excreted into the environment. Once excreted, the by-product can be consumed by other bacterial species that require it. There are many examples of cross-feeding in microbes where partner species trade excreted products (Bull and Harcombe, 2009; Mee *et al.*, 2014).

Second, a similar mechanism that can stabilize mutualistic interactions is partner fidelity feedbacks. In this model, the resources being traded are costly; however, there is a direct link between the amount invested in the mutualism and the benefits received (Weyl *et al.*, 2010). That is, individuals that invest more in providing benefits to their partner will enhance the productivity of their partner and automatically receive more benefits in return. An example of a partner fidelity feedback is the interaction between plants and mycorrhizal fungi that live in plant roots. Plants that invest in carbon to roots infected with fungi increase growth of fungal hyphae that forage for nutrients in the soil. By doing so, the plants gain nutrients and can in turn grow and invest more carbon to fungi. The feedback continues with each partner receiving increased benefits as they trade resources.

A third regulatory mechanism that can prevent overexploitation by mutualist partners are host sanctions where one or both mutualist partners 'punish' partners that defect from the mutualism. A clear example of host sanctions is the mechanism by which yuccas use to regulate the number of pollinator moth eggs placed within flowers (Pellmyr and Huth, 1994). Some female yucca moths use their ovipositors to deposit eggs next to the developing seeds, and as they do so, they often damage the ovules and surrounding tissue. Yuccas selectively abscise flowers with large numbers of wounded ovules, killing all eggs/larvae within them (Marr and Pellmyr, 2003). As a result, yuccas can sanction female yucca moths that lay so many eggs that no seeds would survive.

Finally, partner choice mechanisms regulate mutualisms by allowing mutualists to choose among partners that vary in quality (Bull and Rice, 1991). Mutualists should associate more often with partners that provide the most benefits, thus reducing the costs of the interaction. The mutualism between cleaner wrasse and their ectoparasite-riddled fish hosts is an excellent example of partner choice. Host fish visit cleaning stations where cleaner wrasse feed on the ectoparasites; however, sometimes the cleaners also bite healthy host tissue. Repeated interactions between hosts and cleaners allow the hosts to selectively visit cleaners that remove ectoparasites without biting healthy tissue (Bshary and Schaffer, 2002).

Even with these mechanisms in place, exploitation is a common facet of mutualism. For example, cheater yucca moths do not pollinate, yet lay eggs into yucca fruit and their larvae feed on seeds. Many flowering plant species produce conspicuous flowers that contain a nectar reward that entices potential pollinators to visit the flower and inadvertently transfer and pick up pollen. Numerous pollinator species, however, will also sometimes cut through the base of the flower to rob the nectar (Irwin and Brody, 1998), or are not morphologically adapted to be good pollinators, so they feed on nectar without providing the pollen transfer service. Similarly, some frugivores (fruit feeders) are poor seed dispersers because the plant seeds are damaged or killed during passage through the digestive tract. Further empirical and theoretical

studies are needed to clarify whether the costs of exploitation are normally high or whether exploitation is more likely to have a minimal effect on a mutualistic interaction. The widespread prevalence of exploiters suggests that it might be the latter (Bronstein, 2001).

Mutualism, Specialization, and Coevolution

Another important facet of mutualism is that natural selection can favor the continued interaction of mutualists throughout time. Because of this, mutualists can end up being specialized through local adaptation to their partner(s). Hand in hand with this specialization, is the process of coevolution in which there is reciprocal evolutionary change among species. For example, the long nectar spurs of Madagascan orchids result in the extremely long proboscis of its hawkmoth pollinators (Arditti *et al.*, 2012). A similar pattern is observed among populations of the flowering scroph *Zaluzianskya microsiphon* (Scrophulariaceae) and its long-tongued fly pollinator *Prosoeca ganglbaueri* (Diptera: Nemestrinidae) in South Africa (Anderson and Johnson, 2007). In this example, the length of the floral tube for the plant is highly correlated with the length of proboscis of the fly across many different populations. Thus, specialization and coevolution can occur both within and among populations or across species in interacting lineages (Thompson, 1994). In particular, the geographic mosaic of coevolution championed by Thompson (2005) suggests much of the dynamics of specialization and coevolution occurs among populations within a species. *Greya politella*, another prodoxid moth, is a pollinating seed parasite of prairie stars, *Lithophragma parviflorum*, in the Inland Northwest of the western United States. Depending on the population, the interaction can be antagonistic or mutualistic, yet be a stable coevolutionary hotspot (Thompson and Cunningham, 2002).

Although specialization and coevolution are integral parts of mutualism, there are also many mutualisms in which mutualists are generalists and coevolutionary selection is likely weak (Waser *et al.*, 1996). Many pollination and fruit dispersal mutualisms involve plants that attract a wide variety of pollinators or dispersers. For example, saw palmetto populations that occur along the Lake Wales ridge in Florida attract hundreds of different species of floral visitors, many of which also carry pollen (Deyrup and Deyrup, 2012). A similar pattern has also been found in the mutualism between ectomycorrhizal fungi and trees in which fungal hyphal networks can involve many fungal species that connect to many different tree species (Courty *et al.*, 2010). A major approach to studying specialization and generalism in mutualism has been through examination of mutualistic species interaction networks. These studies describe the patterns of connectedness among mutualists within a community (Bascompte and Jordano, 2013). Interestingly, a repeated pattern of connectedness is found in many mutualistic communities – networks involving interactions of mutualists are nested and asymmetric in the sense that there are generalist mutualist species that interact with a subset of specialist mutualist species (Bascompte and Jordano, 2006). What remains to be tested is why such patterns of nestedness are prevalent, and why is it that some mutualists are specialists whereas others are generalists?

The Community Context of Mutualism

Irrespective of whether a particular mutualism is specialized or generalized, the biological reality is that all mutualisms are embedded in communities of interacting species. These species interact directly or indirectly with mutualists, and so may have the potential to influence the evolutionary ecology of the mutualism (Stanton, 2003). The study of mutualism is expanding to explore how not only other mutualist species but also how herbivores, natural enemies, and competitors of mutualists may shape both the dynamics and the evolution of mutualistic traits (Afkhami *et al.*, 2014). As mentioned earlier, the mutualism between yucca moth pollinators and yuccas is highly specialized, yet both moth and plant species have to contend with other visitors that feed on the flowers of yuccas. The inflorescences of *Yucca filamentosa* populations in the southeastern United States attract the pollinator moth *Tegeticula cassandra* as well as large numbers of the pollen-feeding beetle *Hymenorus densus* and the plant fluid feeding leaf-footed bug *Leptoglossus phyllopus* (Althoff *et al.*, 2013). Feeding by these two species can cause severe damage to yucca flowers and cause them to abscise from the plant. Thus, pollinator moths have to contend with additional egg and larval mortality that may influence the pattern of egg deposition both within a single plant and across many different plants. Furthermore, the effect of the beetle *H. densus* is even more pronounced on the pollinator moths because adult beetles inadvertently feed on pollinator moth eggs that are laid just below the style surface (Segraves, 2008). The beetles could potentially act as indirect mutualists of the plants by limiting the number of moth eggs and reducing the number of seeds eaten.

Mutualisms can also attract a large number of other species that can exploit the resources produced. This is particularly prominent in the mutualism between figs and their pollinating wasps. The fig is in essence an inflorescence that has been turned inside out and is pollinated by specialist agaonid wasps that must crawl through a narrow channel to reach the minute flowers within the fig inflorescence (Weiblen, 2002). As with yucca moths, the female wasp lays eggs into the flowers and then active pollinates each flower. The developing fig contains both mature seeds and pollinator larvae that can be used as resources by other species (Herre *et al.*, 2008). Indeed, in some localities in Australia, there are at least 12 other closely and distantly related wasp species that come to figs to attack the seeds, attack the wasp larvae, or a combination of both (Segar *et al.*, 2014). The presence of these additional community members might be one reason that female pollinator wasps only lay eggs in certain flowers within the fig (Al-Beidh *et al.*, 2012).

Addressing the role of additional community members on mutualism is a daunting task given that there are many different species that can potentially influence a mutualism. This work becomes further complicated by the fact that the effects of different species can be nonadditive and may prevent us from making predictions. In many cases, a single species can have both direct effects and indirect effects, and in other instances, the effect of one species might only be strong when in the presence of additional species. For example, in the defensive mutualism between ants and plants, ants can be very

beneficial by protecting plants from herbivores. Unintentionally, however, ants also deter pollinators from visiting the plants. Thus, when herbivores are rare, ant protection may shift from being very beneficial to less beneficial or even harmful to plants (Ohm and Miller, 2014). Many more studies are needed to examine the role of local community members on the dynamics of mutualism. Only then can we begin to understand and predict how different types of community members may change the ecology and evolution of a focal mutualism.

Synthesis

Mutualism has always attracted considerable attention because of the idea that species may help one another acquire resources or services. This is in part due to the fact that humans are highly cooperative in our day-to-day activities. Research on mutualism, however, is demonstrating that natural selection will always operate to increase the fitness of an organism. In terms of thinking about mutualism, this means that species will continue to interact with their partner species as long as there is a net fitness benefit. To achieve this, there will be evolution to decrease the costs of reciprocating and to increase exploitation. Because of this underlying tension, mutualism is a highly dynamic interaction in which there is likely to be continual evolution and coevolution among partner species. Sometimes this may lead to extreme specialization, but other times it might involve a suite of partner species. Adding to this dynamism is the fact that other community members may directly exploit resources or services provided by mutualists, thus adding to the costs of mutualism. What is perhaps most astonishing is that mutualism has persisted since life began and continues to be an important interaction for generating and maintaining biodiversity.

See also: Coevolution, Introduction to. Commensalism, Amensalism, and Synnecrosis. Geographic Mosaic of Coevolution. Microbiome. Mycorrhizal Fungi, Evolution and Diversification of. Plant–Pollinator Interactions and Flower Diversification. Predation and Parasitism. Symbiosis, Introduction to

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Mycorrhizal Fungi, Evolution and Diversification of

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Glossary

Arbuscular mycorrhiza Mycorrhiza characterized by the formation of arbuscules, or tree-shaped structures, by the fungal hyphae after penetration of the cell wall of host plant root cells.

Arbutoid mycorrhiza Formed by members of the Arbutoideae, a subfamily within the Ericaceae, and representing one type of ectendomycorrhiza, as they exhibit morphological characteristics of both ecto- and endomycorrhiza.

Ascomycota Phylum of fungi characterized by the formation of the ascus, a reproductive structure in which haploid, nonmotile unicellular spores called ascospores are formed, and commonly called the sac fungi.

Basidiomycota Phylum of filamentous fungi that reproduce by the formation of basidia, reproductive structures that typically bear on their tips haploid, nonmotile unicellular spores called basidiospores.

Cavendishioid mycorrhiza Ectendomycorrhizal associations that include a mantle, Hartig net and intracellular growth of hyphae, originally described from *Cavendishia nobilis*, a species in the Ericaceae, and subsequently observed in other members of the Ericaceae.

Ectendomycorrhiza Mycorrhizal associations that exhibit morphological characteristics of both ecto- and endomycorrhiza, in that hyphae form a mantle around the root and may form a distinct Hartig net, while also growing intracellularly.

Ectomycorrhiza Mycorrhizal associations characterized by formation of a sheath of hyphae, called the mantle, on the exterior of the root, and formation of the Hartig net.

Endomycorrhiza Mycorrhizal associations characterized by hyphal penetration of the cell wall of root cells. Typically, the cytoplasms of the fungus and host are kept separate by the plasma membranes of each cell and the cell wall of the fungus.

Endophytic fungi Fungi that live symbiotically within plant cells and generally do not negatively affect plant growth, reproduction or fitness; most plants host endophytic fungi, and many endophytic fungi are beneficial to plants.

Ericaceae Plants in the heath family.

Ericoid mycorrhiza Formed by members of the Ericaceae and characterized by the formation of extensive hyphal coils in root cells.

Glomeromycota A phylum of fungi, the members of which typically form arbuscular mycorrhiza.

Hartig net A structure associated with ectomycorrhizal fungi, formed by a network of hyphae that grow around root cells but do not penetrate the cell wall of the host cells.

Mantle A sheath of hyphae formed by ectomycorrhizal fungi that may partially or completely cover the root surface.

Monotropoid mycorrhiza Mycorrhizal associations formed by members of the Monotropoideae, a subfamily within the Ericaceae. Morphologically similar to the arbutoid mycorrhiza in that they form mantles and Hartig net; however, rather than forming hyphal coils, they form fungal pegs, which cause indentations in the cell wall of host cells but do not penetrate the cell wall.

Mycoheterotrophic plants Plants that receive part or all of their carbohydrates from symbiotic fungi.

Orchid mycorrhiza Formed by members of the Orchidaceae, and characterized by the formation of pelotons.

Pelotons Distinct fungal coils formed by orchid mycorrhizal fungi in the space between root cell walls and plasma membranes of host orchids.

Pyroloid mycorrhiza Mycorrhiza formed by members of the Pyroloideae, a subfamily within the Ericaceae, and morphologically similar to, but distinct from, arbutoid mycorrhiza in the formation of a mantle, hyphal sheath, and intracellular hyphal growth.

Rhyniophytes Members of the class Rhyniopsida, a now-extinct class of plants that arose early in the evolution of plants, and have simple vascular tissue but lack leaves and true roots.

Overview

Mycorrhizal associations, symbiotic associations between fungi and plant roots, likely arose during the initial colonization of land by plants and are nearly ubiquitous in terrestrial habits, occurring in over 90% of plant families (Hibbett *et al.*, 2000; Malloch *et al.*, 1980). Mycorrhizal fungi are highly diverse and are a major contributor to fungal diversity (van der Heijden *et al.*, 2015). Although the mycorrhizal habit likely arose originally in the Glomeromycota, mycorrhizal symbioses arose independently many times within the Ascomycota and Basidiomycota (Hibbett *et al.*, 2014; James *et al.*, 2006; Kohler *et al.*, 2015; Schüßler *et al.*, 2001). Due to convergent evolution of symbioses that arose independently, most mycorrhiza are grouped into two broad types, endomycorrhizas and ectomycorrhizas (**Figure 1**), which reflect whether fungal hyphae penetrate the cell walls of host plant roots during formation of the association. Endomycorrhiza are further classified as arbuscular, ericoid, or orchid, based on the host plants and structural nature of the symbiosis found in each group. Additional types also have been described, including the arbutoid and monotropoid mycorrhiza (**Figure 1**), which form with plants of the Arbutoideae and Monotropoideae subfamilies, respectively, within the Ericaceae (heath family).

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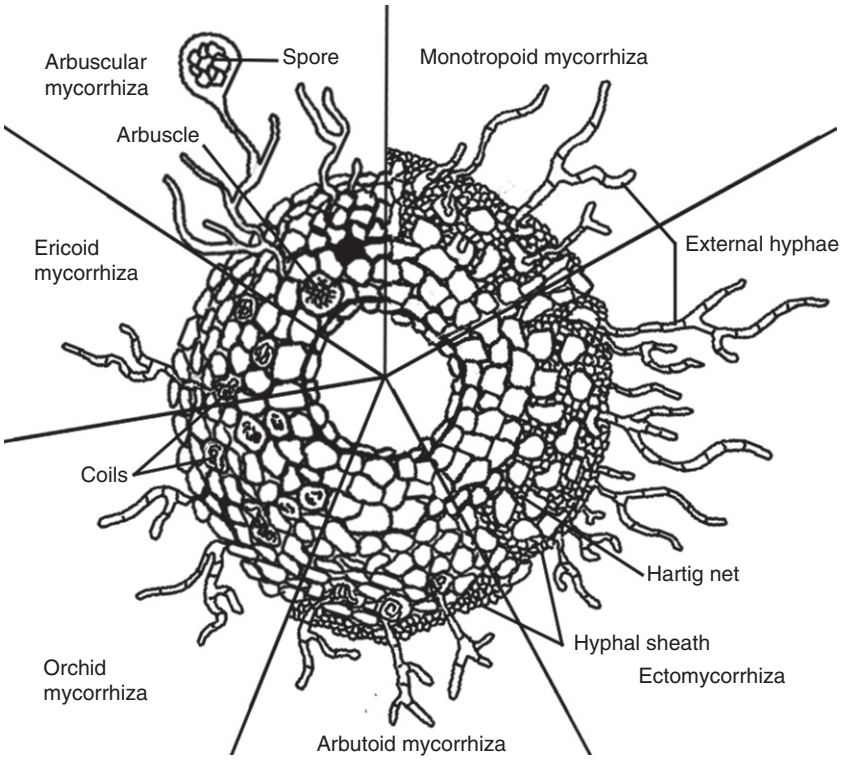

Figure 1 The primary structures of the arbuscular, arbutoid, ecto-, ericoid, monotropoid, and orchid mycorrhiza. Endomycorrhiza share similar structures and include arbuscular, ericoid, and orchid mycorrhiza. Ectendomycorrhiza share structural characteristics with both endo- and ectomycorrhiza, and include arbutoid and monotropoid mycorrhiza. Figure by Aidan Lewis.

As these categories suggest, host specialization is common in mycorrhizal fungi; yet, mycorrhizal fungal diversification patterns generally do not neatly follow plant diversification patterns (Allen *et al.*, 1995; Arnold *et al.*, 2010; Hibbett *et al.*, 2000). For example, although arbuscular mycorrhiza likely evolved first and are found on about 75% of plant species (Brundrett, 2009), only about 300 species of arbuscular mycorrhizal fungi are known (van der Heijden *et al.*, 2015), while there are an estimated 20 000 species of ectomycorrhizal fungal species, despite a limited range of plant host species (Rinaldi *et al.*, 2008; Tedersoo *et al.*, 2010; Allen *et al.*, 1995; van der Heijden *et al.*, 2015). Further, individual plants may simultaneously host many species of mycorrhizal fungi (Avolio *et al.*, 2009; Hibbett *et al.*, 2000; Lewis *et al.*, 2008; Wagg *et al.*, 2015), while individual fungi may form simultaneous associations with plants of different species (Hibbett *et al.*, 2000; Wagg *et al.*, 2015). And, even within a single fungal order, different fungal species may form ecto-, ericoid, and orchid mycorrhizal associations (Oberwinkler *et al.*, 2013). As a result, diversification of mycorrhizal fungi is thought to reflect evolution of the mycorrhizal form multiple times, generally from saprotrophic fungi, followed by convergent evolution both morphologically and physiologically (James *et al.*, 2006; Kohler *et al.*, 2015). Following is a more detailed discussion of the common patterns and key drivers of diversification across mycorrhizal fungi. Specific patterns within each of the major groups of mycorrhizal fungi are then described.

Common Patterns and Key Drivers of Diversification

Mycorrhizal associations generally provide the fungal symbionts with carbohydrates and certain biochemicals, while the plant host generally benefits from increased uptake of certain nutrients, and may receive other benefits, including increased water uptake and protection from pathogens. Fossil and molecular evidence suggest that mycorrhizal symbioses first arose about 460 million years ago between early plants and Glomeromycota, and played a critical role in the colonization of land by plants (Redecker *et al.*, 2013; Schüßler *et al.*, 2001; Selosse and Le Tacon, 1998; see also Desiro *et al.*, 2013). However, there is evidence the Glomeromycota arose earlier than this, and may have formed symbiotic associations with cyanobacteria (James *et al.*, 2006; Schüßler, 2002; Schüßler *et al.*, 2001). If so, the first mycorrhizal fungi may have arisen from fungi that formed mutualistic associations with cyanobacteria or algae, then subsequently formed associations with the earliest land plants (Schüßler *et al.*, 2001).

Although most extant land plants retain the ability to form mycorrhizal associations with Glomeromycota, many species have evolved the ability to form associations with Ascomycota or Basidiomycota. These associations, which most commonly form ectomycorrhiza, ericoid mycorrhiza, or orchid mycorrhiza, arose multiple times during the last 100 million years. Because mycorrhizal associations often fall on a mutualism–parasitism continuum, depending on the relative availabilities of soil nutrients and carbohydrate production, there has been

debate about whether mycorrhizal fungi arose from parasitic or saprotrophic fungi. Molecular evidence suggests ectomycorrhizal, ericoid mycorrhizal, and orchid mycorrhizal fungi typically evolved from saprotrophs (Hibbett *et al.*, 2000; James *et al.*, 2006; Kohler *et al.*, 2015), though there is some evidence that at least some of these fungi evolved from endophytic fungi (van der Heijden *et al.*, 2015).

A common pattern across these independent origins of the mycorrhizal habit is a suite of regulatory changes in the fungi in the form of reductions in decay mechanisms and induction of lineage-specific suites of genes associated with the formation of symbioses (Garcia *et al.*, 2015; Kohler *et al.*, 2015), coupled with the involvement of a set of genes in host plants that are associated with formation of mycorrhizal symbioses and that have been conserved since the early evolution of land plants (Wang *et al.*, 2010). Convergent evolution has also occurred morphologically, in that ectomycorrhizal, ericoid mycorrhizal, and orchid mycorrhizal fungi have distinguishing morphological characteristics that are shared across fungal species within each form, despite the multiple independent origins of fungal species that can form a given type of association.

Following initial evolution of the mycorrhizal habit within a particular lineage, coevolution with host plants is commonly seen. Coevolution between plants and mycorrhizal fungi is perhaps most obvious in mycoheterotrophic plants – plants that receive part or all of their carbohydrates from symbiotic fungi (Hynson *et al.*, 2015). While the general pattern is that mycorrhizal fungi receive carbohydrates from the host plant, most mycoheterotrophic plants have either partially or fully lost the ability to photosynthesize, and are either partially or fully reliant on fungal symbionts for carbohydrates, which, in turn, are obtained by the fungal symbionts from other plants or saprotrophically. Plants that are fully mycoheterotrophic typically are obligate hosts of mycorrhizal fungi, and many form species- or genotype-specific associations with mycorrhizal fungi.

The unique nature of this association and the close coevolutionary relationship between mycoheterotrophic plants and their mycorrhizal fungi has been long-recognized, and has been a model for examining plant–fungal coevolution in mycorrhizal associations. A key finding of this and related work is that although plants and fungal symbionts may coevolve closely, this pattern does not inherently lead to parallel cladograms (Arnold *et al.*, 2010). In fact, as with other mycorrhizal associations, mycoheterotrophic plants may simultaneously form associations with a suite of unrelated fungal species, and these fungi may simultaneously form associations with a suite of host plant species, including both mycoheterotrophic and autotrophic plants. More generally, the common pattern of individual plants hosting multiple fungi and individual fungi colonizing multiple plants often leads to a network of plants interconnected by mycorrhizal fungi, where carbohydrates from one plant may be indirectly transferred to other plants, including autotrophic plants, through the mycorrhizal network. This network may include different mycorrhizal forms, and may include plants that are normally autotrophic but that obtain carbohydrates from other plants via the mycorrhizal network during periods when the plants are heavily shaded or otherwise have carbohydrate demand exceeding supply.

In addition to the role of coevolution with plant hosts, diversification of mycorrhizal fungi also reflects the role of soil characteristics, as these are the key biotic and abiotic factors regulating resource availability for mycorrhizal fungi. As a result, diversification in the mycorrhizal fungi can be viewed as a result of niche partitioning following initial evolution of the mycorrhizal form. Diversification results from differences in the relative costs and benefits of the association among individual fungal species and genotypes within species driven by competition for carbohydrates from host plants and resources from the soil environment. However, although it is clear that both soil characteristics and host interactions play critical roles as drivers of fungal diversity, the relative roles of these factors, and other drivers of fungal diversity, including geographic isolation and drift, remain poorly understood. Ongoing research using molecular approaches to examine underlying evolutionary changes, coupled with research on ecological functions, promises to greatly enhance our understanding of the drivers of diversification in mycorrhizal fungi (Arnold *et al.*, 2010; Comas *et al.*, 2010; van der Heijden *et al.*, 2015). Recent research on specific patterns within each of the major groups of mycorrhizal fungi is described in the following sections.

Diversification of Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhiza are characterized by the formation of arbuscules, or tree-shaped structures, by the fungal hyphae after penetration of the cell wall of host plant root cells. These arbuscules increase the surface area of the fungus–plant interface, increasing the efficiency of resource exchange. However, the fungus and plant cells typically do not exchange cytoplasm, which are kept separate by the plasma membranes and the fungal cell wall. As described above, phylogenetic analyses suggest the earliest arbuscular mycorrhizal fungi arose over 450 million years ago. Fossil evidence of arbuscular mycorrhizae extends back to the Rhyniophytes. Further, the rise of the arbuscular mycorrhizal form early in the evolution of land plants is reflected in the monophyletic evolution of arbuscular mycorrhizal fungi as a distinct phylum, the Glomeromycota (Schüßler *et al.*, 2001). The close association between the early evolution of arbuscular mycorrhizal fungi and plants, coupled with the radiation of plants to roughly 300 000 species, has led the arbuscular mycorrhizal association to be the most common mutualism formed by plants. However, despite the long evolutionary history of arbuscular mycorrhiza, and the concomitant diversification of plants, the diversity of arbuscular mycorrhizal fungal species is more than an order of magnitude lower compared with ectomycorrhizal and orchid mycorrhizal fungi.

Fossil evidence suggests that much of the extant diversity in arbuscular mycorrhizal fungi had already arisen over 400 million years ago, with limited speciation since then. The low species diversity of arbuscular mycorrhizal fungi despite their ancient origins and broad host range likely reflects at least in part a high degree of genetic diversity within fungal species coupled with low host specificity. Also, arbuscular mycorrhizal fungi host their own endosymbionts, mycoplasma-related endobacteria, which may play a critical role in the

maintenance of arbuscular mycorrhiza, and appear to have a high degree of genetic plasticity (Kuo, 2015; Naito *et al.*, 2015; Torres-Cortés *et al.*, 2015). Further, the Glomeromycota are asexual and have comparatively simple morphologies, making morphological distinctions difficult even among taxa identified as distinct based on molecular tools. As a result, molecular evidence is increasingly being used to assess the diversity and evolution of the Glomeromycota (Hart *et al.*, 2015; Krüger *et al.*, 2012; Redecker and Schüßler, 2014). Evidence from molecular analyses of field samples suggests that many species may not yet have been described, but careful assessment will be required in the further development of the classification of this phylum due to the unique challenges of identifying obligate symbionts (Redecker *et al.*, 2013).

Diversification of Orchid Mycorrhizal Fungi

In addition to the formation of pelotons, which are distinct fungal coils in the space between root cell walls and plasma membranes, orchid mycorrhizal associations differ from most other mycorrhizal associations in that orchids are mycoheterotrophic during germination and initial seedling growth, relying on mycorrhizal fungi as a carbohydrate source (Dearnaley *et al.*, 2012). Most orchids subsequently transition from mycoheterotrophy to autotrophy once the first leaves emerge and become photosynthetic, and, hence, they are mixotrophic. However, some orchids remain mycoheterotrophic throughout their life cycle. These parasitic plants typically acquire carbohydrates from fungi that are saprotrophic or form ectomycorrhizal associations with other plants. As a result, while many fungi only form associations with orchids, many ectomycorrhiza-forming fungi also form associations with orchids.

Orchid mycorrhizal fungi have been estimated to include about 25 000 species in the Ascomycota and Basidiomycota (Dearnaley *et al.*, 2012; van der Heijden *et al.*, 2015), forming associations with most orchid species, which represent about 10% of plant species (Brundrett, 2009). In contrast to the arbuscular mycorrhizal fungi, host specialization appears to have played a key role in the diversification of orchid mycorrhizal fungi. Closely related orchids often share specific combinations of mycorrhizal fungi, with some plant–fungal associations suggesting species- (Okayama *et al.*, 2012; Xing *et al.*, 2015), and even genotype-level (Taylor *et al.*, 2004; Okayama *et al.*, 2012), specificity. Despite this high degree of host specificity, recent phylogenetic analyses suggest orchid mycorrhizal fungi have independently arisen many times, typically from saprotrophic fungi and fungi that form ectomycorrhiza (Schüßler *et al.*, 2001), and there is evidence that some orchid mycorrhizal fungi may have evolved from arbutoid (Okayama *et al.*, 2012) and endophytic (Oberwinkler *et al.*, 2013) fungi. This pattern of high host specificity coupled with multiple independent origins suggests orchid mycorrhizal fungi may rapidly evolve host specificity, at least in some circumstances.

Diversification of Ericoid Mycorrhizal Fungi

Ericoid mycorrhizal fungi are characterized by the formation of distinctive hyphal coils within root cells during establishment of mycorrhizal associations with plants in the Ericaceae. Molecular evidence suggests ericoid mycorrhizae arose independently several times. In addition to arising from saprotrophic lineages, at least some ericoid mycorrhizal fungi apparently diversified from within clades of ectomycorrhizal fungi, and some may have arisen from endophytic fungi. As with orchid mycorrhizal fungi, host specialization appears to have played a key role in the evolution of ericoid mycorrhizal fungi, as these fungi are specific to this family. However, similar to ectomycorrhizal fungi, ericoid mycorrhizal fungi studied to date range greatly in host specialization, from narrow to very broad host ranges within the Ericaceae. In at least some cases, host specialization within the ericoid mycorrhizal fungi in the Ascomycota and Basidiomycota has led to the evolution of distinct mycorrhizal types, including the arbutoid and monotropoid, which are discussed below.

Diversification of Ectomycorrhizal Fungi

In contrast to the endomycorrhizal fungi, ectomycorrhizal fungi are characterized by lack of intracellular hyphae, coupled with the formation of a hyphal mantle on the root surface and a network of intercellular hyphae, referred to as the Hartig net, within the root. Highly diverse, ectomycorrhizal associations are estimated to have arisen independently at least 80 times in the Ascomycota and the Basidiomycota (Tedersoo and Smith, 2013). Molecular evidence suggests ectomycorrhizal fungi typically have arisen from saprotrophs, but there is some evidence suggesting that some ectomycorrhizal fungi have evolved from plant endophytes. Convergent evolution has led to morphological and physiological similarities among these lineages of ectomycorrhizal fungi, with some shared suites of gene changes related to decay function and mycorrhizal formation, but there also is evidence that independent origins of the symbiosis are associated with unique physiological changes in ectomycorrhizal fungi from a given lineage. The repeated independent origins of ectomycorrhizal fungi, coupled with these unique physiological adaptations and the combined effects of coevolution with host plants and evolution in response to environmental factors, likely play a key role in the high diversity of ectomycorrhizal fungal species.

Compared with arbuscular and orchid mycorrhizal fungi, ectomycorrhizal reflect a middle ground in the roles of environmental factors and host specialization in driving speciation. Host specialization in ectomycorrhizal fungi runs the gamut from cosmopolitan species that form associations with most, if not all, of the plants species that host ectomycorrhizal fungi, to fungal species that are only known to form associations with a narrow range of host species. Further, within the more generalist fungi, some genotypes may exhibit greater host specialization than others. While the typical ectomycorrhizal association is with autotrophic plants, some ectomycorrhizal fungi form associations with both mycoheterotrophic and autotrophic plants, though the effects of these interactions on fungal diversity and distributions are unclear. Ectomycorrhizal fungi typically have more restricted geographic distributions than arbuscular mycorrhizal fungi (Allen *et al.*, 1995). While this pattern

reflects in part effects of host specificity, a wide range of abiotic factors, including soil characteristics, such as nitrogen and phosphorus concentration, and successional dynamics, also affects ectomycorrhizal fungal distributions. Taken together, diversification in response to both host specialization and environmental factors has resulted in a high degree of niche differentiation and specialization among ectomycorrhizal fungal species.

Specialized Mycorrhizal Forms Within the Ericaceae

Given the long evolutionary history of mycorrhizal fungi, and the unique associations with certain plant families, it is perhaps not surprising that coevolution with host plants and further host specialization have occurred within at least some mycorrhizal fungal lineages. In particular, coevolution between certain fungi and certain subfamilies of plants within the Ericaceae has led to the development of mycorrhizal forms distinct from the common ericoid type, such as the arbutoid, monotropoid, cavendishioid, and pyroloid (Oberwinkler *et al.*, 2013). As more research is conducted, additional forms may be described; here, diversification in two of the most-studied forms is briefly discussed.

Diversification of Arbutoid Mycorrhizal Fungi

Arbutoid fungi fall somewhere between endo- and ectomycorrhizal associations, as they are characterized by the formation of a hyphal sheath while also growing intracellularly. As a result, this form often is referred to as ectendomycorrhiza. In addition to arbutoid fungi, other fungi that form ectendomycorrhizal associations include pyroloid fungi (Oberwinkler *et al.*, 2013). Arbutoid fungi have a very narrow host range, only forming associations with plants in the subfamily Arbutoideae. Nonetheless, the arbutoid mycorrhizal association arose multiple times within both the Ascomycota and the Basidiomycota, generally within taxa associated with ectomycorrhizal formation. Molecular evidence also suggests some arbutoid mycorrhizal fungi may have arisen from lineages of ericoid mycorrhizal fungi or endophytic fungi. As with ericoid and ectomycorrhizal fungi, arbutoid mycorrhizal fungi reflect convergent evolution both physiologically and morphologically across the disparate lineages.

Diversification of Monotropoid Mycorrhizal Fungi

Monotropoid mycorrhiza share some similarities to orchid mycorrhiza, in that plants are mixotrophic or heterotrophic, and monotropoid mycorrhizal fungi have a very narrow host range, only forming associations with plants in the subfamily Monotropoideae in the Ericaceae. However, monotropoid mycorrhiza are morphologically distinct from orchid mycorrhiza, in that they form a mantle and Hartig net, and evolved independently of orchid mycorrhizal fungi. Monotropoid mycorrhiza also are morphologically distinct from arbutoid mycorrhiza, in that they do not form the hyphal coils commonly observed in arbutoid mycorrhiza; rather than penetrating the cell wall, the hyphae form pegs that cause indentations in the cell wall without actual penetration. Molecular evidence suggests monotropoid mycorrhizal fungi arose multiple times within both the Ascomycota and the Basidiomycota, roughly paralleling evolution of arbutoid mycorrhizal fungi in that monotropoid mycorrhizal fungi appear to have arisen from ectomycorrhizal fungi, ericoid mycorrhizal fungi, and perhaps endophytic fungi, followed by convergent evolution.

Summary

Mycorrhizal fungi account for a significant fraction of fungal diversity. Coevolution with plants has played a significant role in diversification of mycorrhizal fungi, but fungal evolution does not parallel plant evolution. Although arbuscular mycorrhiza evolved first, during the initial invasion of land by plants, ectomycorrhizal and orchid mycorrhizal fungi are the most speciose groups of mycorrhizal fungi. Each of these groups, as well as the ericoid mycorrhiza, is the result of multiple independent evolutionary events, typically from saprotrophic fungi, followed by convergent evolution.

See also: Commensalism, Amensalism, and Synnecrosis. Endophytic Microbes, Evolution and Diversification of. Mutualism, the Evolutionary Ecology of. Symbiosis, Introduction to

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Natural Selection, Introduction to

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Glossary

Absolute fitness A measure of fitness given by the number of offspring.
Density dependence Selection pressures in the population is affected by the population density.
Deterministic Not dependent of random events.
Fitness landscape A map of fitness given as a function of either the genotype or phenotype.
Genetic drift Random sampling of alleles within a population.
Geometric mean An average taken over the sample using the product rather than the sum of the values.
Heritability The degree to which offspring have the same traits as their parents.
Relative fitness The fitness advantage of an individual relative to that of others within a population.
Stochastic Influenced by random events.

One of Charles Darwin's most fundamental contributions to modern biology was his clear description of how 'natural selection' could result in changes in populations over time that would lead to the observed fit between the abilities of plants and animals to survive in the conditions that they are found in nature. We now understand that there is a range of forces that cause change in evolving populations over time (genetic drift, migration, mutation, gene conversion, demographic stochasticity, phenotypic plasticity), but selection is often considered the primary mechanism driving evolutionary change. Selection is a consistent, directional force that acts on the frequencies of alleles in populations and results in the change of the average phenotype within the population over successive generations.

Darwin presented an elegant argument that described when selection will act. He identified four factors, which, if present, imply that populations will change from generation to generation (Darwin, 1859; see the summary of chapter IV for a succinct passage). These assumptions are:

1. Individuals within populations vary in terms of their *traits*. For example, individual finches may have different bill sizes.
2. These traits are related to the ability to survive and reproduce. This ability if often called *fitness*. For example, finches with just the right bill size may have an advantage in finding insects to eat.
3. Offspring have traits that are similar to their parents. This is the notion of *heritability*. Finches with longer than average bills tend to have offspring that also have longer than average bills.
4. There are not enough resources for all offspring produced to survive. This can also be described as *density dependence*: when a population increases in size it will eventually exhaust resources, which will increase the rate of death in the population.

These simple assumptions lead to the conclusion that the population will change over the course of generations in terms of the traits that individuals bear and that the population will become more 'adapted' over time. Adaptation occurs when traits that increase fitness become more common. Darwin's elegant idea did not require an understanding of how traits are passed on to offspring, and in fact it would be almost a century before a detailed understanding of the molecular basis of genetic inheritance was developed.

While Darwin's simple set of assumptions lead to the conclusion that selection will lead to a change in populations over generations, a much more detailed theory of selection was developed to take into account the details of inheritance and population biology. A major advance was due to Fisher (1930) and is sometimes called the Fundamental Theorem of Natural Selection. This theorem states that populations will become more adapted (i.e., will increase in fitness) at a rate that depends on how much heritable variation is present in the population, how large the effect of differences in trait is on fitness, and whether fitness depends on the population or environment in a trait specific way. The main point is that adaptation will increase more quickly when there is a range of traits present in the population and there are large heritable differences in the reproductive output of individuals with different traits (Edwards, 2014).

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Genotype versus Phenotype

To understand how selection works on biological organisms, it is important to distinguish between the genotype and the phenotype. The genotype is the genetic makeup of an organism, and it serves two purposes at the same time: it is both the code that gets copied and inherited between the generations and the code from which the phenotype is constructed as it interacts with the physical environment. The phenotype is the composition of the organism's traits or observable characteristics, and it is on the phenotype that selection acts, as it is the phenotype that interacts directly with the environment. Thus, what matters for selection in biological evolution are the changes that take place in the genotype and how those changes affect the phenotype. Changes to traits that are encoded in the genotype can be passed on to offspring, while traits acquired during the lifetime of the organism cannot.

Fitness and Strength of Selection

Fitness is a measure of an organism's long-term reproductive success and encompasses both its ability to survive and the rate at which it reproduces. The definition of fitness can be complicated and depends on the ecological context (Proulx and Adler, 2010). In the most general sense, the 'absolute fitness' of a phenotype or behavioral strategy can be measured by the number of offspring that a single individual with that phenotype produces over the course of their lifetime. The long-term measure of fitness for an allele is simply the increase in the number of individuals carrying the allele that are present in the population at a distant time in the future.

The strength of selection depends on this fitness, and is given by the 'selection coefficient,' s. The selective advantage of an allele or different phenotype is the new fitness, W', relative to the wild-type fitness, W, $s = W'/W - 1$. Beneficial mutations that cause an increase in fitness thus have a positive selection coefficient, while those of deleterious mutations are negative. This measure of fitness relative to other individuals is called 'relative fitness.'

The effect of relative fitness and the strength of selection also depend on the population size. Stochasticity in survival and reproduction has a smaller effect the larger the population is, while random events have a greater influence on the evolutionary dynamics in small populations leading to genetic drift becoming dominant. As the role of genetic drift increases, the greater the selection coefficient has to be for selection to be able to distinguish between organisms with different fitness. This dependence on population size means that small populations can better tolerate deleterious mutations than larger populations. This effect may enable smaller population to explore the space of possible genotypes and phenotypes it led Wright to propose his Shifting Balance Theory (Wright, 1932), in which a large population splits into smaller demes that are less affected by selection and can therefore explore phenotypic space and find other peaks in the 'fitness landscape' – the mapping from genotype or phenotype onto fitness. In larger populations, selection is stronger and smaller fitness differences can be detected. This increases the probability that advantageous alleles will become established in the population and eventually go to fixation.

Effects of Selection

Selection can be divided into natural and artificial selection, with the former being caused by nonhuman agents and the latter influenced by human interference. In principle, there is no difference in terms of the mechanism, as the population in question responds to any selection pressure in the same way, no matter what the origin is. However, while both types of agents can cause a wide range of changes in fitness, compared to natural selection, artificial selection can be persistently severe, and thereby faster than perhaps most instances of natural selection. For example, under the control of human breeders using artificial insemination, one stallion can potentially have thousands of offspring, which would not be possible under natural conditions. Breeding practices can thus severely affect fitness, and yet the mechanism of selection remains the same.

Natural selection is often further divided into ecological and sexual selection, distinguishing between changes in fitness due to both biotic and abiotic environmental agents leading to ecological selection, and competition for mating between conspecific individuals leading to sexual selection.

In terms of the phenotype, selection can have a number of effects. Stabilizing selection results from agents of selection that favors the current phenotype, with any changes leading to decreases in fitness, thereby stabilizing the population phenotype at its current value. Directional selection occurs when changes in the phenotype are favored, with individuals in the tail end of the phenotypic distribution having higher fitness. This drives the population away from the current phenotypic mean resulting in adaptive evolution. Disruptive selection is similar to directional selection in that the most abundant phenotype is not the most favored, but in this case there are more than one phenotypic direction that increases fitness. This can lead to the population splitting up into two or more subpopulations with different phenotypes. However, if there is no mechanism present to decrease competition between these subpopulations, this phase will be transient, and competition will cause the population to converge on just one of the new phenotypic values. On the other hand, if competition between segregating populations can be mitigated, then they may coexist in what is termed balancing selection. This can be caused by negative frequency-dependent selection, which results when rare phenotypes have a fitness advantage over abundant phenotypes (Ayala and Campbell, 1974). When one phenotype increases in abundance, the rarer type increases in fitness, which leads to an increase in the rare type and a decrease in the abundant type, thereby leading to balancing selection.

It is useful to illustrate these effects of selection in terms of the fitness landscape (Østman and Adami, 2014). The fitness landscape is a mapping of either genotype or phenotype onto fitness. For every unique type there is a corresponding fitness (this scalar value may change over time), and this multidimensional function constitutes the fitness landscape. In rugged fitness landscapes (a landscape with many distinct local maxima) populations located on a peak will experience

stabilizing selection, as every direction in phenotypic space away from the current position leads to a decrease in fitness. If the population is located on an incline in the fitness landscape, it will be under directional selection and will most likely evolve in the direction of the highest fitness increase. If the population is located in a valley with paths leading to different peaks, it will experience disruptive selection, and if the population is also under balancing selection, more than one peak can be climbed and populated for an indefinite amount of time. In a static fitness landscape where the fitness of every type is constant in time, disruptive selection can only be transient. Balancing selection through negative frequency-dependent selection implies that the fitness landscape fluctuates in response to the abundance of different phenotypes.

Stochasticity

It is important to appreciate the role stochasticity plays in evolutionary dynamics. Which organisms survive and which of those actually reproduce depends not only on the organism's phenotype, but also on random events. Random events may include predation, discovery of nest sites, and fluctuations in local weather. If every individual within a population has the same fitness, then obviously selection plays no role in the evolutionary outcome. On the other hand, a single fitter mutant has a higher chance to go to fixation than any other individual, with this probability being dependent on the selection coefficient (**Figure 1**). The specific details of the interplay between selection and random effects is complex, but in large populations competition between established alleles is largely deterministic: the allele with the higher average fitness almost certainly becomes more common (see Video 1).

However, when a mutation occurs the new allele will be represented by only one or a few copies. This means that a string of bad luck affecting all copies of a rare allele can remove it from the population, even when it has higher fitness on average than the common alleles in the population. This effect can be measured by considering the probability of fixation: the probability that a mutant allele present as a single copy eventually comes to be only allele present in the population. Without stochasticity, we might expect that alleles with higher fitness always come to dominate the population, meaning that their probability of fixation would be 1. A variety of mathematical models have been developed to study this process, but a simple result argues that the probability of fixation should be approximately $2 \times s$ in a large population (first derived by Haldane, reviewed in Patwa and Wahl, 2008). This is somewhat surprising since it means that even a mutation with a 5% advantage is lost from the population about 90% of the time. This approximation is valid only if the selection coefficient is small, in part because the fixation probability can never be greater than 1 (see **Figure 1**).

In smaller populations, particularly when there are small differences in fitness, random components tend to overwhelm the directional nature of selection. This means that alleles that have lower than average fitness may still spread in the population leading to a loss of adaptation over evolutionary time.

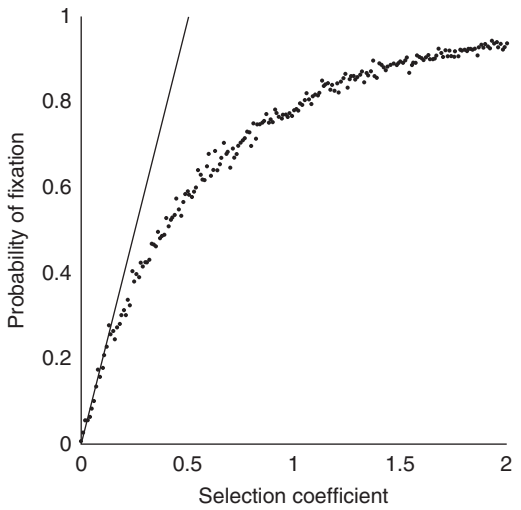

Figure 1 Probability of fixation as a function of selection coefficient. The dots are simulation results where a single mutant individual was introduced into an established population. Each simulation was run until only one allele was left, meaning that either the mutant was lost from the population or spread and became fixed. Each dot represents 1000 simulations with their results averaged. The straight line is the $2s$ prediction that is valid for small values of s. Note that the simulated results (dots) show some random variation around this prediction up until about $s=0.15$. For larger values of s, the approximate result is no longer valid. Data are from a simulation with a constant population size of $N=100$, nonoverlapping generations, asexual reproduction, chance of reproducing is equal to the normalized fitness, which is 1 for 99 individuals and $1+s$ for one individual.

Factors Affecting the Action of Selection

The strength of selection depends on fitness differences among phenotypes, but several factors can complicate the link between the long-term measure of fitness and the number of offspring carrying the parental genes.

1. Genetic relatedness: the offspring must bear the genes that were responsible for the phenotype that the parents developed. This is clearly not the case in situations where offspring are physically replaced, as is the case for brood parasites like the cuckoo. A similar effect can occur when genetic parasites are present that cause biased genetic transmission (see meiotic drive).
2. Offspring quality: while producing more genetic offspring does increase the number of individuals carrying the focal allele in the next generation, it will only lead to a long-term spread if those offspring also have the same ability. A strategy that results in increased offspring production but a decrease in offspring quality may therefore not have high fitness. This is an example of an intergenerational fitness effect (e.g., Hoyle and Ezard, 2012).
3. Sexual selection: offspring quality can vary because of parental investment, or because of genetic effects. Choosing a mating partner may influence the genetic quality of offspring without changing the total number of offspring.

This affects the genes present in the parent because genes that become associated with higher genetic quality will leave more offspring themselves, increasing the genetic representation of the parental allele in future generations.

4. Sex ratio: in many species, the sex of offspring is determined directly by the parent or by some combination of parental influence and environmental influences (genetic sex determination, as in humans and most other mammals, is rare across plants and animals as a whole).
5. Frequency dependence: the important measure of fitness is relative fitness, which relates the offspring production of one phenotype to the average offspring production in the population. When there is frequency dependence, the fitness of a phenotype depends on the phenotypes that it is competing against. For example, tree height is an important parameter to determine light competition and seed dispersal ability. The benefit of growing taller, however, depends on how tall the competitors are. This can lead to counterintuitive effects where total offspring production goes down over evolutionary time because more resources are devoted to competition (growing more wood) as compared with reproduction.
6. Population dynamics: the representation of an allele in future generations can depend on the timing of reproduction as well as the total amount of reproduction. In a growing population, it is generally better to reproduce earlier (for the same reason that investing in retirement early provides a higher return), whereas in a declining population it actually makes sense to reproduce later.
7. Environmental variability: because the performance of phenotypes depends on the environment that they experience, one allele may be favored in some environments but disfavored in others. Under environmental variability, the fitness of a phenotype depends on the type of variability in the environment. When variability affects each individual directly (e.g., variability in local resource density) then the appropriate measure of fitness is the arithmetic mean of fitness. However, when variability affects all individuals the same way (e.g., year-to-year variance in temperature) then the appropriate measure of fitness is the geometric mean of fitness. While the arithmetic mean is equally influenced by high and low values, the geometric mean is disproportionately affected by low values. Therefore, fitness can be greatly decreased when there is variability between generations.

Acknowledgments

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See also: Neutral Evolution, Population Genetic Tests of. Neutral Models of Genetic Drift and Mutation

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Natural Selection, Measuring

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Glossary

Fitness function The quantitative relationship between phenotype and fitness. Measuring selection requires estimating this function.

Opportunity for selection (I) Variance in relative fitness.

Relative fitness (ω) Individual fitness divided by population mean fitness.

Selection differential (S or C) Covariance between relative fitness and phenotype. S describes the strength of linear selection, and C, the strength of nonlinear selection.

Selection gradient (β or γ) Partial regression of relative fitness on phenotype independent of relationships with other (measured) phenotypes. β describes the strength of linear selection, and γ, the strength of nonlinear selection.

Natural selection (hereafter, 'selection') is the main driver of adaptive phenotypic evolution (Falconer and Mackay, 1996). Measuring selection is therefore foundational to our understanding of adaptation. Studying patterns of selection allows evolutionary biologists to identify the specific traits that drive variation in survival and reproductive success, provides the quantitative measurements used to predict evolutionary change, and pinpoints the ecological agents that shape evolution in natural populations. The measurement of selection has advanced the study of questions as diverse as the fitness consequences of trait integration (Brodie, 1992), the evolution of phenotypic plasticity (Baythavong and Stanton, 2010), and adaptation to changing environmental conditions (Etterson, 2004). Here we provide an overview of the most common statistical and analytical methods used to measure and interpret patterns of selection in natural populations.

What Is Selection?

Selection occurs when individuals with different characteristics ('phenotypes' or 'traits') differ in their survival or reproductive success ('fitness'). In other words, selection is a statistical association – the covariance – between phenotype and fitness (Robertson, 1966; Price, 1970; Lande and Arnold, 1983; Endler, 1986). Covariances (unscaled correlations) describe the degree to which two variables change together. This relationship is foundational in quantitative genetics because regardless of the complexity of the fitness function, larger covariance between phenotype and fitness indicates stronger selection (Lynch and Walsh, 1998). All of the methods of measuring selection described in this article are based on this fundamental relationship, though they may differ in the statistical approach used to estimate the function that relates fitness to phenotype.

The definition of selection as the covariance between phenotype and fitness highlights three crucial points. First, selection requires variation in both phenotype and fitness: two variables cannot covary unless both vary (Endler, 1986). Second, like correlation, covariance does not imply causation: covariance between a phenotype and fitness does not indicate why selection acts on that particular phenotype. For example, Fishman and Willis (2008) found that *Mimulus* plants with wide flowers produce more seeds, but pinpointing pollinators as the ecological agent of selection on floral width required a comparison of open-pollinated plants and plants that were caged to exclude pollinators. Finally, selection is a population-level process that affects characteristics of phenotype distributions – means, variances, and covariances (Endler, 1986; Brodie *et al.*, 1995).

The Data: Phenotypes and Fitness

To measure selection, phenotype and fitness data must be collected on the same set of individuals. Selection can be measured on any phenotype (measurable property) of an organism. In this article we focus on quantitative traits that vary continuously among individuals (Falconer and Mackay, 1996). Selection acts on phenotypes irrespective of their genetic basis, but selection results in evolution only when the phenotypic differences between individuals are heritable. This distinction between selection and evolutionary response allows biologists to study selection without knowing the genetic basis of a trait (Brodie *et al.*, 1995).

Fitness is estimated empirically as the number of offspring that an individual contributes to the next generation. However, total lifetime fitness is notoriously difficult to measure, especially for long-lived organisms, and few empirical studies are able to assess it (Kingsolver and Pfennig, 2007). Instead, most analyses use multiplicative fitness components – measurements that contribute to lifetime reproductive success – in lieu of total lifetime fitness (Conner and Hartl, 2004; Kingsolver and Pfennig, 2007). Commonly measured fitness components include survival, mating success, and fecundity. Different fitness components often drive very different patterns of selection. A single trait, for example, can be subject to opposing selection via survival ('viability selection') and via mating success ('sexual selection'). These episodes of selection can be combined to estimate lifetime selection, although care must be taken when dealing with complex fitness functions (Arnold and Wade, 1984; Wade and Kalisz, 1989; McGlothlin, 2010; Shaw and Geyer, 2010). Even when it is possible to measure total fitness, estimating the number of offspring an individual contributes to the next generation is complicated by the fact that populations are often age- or stage-structured, which blurs generation boundaries (Lande, 1982; Barfield

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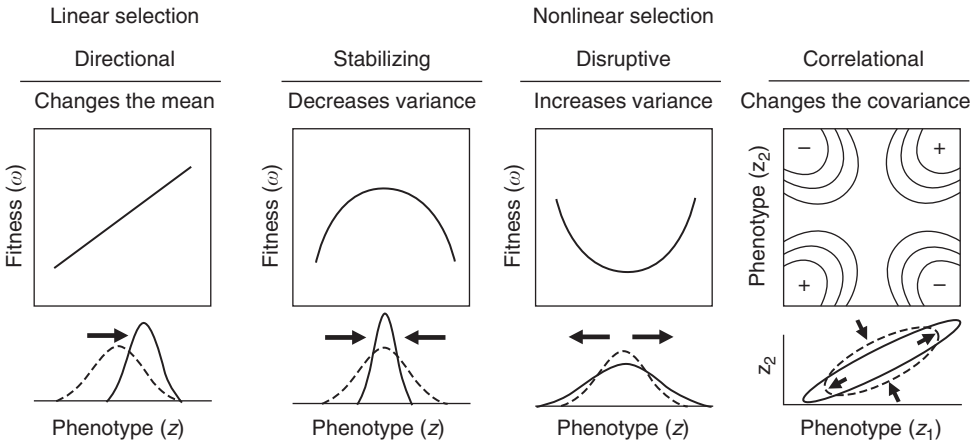

Figure 1 The modes of selection. They are characterized by the shape of the fitness function (top row) and their corresponding effect on the phenotypic distribution (bottom row). The fitness function for correlation selection is shown as a contour plot with isoclines of equal fitness.

et al., 2011; Moorad, 2014). Under these circumstances, an individual's reproductive value at birth (Moorad, 2014) or the 'sensitivities' of population growth to changes in age-specific fitness of individuals of different phenotypes (Lande, 1982) can be used to estimate fitness and selection on the relevant phenotypes.

Regardless of the fitness component, relative fitness rather than absolute fitness is always used to measure selection (Conner and Hartl, 2004). An individual's relative fitness is its absolute fitness divided by the population mean fitness. Relative fitness captures an individual's success compared to others in its population, and is used because selection operates on differences in fitness among individuals (Conner and Hartl, 2004). The variance in relative fitness sets a limit on the strength of selection on any phenotype, an upper bound known as the opportunity for selection, I (Arnold and Wade, 1984; Arnold, 1986). This limit exists because selection requires fitness differences among individuals that differ in phenotype, so the more variation there is in fitness, the greater the potential for strong selection. It is important to recognize that the opportunity for selection does not measure the actual strength of selection on any given phenotype, because selection requires a relationship between fitness and a specific phenotype.

Modes of Selection

Selection is described by a 'fitness function,' $\omega(z)$, that relates phenotype (z) to expected relative fitness ($\hat{\omega}$). Fitness functions can take many forms, or 'modes' (Phillips and Arnold, 1989; Brodie *et al.*, 1995). Selection can be conceptually decomposed into four modes: directional, stabilizing, disruptive, and correlational (**Figure 1**). The shape of the fitness function and its effect on the phenotype distribution distinguishes these modes. Directional selection occurs when individuals with phenotypes larger or smaller than the mean have higher expected relative fitness. Stabilizing or disruptive selection occurs when individuals with phenotypes closest to (stabilizing selection) or farthest from (disruptive selection) the mean have the highest expected relative fitness. Correlational selection describes selection on multiple traits simultaneously. It occurs when individuals with certain combinations of two or more phenotypes (e.g., large values of both) have the highest expected relative fitness (Brodie, 1992). Fitness functions observed in natural populations typically are complex mixtures of these four modes.

Because a selection function might include elements of multiple modes, it is often useful to classify selection as linear (directional) when the fitness function is not curved and nonlinear (stabilizing, disruptive, correlational) when the fitness function is curved (Phillips and Arnold, 1989). Linear and nonlinear selection affect different aspects of the phenotype distribution (**Figure 1**). Linear (directional) selection affects the mean, whereas nonlinear selection affects the variance (stabilizing or disruptive selection) or the covariance between two traits (correlational selection).

How to Measure Selection

A rigorous selection analysis involves three steps: collecting the data, estimating selection, and interpreting the results (Lande and Arnold, 1983; Brodie *et al.*, 1995). Details vary depending on the question and system, but selection analyses on two or more phenotypes generally include these fundamental elements:

Anatomy of a selection analysis on two (or more) phenotypes

Collect the data	Measure fitness and at least two phenotypes on each individual. Fitness is converted to relative fitness $\left(\frac{w}{\bar{w}}\right)$. Each phenotype is expressed as a deviation from the mean ($\tilde{z}=z-\bar{z}$) and standardized to unit variance $\left(\frac{\tilde{z}}{\sigma}\right)$. This standardization expresses traits in standard deviation units and allows comparison of selection between species or between traits measured on different scales (Hansen and Houle, 2008)
Estimate linear selection	Estimate selection using multiple regression of relative fitness on phenotypic values. The regression model to estimate linear selection includes fitness as the dependent variable (ω) and

	the two standardized phenotypes as independent variables ($\tilde{z}_1$ and $\tilde{z}_2$); ε is residual error: $\omega = \beta_1\tilde{z}_1 + \beta_2\tilde{z}_2 + \varepsilon$ β'_1 and β'_2 are estimates of linear selection on $\tilde{z}_1$ and $\tilde{z}_2$. They are known as linear selection gradients (primes are often used to note that they have been estimated on standardized traits). Positive βs indicate selection favoring larger values of phenotypes; negative βs indicate selection favoring smaller values of phenotypes
Estimate nonlinear selection	Estimate nonlinear selection by adding three quadratic forms of the original traits (expressed as deviations from the mean) to the regression: $\omega = \beta_1\tilde{z}_1 + \beta_2\tilde{z}_2 + \frac{1}{2}\gamma_{11}\tilde{z}_1^2 + \frac{1}{2}\gamma_{22}\tilde{z}_2^2 + \gamma_{12}\tilde{z}_1\tilde{z}_2 + \varepsilon$ γ'_{11}, γ'_{22}, and γ'_{12} are known as the nonlinear selection gradients. γ'_{11} and γ'_{22} measure the strength of stabilizing (negative γ) or disruptive (positive γ) selection on $\tilde{z}_1$ and $\tilde{z}_2$. γ'_{12} measures the strength of correlational selection on the simultaneous combination of $\tilde{z}_1$ and $\tilde{z}_2$. Positive γ_{12} reflects selection favoring similar combinations of the two traits, and negative γ_{12} reflect selection favoring opposite combinations. Linear terms from this quadratic regression model are ignored due to nonindependence with the nonlinear terms (Lande and Arnold, 1983; Brodie *et al.*, 1995)
Interpret the results	Test significance of the estimated selection gradients (i.e., whether βs and γs are statistically different from zero), accounting for statistical assumptions. Visualize, the relationship of phenotype and fitness to interpret selection estimates, and to identify peaks and valleys in the fitness function

The Details of a Selection Analysis

The approach outlined above is powerful because the parameters it produces – selection gradients and a related metric, the selection differential – are exactly what are required to quantitatively predict evolutionary change (**Table 1**). Both gradients and differentials can be plugged directly into the breeder's equation, which describes the change in a trait due to selection in a single generation (Lande and Arnold, 1983).

Selection gradients (β and γ): Direct selection on a trait

Total selection on a trait is composed of direct selection on that trait (i.e., its causal effect on fitness) and indirect selection on correlated traits. The selection gradients estimated using multiple regression (above) measure the strength of direct selection acting on a trait, independent of any correlated traits that are included in the model. Each partial regression coefficient measures the relationship between a single trait and relative fitness, controlling for the effects of all measured correlated traits on fitness (Lande and Arnold, 1983).

The linear selection gradients (β) are linear partial regression coefficients. These gradients measure the average slope of the fitness function around the population mean (directional selection) (**Figure 2**). The nonlinear selection gradients (γ) are quadratic partial regression coefficients that describe the average curvature of the fitness function around the population mean (stabilizing, disruptive, and correlational selection). The partial regression coefficients corresponding to stabilizing and disruptive selection (γ_{11} and γ_{22} above) estimated from the model must be doubled to estimate the stabilizing/disruptive gradients (Stinchcombe *et al.*, 2008).

Selection differentials (S and C): Total selection on a trait

The selection differential measures the strength of total selection on a trait (both direct and indirect) within a generation (Lande and Arnold, 1983; Brodie *et al.*, 1995), and is equivalent to the covariance of the trait with fitness, cov(ω, z). For viability selection, the linear selection differential (S) is the difference in the trait mean before and after selection (a weighted analog holds for other measures of fitness)

Table 1 Measures of selection, their interpretation, and method of estimation

Mode of selection		*Parameter*	*Interpretation*	*Method of estimation*
Selection gradients: direct selection on a phenotype				
Linear	Directional	β_i	Average slope of the fitness function	Partial regression coefficient from a regression of fitness on deviations from the mean phenotype
Nonlinear	Stabilizing/disruptive	γ_{ii}	Average curvature of the fitness function	Partial regression coefficient from a regression of fitness on squared deviations from the mean phenotype
	Correlational	γ_{ij}	Average curvature of the fitness function	Partial regression coefficient from a multiple regression of fitness on the cross-product of deviations from the mean phenotype
Selection differentials: total selection on a phenotype				
Linear	Directional	S_i	Change in the phenotypic mean due to selection	Covariance between deviations from the mean phenotype and relative fitness
Nonlinear	Stabilizing/disruptive	C_{ii}	Change in the phenotypic variance due to selection	Covariance between squared deviations from the mean phenotype and relative fitness
	Correlational	C_{ij}	Change in the phenotypic covariance due to selection	Covariance between the cross-product of deviations from the mean phenotype and relative fitness

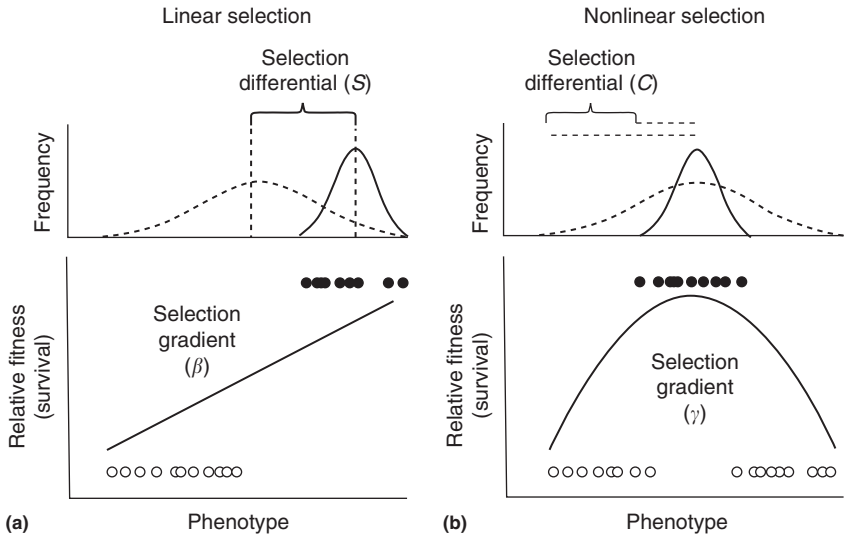

Figure 2 Illustration of selection gradients and differentials, in the case of survival selection on a hypothetical phenotype. The relationship between phenotype and fitness is shown in the panels below. The frequency distributions of the phenotype before and after selection are shown in the panels above. (a) Linear selection. The selection differential (S) is the difference in the phenotype means before and after selection. The selection gradient (β) is the regression coefficient from the regression of fitness on phenotype. (b) Nonlinear selection. The selection differential (C) is the difference in the phenotypic variance before and after selection. The selection gradient (γ) is the regression coefficient from the regression of fitness on squared deviations from the phenotype mean.

(Figure 2). Linear selection differentials can be compared across phenotypes or species if they are estimated on standardized traits (above), or by dividing the unstandardized differential by the phenotypic standard deviation. Such standardized linear differentials are known as selection intensities, i (Falconer and Mackay, 1996).

The nonlinear selection differential (C) is the difference in the trait variance or covariance before and after selection, after removing the effect of linear selection (Figure 2). As with linear selection differentials, nonlinear selection differentials can be estimated as covariances and standardized for comparison (Brodie *et al.*, 1995). The univariate nonlinear differential, which measures stabilizing and disruptive selection, is the covariance between relative fitness and squared deviations from the phenotype mean, $\mathrm{cov}(\omega, z^2)$. The bivariate nonlinear differential, which measures correlational selection, is the covariance between relative fitness and the cross-product of deviations from the phenotype means, $\mathrm{cov}(\omega, z_1 z_2)$.

Limitations and Underlying Assumptions

The application of multiple regression to the measurement of selection is powerful, but it is important to recognize that it is subject to the limitations and underlying assumptions of parametric statistics (Mitchell-Olds and Shaw, 1987). First, sample size limits the statistical power of a selection analysis. Robust quantitative estimates of selection require at least 200 individuals, so most selection gradients are associated with large standard errors (Kingsolver *et al.*, 2001, 2012). Second, the interpretation of a selection gradient as independent selection directly on a trait is strictly true only when all traits that affect fitness were included in the analysis (Lande and Arnold, 1983). Third, it is difficult to statistically disentangle the effects of extremely tightly correlated traits on fitness (a problem known as 'multicollinearity'; Mitchell-Olds and Shaw, 1987). Finally, fitness data frequently violate statistical assumptions, so significance testing of selection analyses often employs statistical techniques that account for non-normal data (e.g., generalized linear models; Bolker *et al.*, 2009).

Exploring the Complexity of Fitness Functions

The relationship between phenotype and fitness can be substantially more complex than the two-trait example outlined above. The strength, direction, and mode of selection depend on the biotic and abiotic environmental conditions that underlie the relationship between phenotype and fitness (MacColl, 2011). As a result, selection may vary substantially in space and time, which is easily missed in selection analyses conducted in a single year or in a single population (Kingsolver *et al.*, 2001, 2012; Siepielski *et al.*, 2009).

Even in a single selection analysis, the true fitness function may contain peaks and valleys that cannot be captured by quadratic selection gradients and differentials. Moreover, fitness is often determined by complex interactions between multiple phenotypes, making it challenging to disentangle their separate effects. The following approaches are useful ways to uncover and interpret complex fitness functions.

Rugged Fitness Functions

Perhaps the most fundamental assumption of the multiple regression approach is that a smooth linear or quadratic function is a good approximation of the true fitness function. Selection gradients are powerful evolutionary predictors because they measure the average slope and curvature of the fitness function, but this 'average' characterization does not

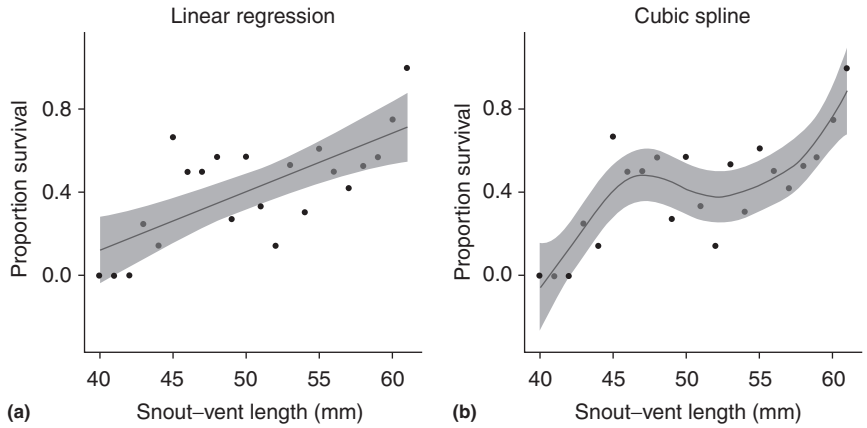

Figure 3 Alternative visualizations of the same data. Cubic splines identify peaks and valleys in rugged fitness functions that are undetected by linear regression. (a) Linear regression of survival on body size in male *Anolis* lizards suggests purely directional selection. (b) The use of a cubic spline to estimate selection reveals an intermediate local peak of high fitness not detected by linear regression. Data from Figure 4 in Cox, R.M., Calsbeek, R., 2010. Sex-specific selection and intraspecific variation in sexual size dimorphism. Evolution 64, 798–809.

reflect the idiosyncracies of rugged functions that contain many peaks and valleys (Schluter, 1988).

Cubic splines provide a nonparametric visualization of the fitness function that accurately reflects its rugosity (**Figure 3**). Splines derive their name from the flexible curves used in architectural drafting and engineering to interpolate between fixed points (Schluter and Nychka, 1994). Fitting a spline is similar to fitting a line using a moving average: the slope of the spline at any point reflects the local relationship between phenotype and fitness (Schluter, 1988). As a result, splines reveal fitness peaks and valleys that go undetected by quadratic regression.

Visualizing the spline is a powerful way to assess whether a linear or quadratic approximation of the average fitness function sufficiently captures the nuances of the relationships between traits and fitness. Spline interpolation is best employed as a complement of, rather than a replacement for, parametric estimates of the fitness function, because the equations used to predict adaptive evolutionary change rely on the coefficients obtained from parametric regression (i.e., linear and quadratic gradients and differentials; Brodie *et al.*, 1995).

Selection on Many Traits at Once

The multiple regression approach used to estimate selection gradients allows biologists to measure selection acting on many traits simultaneously. This property is essential because selection acts on organisms composed of integrated suites of traits, rather than on individual traits in isolation. In other words, each individual has many traits but only one fitness, so it is necessary to capture how all of its traits interact to determine its fitness. However, interpreting estimates of selection becomes difficult with large numbers of traits because each trait introduces a new dimension to the analysis. It is challenging to visualize the complete fitness function with more than two traits in the analysis, a critical step in interpreting the pattern of selection described by the shape of the fitness function. This problem is especially pronounced with nonlinear selection gradients. The addition of a trait to an analysis of n traits adds one linear selection gradient and $n+1$ nonlinear selection gradients: the stabilizing/disruptive gradient on the trait itself, plus correlational selection gradients with all other traits in the analysis. Moreover, the precise shape of the nonlinear fitness function depends on the relative strength of stabilizing, disruptive, and correlational selection gradients (Phillips and Arnold, 1989).

Dimensionality of the selection analysis is generally reduced in one of two ways: focusing on the trait combinations that contain the most variation or focusing on the trait combinations that experience the strongest selection.

Identifying the trait combinations containing the most variance

Principal components analysis identifies new variables ('principal components') comprising the trait combinations that contain the most variation. Principal components analysis produces the same number of principal components as original traits, but in most biological datasets, the first few principal components contain the majority of variation found in the original traits. Therefore, the selection analysis can be performed using only the first few principal components, decreasing the number of variables included in the analysis. The disadvantage of this approach is that the trait combinations that contain the most variation may not necessarily correspond to the traits that experience the strongest selection (Brodie *et al.*, 1995). It can also be challenging to interpret selection on principal components, because they are abstracted away from the original measured traits, the choice of which was usually motivated by a biological hypothesis about their effects on fitness (Brodie and McGlothlin, 2007).

Identifying the trait combinations under the strongest selection

One alternative is to focus on the trait combinations experiencing the strongest selection, which is accomplished with a procedure known as canonical analysis (**Figure 4**). Canonical

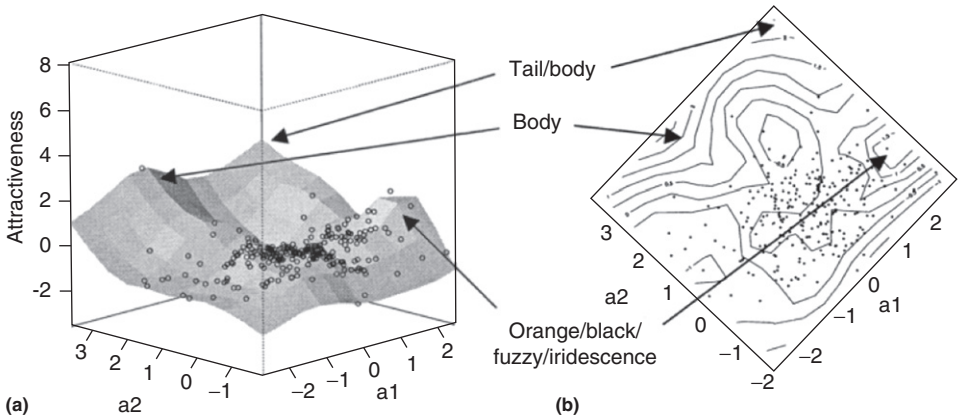

Figure 4 Selection on a suite of morphological and color traits in guppies. (a) Three-dimensional surface visualization of the fitness function, fitted with a thin-plate spline. (b) A top-down view of the same fitness function, where the lines are isoclines of equal fitness. The a1 and a2 axes represent combinations of traits under the strongest selection identified by projection pursuit regression. Peaks in the surface indicate combinations of traits that confer high fitness ('attractiveness'). Reproduced from Blows, M.W., Brooks, R., Kraft, P.G., 2003. Exploring complex fitness surfaces: Multiple ornamentation and polymorphism in male guppies. Evolution 57, 1622–1630, with permission from John Wiley and Sons.

analysis is most often used to identify the trait combinations that experience the strongest nonlinear selection, in order to reduce the number of nonlinear gradients in a selection analysis (Phillips and Arnold, 1989; Blows and Brooks, 2003). Canonical analysis is similar to principal components analysis (described above), but instead of identifying new variables composed of the trait combinations containing the most phenotypic variation, canonical analysis finds the trait combinations that explain the most variation in the relationship between phenotypes and fitness. Selection gradients are then calculated on these new composite traits (Phillips and Arnold, 1989). These composite traits tend to be under stronger nonlinear selection than the original traits, and the resulting gradients are more tractable to interpret because canonical analysis nearly always produces fewer nonlinear coefficients than the original selection analysis (Phillips and Arnold, 1989; Blows, 2007).

Canonical analysis still relies on parametric statistics to estimate nonlinear selection on the new trait combinations that it identifies. There are nonparametric analogs that can be used to distill rugged fitness functions that are insufficiently described by quadratic selection gradients. The nonparametric alternative to canonical analysis is projection pursuit regression, which estimates a function composed of univariate cubic splines (Schluter and Nychka, 1994). Projection pursuit regression identifies the trait combinations that experience the strongest overall selection, both linear and nonlinear. The multivariate extension of cubic splines – thin-plate splines – can be used to visualize the multivariate fitness surface (Blows *et al.*, 2003).

Complex Causal Relationships between Phenotypes and Fitness

Not all traits influence fitness directly. The effect of a trait on fitness is often mediated by other traits. Path analysis is used to estimate selection gradients on groups of phenotypes with

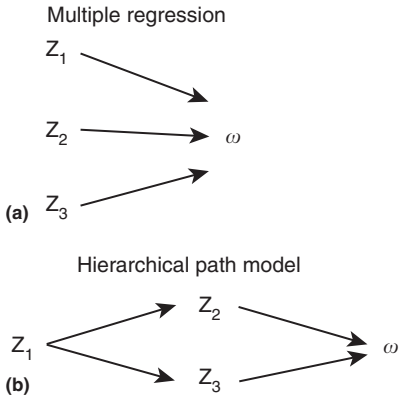

Figure 5 Path models capture complex relationships between phenotype and fitness. (a) A typical multiple regression model assumes all three phenotypes (z_1, z_2, and z_3) causally affect fitness directly. (b) Path models allow hierarchical relationships between traits and fitness. In this example, one phenotype (z_1) does not directly affect fitness. Instead, it affects two other phenotypes (z_2 and z_3) that in turn influence fitness.

complex effects on each other and on fitness (Lynch and Walsh, 1998; Morrissey, 2014). Path analysis is an extension of multiple regression that allows some phenotypes to mediate the effect of others on fitness (**Figure 5**). The coefficients estimated in path analysis are partial regression coefficients, just like the selection gradients from a multiple regression (Scheiner *et al.*, 2000).

Path analysis is a powerful tool to measure selection on suites of morphological and performance traits because the effect of morphological traits on fitness is mediated through their effect on overall performance (Arnold, 1983). Arnold (1983) applied path analysis to investigate the effect of skull morphology on fitness in snakes, a gape-limited predator. He used it to partition selection into two gradients: the effect of skull morphology on swallowing performance (the

'performance gradient'), and the effect of swallowing on fitness (the 'fitness gradient').

Targets and Agents of Selection

Selection gradients obtained from regression analysis identify traits that are the likely targets of direct selection, but purely statistical approaches are unable to demonstrate a causal relationship between a phenotype and fitness (Wade and Kalisz, 1990). These approaches give us quantitative descriptions of a pattern, but cannot tell us how or why selection acts on a particular trait. To these ends, measuring selection on naturally occurring phenotypic variation in unmanipulated populations must be coupled with experimental manipulation in order to conclusively identify the agents and targets of selection. A holistic understanding of adaptive evolution ultimately requires linking the environmental factors causing selection (selective 'agents') to the phenotypes on which they directly act (selective 'targets') (Conner and Hartl, 2004).

Targets of Selection

Phenotypic engineering – the manipulation of phenotypes – is a common technique used to confirm targets of selection identified by selection analyses (Conner and Hartl, 2004). Phenotypic engineering can be used to decouple correlated traits, facilitating the measurement of their independent effects on fitness. Such manipulation is especially important when two traits are so tightly correlated that their independent effects on fitness are impossible to disentangle statistically (e.g., Veiga and Polo, 2008; Sekii *et al.*, 2013). Second, phenotypic engineering is useful for expanding natural phenotypic distributions by creating more extreme phenotypes (Hotzy *et al.*, 2012). A perennial limitation on detecting selection on quantitative traits is that they tend to be normally distributed and occupy a relatively small region of phenotypic space. Most individuals exhibit phenotypes close to the mean, resulting in few samples and low statistical power at the extremes of the phenotype distribution. The current distribution is likely a by-product of past selection that eliminated phenotypes far from the optimum, confining the phenotypic distribution to a small flat region of the adaptive landscape. As a result, it is often necessary to artificially create phenotypes beyond the natural limits of the distribution to estimate the slope and curvature of the true fitness function. Sinervo *et al.* (1992) employed phenotypic engineering to circumvent these challenges and assess selection on offspring size in side-blotched lizards. They manipulated yolk investment to increase the frequency of extremely small and extremely large hatchlings, and demonstrated that selection favoring large offspring was not universal, but varied among years and between sexes.

Environmentally induced covariance between phenotype and fitness is a pervasive impediment to pinpointing the target of selection. It occurs when an underlying environmental factor influences both the expression of a trait and fitness, generating a spurious relationship between the two (Mauricio and Mojonnier, 1997; Pemberton, 2010). This problem can be solved experimentally by phenotypic engineering or by measuring selection under controlled environmental conditions. Alternatively, selection can be estimated by regressing fitness on genotypic (breeding) values rather than phenotypic values (Rausher, 1992). However, the latter is not always a practical solution, because it can be prohibitively difficult to estimate breeding values in natural populations.

Agents of Selection

Ultimately, questions about natural selection revolve around the functional relationship between organisms and their social, biotic, and abiotic environments (Wade and Kalisz, 1990; Boughman, 2001). Selection for larger bills in Galápagos finches, for example, is due to the fact that birds with larger bills are more successful foragers because they are able to crack larger seeds (Grant and Grant, 1995, 2011). However, this connection between an ecological agent and selection remains difficult to draw in the wild for many reasons (MacColl, 2011). A single trait can have many putative selective agents that are difficult to distinguish experimentally, and are challenging to manipulate without perturbing other aspects of a species' ecology. These experiments are logistically difficult to execute because identifying the agents of selection requires multiple estimates of selection under different ecological conditions.

Correlational, observational, and experimental approaches can be used to fill this gap (MacColl, 2011). Correlational studies exploit naturally occurring environmental differences between populations to ask whether the environment is associated with predictable differences in the fitness function (Wade and Kalisz, 1990). In some cases, field observation and attention to natural history can reveal the agents of selection. Young *et al.* (2004) identified loggerhead shrikes as the agent of selection on horn length in *Phrynosoma* lizards by comparing the length of the horns on live lizards to those on dead lizards that shrikes had impaled on trees. Alternatively, the putative environmental agents of selection can be manipulated. For example, although body size and performance traits are consistently targets of selection in lizards in the *Anolis* radiation, the mechanism responsible for the relationship between phenotypes and fitness is usually disregarded. Calsbeek and Cox (2010) addressed this question by manipulating both predation and intraspecific competition in *Anolis sagrei* and demonstrated that competition generates stronger selection on these phenotypes than predation.

Conclusion

The best selection analyses employ a holistic approach to characterize patterns of selection in natural populations. Measurements of selection from rigorous analyses have allowed us to identify general patterns in the causes and consequences of selection from studies performed in different sexes and species, or different habitats and years. Knowledge of the patterns and process of selection in natural populations is the foundation of our understanding of microevolutionary change.

See also: Artificial Selection. Evolution and Agriculture II. Evolutionary Applications to Breeding

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Neutral Evolution, Population Genetic Tests of

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Glossary

Background selection The effect of negative selection on diversity at linked neutral sites.
Balancing selection A type of selection that results in the maintenance of two or more alleles at a locus over some long period of time.
Divergence The genetic differences between two sequences sampled from two species.
Haplotype A particular combination of alleles found on the same chromosome.
Infinite island model A conceptual model for population structure and gene flow under which an infinite number of identical subpopulations (demes) exchange migrants with each other. Each subpopulation experiences an equal amount of gene flow from all other subpopulations.
Negative/purifying selection Selection against a mutation that reduces fitness.
Neutral theory The theory that segregating genetic variation is predominantly neutral and shaped by mutation and random genetic drift.
Nonsynonymous mutation A point mutation in the coding region of a gene that leads to a change of an amino acid in the corresponding protein.
Nucleotide diversity (θ_π) The average number of nucleotide differences per site between any two sequences chosen randomly from the population. Under the SNM, it is an unbiased estimator of θ.
Population mutation rate (θ) The per-generation mutation rate multiplied by four times the population size.
Positive/directional selection Selection favoring a mutation that increases fitness.
Segregating sites Sites where multiple alleles can be found in a sample of sequences. Its is equivalent to polymorphic sites.
Selective sweep The process by which positive selection eliminates neutral variation at linked sites.
Site frequency spectrum (SFS) A histogram describing the number (or proportion) of segregating sites that have different frequencies in the sample.
Standard neutral model (SNM) A simple model of the neutral theory, where mutation and drift act in a randomly mating population of constant size.
Stepping stone model A class of models that restricts gene flow to subpopulations that are adjacent or nearby in one or two dimensions. It is used to model isolation by distance, the increase in genetic differentiation with physical distance.
Synonymous mutation A point mutation in the coding region of a gene that does not alter the amino acid sequence of the corresponding protein.
Tajima's D A test statistic computed as the difference between two estimates of genetic diversity, θ_W and θ_π, divided by the standard deviation of the difference. Under the SNM, its average value is zero.
Watterson's θ (θ_W) An unbiased estimator of θ under the SNM. It is based on the number of segregating sites and is not influenced by the frequencies of the polymorphisms.

Introduction

Since the advent of DNA sequencing in the early 1980s, a major focus in empirical population genetics has been determining whether a particular DNA sequence has been neutrally evolving, or has been affected by natural selection (Hudson *et al.*, 1987; Kreitman, 1983; McDonald and Kreitman, 1991; Tajima, 1989). One way to do this is to assess whether patterns in the sequence of interest are compatible with predictions from standard neutral models (SNMs) of evolution (Kimura, 1983). This is typically done using a test of neutrality. Originally, these tests had been applied to a small number of candidate genes, but in more recent times, they have been applied to whole genomes (Akey *et al.*, 2002; Carlson *et al.*, 2005; Chávez-Galarza *et al.*, 2013; Huber *et al.*, 2014; Kelley *et al.*, 2006; Kimura *et al.*, 2007; Long *et al.*, 2013; Moon *et al.*, 2015; Qanbari *et al.*, 2014, 2012; Ramey *et al.*, 2013; Sabeti *et al.*, 2007; Tang *et al.*, 2007; Voight *et al.*, 2006; Wang *et al.*, 2006; Williamson *et al.*, 2007; Xia *et al.*, 2009). The advantage of scanning the genome for signatures of selection is that it provides an unbiased survey of genes that have been important throughout evolution, without external knowledge of what the genes do or the particular phenotype(s) involved. These selection scans are becoming an important part of evolutionary genetic studies across a wide array of model and non-model systems.

Here we will review basic tests of neutrality. These tests can be divided into several categories based on the information from the data that they use (**Table 1**). We will describe each type of test and highlight some strengths and weaknesses of the approach.

Site Frequency Spectrum-Based Methods

One class of tests for detecting deviations from neutrality is based on the site frequency spectrum (SFS). The SFS tabulates the number of mutations ξ_i that exist in a frequency of $x_i = i/n$ for $i = 1, 2, ..., n-1$, in a sample of size n (Nielsen, 2005), i.e., it summarizes the number of mutations segregating at particular frequencies in the sample. In a model of a single randomly mating population with constant population size N

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Table 1 Comparison of tests of neutrality

Test	*Number of loci*	*Type of sites/regions*	*Number of species*	*Sensitivity to demography*	*Most power to detect*	*Direct or indirect selection*
Tajima's *D*	1	Any genomic region	1	Yes	Recent positive selection at a single site	Indirect
Fay and Wu's *H*	1	Any genomic region	2	Yes	Recent positive selection at a single site	Indirect
HKA test	2	Neutral and selected region	2	Yes	Recurrent positive selection at multiple sites	Indirect
MK test	1	Synonymous and nonsynonymous sites	2	No	Recurrent positive selection at multiple sites	Direct
Haplotype-based tests	Genome-wide	Any genomic region	1	Yes	Recent positive selection	Indirect
Population differentiation	Genome-wide	Any genomic region	1; but multiple populations	Yes	Population-specific positive selection	Direct & indirect

(SNM), the expected value of ξ_i equals θ/i (Fu, 1995), where θ is the population scaled mutation rate defined as $4N\mu$. The classic neutrality tests quantify deviations of the empirical SFS from this expectation.

One of the earliest tests of neutrality, Tajima's D (Tajima, 1989), contrasts the proportion of segregating sites at low and high frequency with the proportion of sites at intermediate frequency. It does this by calculating the standardized difference between two different estimators of θ, Watterson's θ (θ_W) and nucleotide diversity (θ_π). θ_W is based on the number of segregating sites in the sample, while θ_π is the average number of nucleotide differences between pairs of sequences in a region. Under the SNM, these two estimates of θ should be equal to each other, making the expectation of Tajima's D approximately zero. In the case of negative selection acting directly on sites within the region, or a close recent selective sweep, there is an excess of rare alleles compared to the SNM. Rare alleles contribute as much as common variants do to increase the value of θ_W, but contribute less than common variants to increase the value of θ_π. As such, Tajima's D becomes negative. Based on coalescent simulations under a neutral model, a p-value can be computed. Tajima's D was, for example, used to scan for selective sweeps in genome-wide human data (Carlson *et al.*, 2005; Kelley *et al.*, 2006). It is sensitive to demographic histories that also lead to an increase of rare variants, like a recent population expansion (Tajima, 1993). Such events might therefore lead to rejection of the SNM, but not because of a selective sweep, but because of the nonconstant population size. An excess of common variants relative to rare variants will lead to positive Tajima's D values. This may be seen for balancing selection, but also for population structure with equal sampling from different subpopulations.

If outgroup information is available (e.g., chimpanzee in an analysis of human genetic data) it can be used to polarize polymorphisms to test for an excess of alleles that are new (derived) and at high frequency in the population (Fay and Wu, 2000; Fu, 1996; Fu and Li, 1993). For example, Fay and Wu's H contrasts θ_π with θ_H, an estimate of θ that is sensitive to high frequency derived alleles (Fay and Wu, 2000). Negative values of H indicate an excess of high frequency derived alleles in the tested region. Tests based on the derived allele frequency seem to be less sensitive to population size changes when testing for the signatures of a recent selective sweep. However, this sweep signal is relatively short-lived and still suffers from false positives from population subdivision (Przeworski, 2002).

The classical neutrality tests discussed above can be expressed as a linear combination of ξ_i (Achaz, 2009), where the weights represent the sensitivity of the test to deviations from neutrality at different frequency classes of the SFS. They are not optimized to perform well under a specific alternative hypothesis. More recent methods, like SweepFinder, compare the SFS in a region of the genome with that predicted under a model of a recent selective sweep versus. the genome-wide background SFS (Nielsen *et al.*, 2005). By explicitly comparing to an alternative model including natural selection and using the genome-wide background SFS, this procedure is thought to be more powerful and robust to demography than the neutrality tests discussed above. Explicit models of selection in a similar framework were also developed to test for local selective sweeps (Chen *et al.*, 2010) and balancing selection (DeGiorgio *et al.*, 2014).

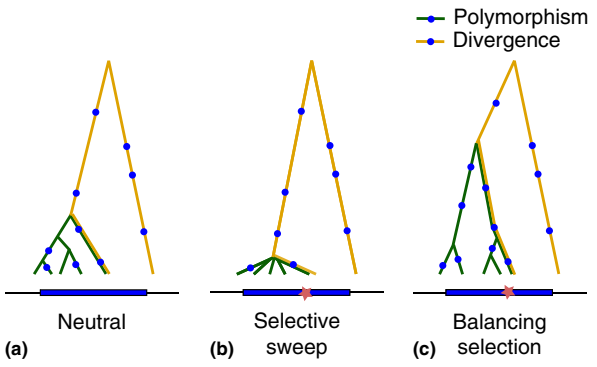
Faced with the problems of heterogeneous data and confounding demography, researchers have avoided specifying a model and instead take a pure outlier approach, assuming that most loci in the tails of the distribution are likely targets of selection (Teshima *et al.*, 2006). However, outlier selection scans do not provide an explicit test of a neutral model. Rather, they simply determine whether a particular region of the genome is unusual compared to the rest of the genome. Even with no selection, one region will always be the most extreme. The overall performance of these approaches will depend on

the demography and the proportion of the genome under positive selection (Kelley *et al.*, 2006; Teshima *et al.*, 2006; Thornton *et al.*, 2007).

HKA

The HKA test of Hudson *et al.* (1987) was the first test to utilize the positive association between polymorphism within a species and divergence between different species as a basis for a statistical test of neutrality. To apply the HKA test, one needs data from at least two different genomic regions in two (or more) species (Hudson *et al.*, 1987; Wright and Charlesworth, 2004). One of these regions needs to be neutrally evolving while the other may be subjected to natural selection. The HKA test compares the ratio of polymorphism to divergence of the neutral with the tested regions (**Figure 1**). Given an infinite sites model and assuming neutrality, it can be shown that the expected values for both polymorphism and divergence are a function of the time since the split, the effective population sizes of the two species, and the mutation rate for each region. As such, the ratio of polymorphism to divergence should, on average, be equal between all regions.

Although the mutation rate can vary between the regions, the ratio between diversity to divergence is unaffected by changes in the mutation rate. This is a consequence of the fact that both diversity and divergence scale linearly with mutation rate, and hence changes in mutation rate cancel out in both estimates. Any significant difference in the ratio between the tested and the neutral region indicates a violation of the assumption of neutrality in the tested region. Positive selection increases divergence relative to diversity (**Figure 1(b)**) while balancing selection (**Figure 1(c)**) and negative selection show the opposite pattern (Charlesworth and Charlesworth, 2010, p. 279).



	Neutral	Selective sweep	Balancing selection
Polymorphism	75	30	120
Divergence	105	105	105
χ^2		4.384	1.025
P-value		0.036	0.311

(d)

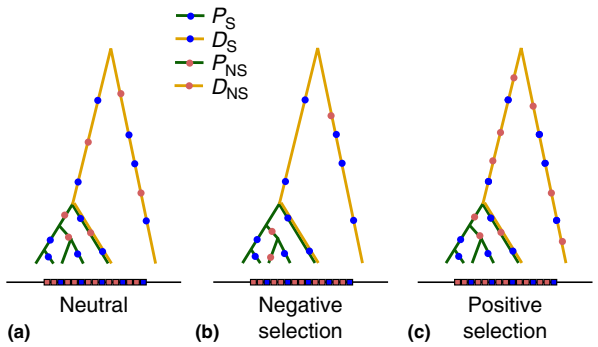
Figure 1 Example genealogies and data for the HKA test. χ^2 Statistic and *p*-value were calculated using HKAdirect (Esteve-Codina *et al.*, 2013).

The HKA test was shown to have high power for testing the null hypothesis of neutrality when multiple sites have been repeatedly targets of positive selection in the selected region. There is only little power for detecting weak purifying selection (Zhai *et al.*, 2009). Importantly, there is no power to detect strong purifying selection, because it affects both diversity and divergence proportionally, leading to a pattern equivalent to a lower mutation rate in the selected region. Inference about strong purifying selection can be made only when the neutral mutation rate for both regions is known.

Note that a significant HKA test only indicates a significantly larger or smaller diversity relative to divergence in one region compared to the other region. In the ideal case, one region is thought to be neutrally evolving, but often evidence of this is ambiguous. From the HKA test itself, there is no indication which of the two regions led to the deviation, and in fact, both regions might violate neutrality, but to a different degree or in different directions. For example, one region might be under the influence of balancing selection, leading to increased levels of diversity than expected, whereas the other region might be affected by a recent selective sweep, leading to a reduction of diversity. In such a case, there might be strong (and correct) support against the neutral model, but it is unclear if one or both regions are responsible for the deviation. Alternatively, it could be that both regions are affected by background selection to a similar degree, leading to a (misleading) non-significant HKA test. Note that a large portion of the genome can be under the influence of either direct or indirect selection. For example, about 40–70% of the noncoding DNA of *Drosophila melanogaster* are evolutionary constrained relative to synonymous sites (Andolfatto, 2005), and most, if not all, sites in the *Drosophila* genome are influenced by the indirect effects of deleterious mutations, i.e., background selection (Comeron, 2014). Finally, the HKA test has been extended to be applied to more than two genes (Wright and Charlesworth, 2004). In conclusion, although power of the HKA test is often superior to other methods (Zhai *et al.*, 2009), the necessity of defining a neutral region makes practical application of the HKA test challenging.

MK

The McDonald–Kreitman (MK) test (McDonald and Kreitman, 1991) is based on the same principle as the HKA test, except that instead of dividing the genome into neutral and nonneutral regions, it divides a region into interspersed neutral and selected sites, most commonly synonymous and nonsynonymous sites. Therefore, the MK test is often applied to protein-coding sequence (and we will discuss it in this context), but can also be used in situations where non-coding sites can be classified into interspersed neutral and putatively selected sites (Andolfatto, 2005). The basic premise is similar to that of the HKA test: Under neutrality, the synonymous ratio of diversity to divergence is expected to be the same as the nonsynonymous ratio (**Figure 2(a)**). Selection on nonsynonymous mutations generates a deviation from this proportionality that can be tested by any conventional test of independence (χ^2 test or Fisher's exact test, see Sokal and Rohlf, 2011). An excess of nonsynonymous divergence (i.e., a



	Neutral		Negative selection		Positive selection	
	S	NS	S	NS	S	NS
Polymorphism	75	45	75	30	75	45
Divergence	105	60	105	15	105	105
χ^2		0.0001		8.1		4.3
P-value		0.9936		0.0045		0.0376

(d)

Figure 2 Example genealogies and data for the MK test. χ^2 Statistic and *p*-value where calculated in *R* using 'chisq.test'. P_S = synonymous polymorphism, D_S = synonymous divergence, P_{NS} = nonsynonymous polymorphism, D_{NS} = nonsynonymous divergence. Red and blue squares represent nonsynonymous and synonymous sites of a gene, respectively.

too small diversity to divergence ratio at nonsynonymous sites compared to synonymous sites) is generally interpreted as evidence for positive directional selection (**Figure 2(c)**), whereas a deficit of nonsynonymous divergence is interpreted as evidence for weak purifying selection (**Figure 2(b)**). The MK test was found to be better at detecting negative selection than the HKA test (Zhai *et al.*, 2009). Again, very strong purifying selection does not lead to a significant MK test because it affects both diversity and divergence proportionally, leading to a pattern equivalent to a lower mutation rate at the nonsynonymous sites.

Comparison of the HKA and MK Tests

The crucial difference between the MK test and the HKA test is the spatial organization of neutral and selected sites. For the MK test, the two classes of sites are homogeneously interdigitated, whereas for the HKA test the two classes of sites are in separate regions with an arbitrary genetic distance in between. Although it seems like a trivial disparity, it has several important consequences. One has to do with the effect of negative selection at linked neutral sites. The classical model of the effect of negative selection on linked neutral sites, background selection, can be formulated as a local reduction in effective population size that is a function of the density of functional elements, selection coefficient, mutation rate, and recombination rate (Charlesworth *et al.*, 1993, 1995; Hudson and Kaplan, 1995, 1994; Nordborg *et al.*, 1996). Because background selection leads to a reduction in N_e at both synonymous and nonsynonymous sites, such indirect effects of selection do not affect the MK test. In contrast, the HKA test deviates from the nominal significance level when one of the regions is more strongly affected by background selection than the other regions (Williford and Comeron, 2010). A similar argument can be formulated for the effect of a recent selective sweep. Put differently, a significant MK test implies that the sites themselves are under selection, whereas a significant HKA test would also detect selection 'at a distance' (background selection, selective sweep), but does not necessarily provide information about the genes under selection (Nachman, 2006). Note that SFS-based methods discussed above fall into the same category as the HKA test by also being sensitive to indirect effects of selection.

A more subtle statistical issue relates to the theoretical distribution of the test statistic for sequences under recombination. The 2×2 test of independence of the MK test is based on the assumption of no recombination, so that the frequencies of different observations, i.e., the numbers in the contingency table, follow a multinomial distribution (Charlesworth and Charlesworth, 2010, p. 284). However, the MK test is relatively robust to recombination in the tested coding region, because synonymous and nonsynonymous sites share the same (local) genealogical history (Andolfatto, 2008). For the HKA test, on the other hand, neutral and selected sites do not share the same genealogy when the two regions are separated by recombination, or when there is recombination within the regions. This results in shorter or longer gene genealogies in one region over the other, and therefore more or less diversity due to chance. It adds another source of variance to the HKA test compared to the mutational variance accounted for in the MK test: the evolutionary variance of the gene genealogies. The MK test can be performed with any conventional test of independence (χ^2 test or Fisher's exact test, see Sokal and Rohlf, 2011), whereas the HKA test has to incorporate the evolutionary variance into the null distribution of its goodness-of-fit statistic. To do this, the HKA test assumes no recombination within the regions and no linkage between the regions, which are the most conservative assumptions one can make regarding linkage (Hudson *et al.*, 1987). However, to account for evolutionary variance, the HKA test makes three strong demographic assumptions: (1) constant population size, (2) random mating, and (3) ancestral population size equal to current population size (Hudson *et al.*, 1987).

In summary, the MK test is robust to any demographic assumption but cannot detect indirect effects of selection, like selective sweeps or background selection. The HKA test has power to detect indirect effects of selection and is more powerful to detect positive selection, but violation of the demographic assumptions, like population structure or bottlenecks, lead to an increased level of false positives (Andolfatto, 2008; Ford, 1998; Ingvarsson, 2004; Innan, 2006; Zhai *et al.*, 2009).

Other Tests of the Neutral Model

Haplotype-Based Tests

Another type of test uses information from haplotype patterns. The length of a haplotype is indicative about its age. Younger alleles tend to sit on longer haplotypes because they have not been disrupted by recombination. It is thought that positive

selection will bring young alleles to unusually high frequency (Hudson *et al.*, 1994). This idea of searching the genome for unusually long and common haplotypes as a test of selection has been implemented in the extended haplotype homozygosity (EHH; Sabeti *et al.*, 2002), cross population extended haplotype homozygosity (XP-EHH; Sabeti *et al.*, 2007), and integrated haplotype score (iHS; Voight *et al.*, 2006) tests and has been widely applied. Those methods are designed to discover incomplete (partial) sweeps, where the sweep haplotype is not fixed, but has reached a frequency $>10\%$. It has been argued that mutations that appeared after the human out-of-Africa migration are unlikely to have reached fixation today, even in case of a strong selective advantage (Coop *et al.*, 2009; Vitti *et al.*, 2013). In such a context, haplotype-based tests might provide useful information about adaptations to new environmental challenges. Recently, tests have been developed to detect soft sweeps, i.e., a pattern caused by multiple haplotypes increasing in frequency that can be the result of adaptation from standing genetic variation (Ferrer-Admetlla *et al.*, 2014; Garud *et al.*, 2015). Currently, rather little is known about performance of haplotype-based tests and their robustness to different demographic assumptions (but see Crisci *et al.*, 2013; Ferrer-Admetlla *et al.*, 2014).

Population Differentiation

A final category of test of the neutral model is based on population differentiation. It is predicted that population-specific positive selection will yield single nucleotide polymorphisms (SNPs) that show large differences in allele frequency across populations. A popular approach involves examining the distribution of F_{ST}, a summary of genetic variation, across the genome (Akey *et al.*, 2002; Oleksyk *et al.*, 2008). SNPs showing high values of F_{ST} may be candidates for positive selection (Beaumont and Balding, 2004; Foll and Gaggiotti, 2008; Guo *et al.*, 2009). A difficulty is that this outlier approach is not a formal test of neutrality, as there will always be outlier variants, even under neutrality. Thus, a more rigorous approach involves testing whether the differentiation at a candidate SNP is unusual under a null model of demography. The classical test is based on an infinite island model with symmetric migration and equal population sizes (Lewontin and Krakauer, 1973), and was shown to be quite robust against models of recent divergence and growth, or two-dimensional stepping stone models (Beaumont and Nichols, 1996). Importantly, the test is not robust against models with hierarchical population structure, i.e., when demes are grouped and exchange more migrants within groups than between groups (Excoffier *et al.*, 2009; Hermisson, 2009). These problems can arise due to historical population branching, or due to barriers to gene flow that isolate populations in different parts of a species range. Recent methods have extended the Lewontin–Krakauer test to account for hierarchical population structure (Bonhomme *et al.*, 2010; Excoffier *et al.*, 2009; Fariello *et al.*, 2013). Scanning the genome for sites with unusual population differentiation has yielded important candidate genes for adaptation in humans related to altitude (Bigham *et al.*, 2010; Simonson *et al.*, 2010), disease resistance (Barreiro *et al.*, 2008; Cagliani *et al.*, 2011; Fumagalli *et al.*, 2011, 2010), or climate (Lao *et al.*, 2007; Rees and Harding, 2012).

See also: Adaptive Molecular Evolution: Detection Methods. Directional Selection and Adaptation. Natural Selection, Introduction to. Neutral Models of Genetic Drift and Mutation. Selective Sweeps

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Neutral Models of Genetic Drift and Mutation

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Glossary

Allele Specific molecular variant at a locus.
Demography History of population size changes over time.
Diffusion process Continuous-time stochastic process describing the random movements of particles.
Haplotype Combination of individual molecular markers that tend to be inherited together.
Locus Position in the genome where an allele resides.
Markov process Stochastic process in which the transition rates depend only on the previous state.
Null model Baseline model used to test whether additional processes need to be considered.
Ploidy Number of sets of chromosomes present in a cell (one set: haploid; two sets: diploid).
Poisson process Continuous-time stochastic process for modeling events that occur at a specified rate.
Segregating site Genomic position at which more than one allele is present in a population sample.
Substitution Mutation that has become fixed and is now present in every individual in the population.

Random Genetic Drift

In a finite population, the number of individuals that carry a specific allele may change over time merely due to stochastic variation in the reproductive success among individuals. This effect is termed random genetic drift. For studying genetic drift in statistically meaningful ways, we rely on idealized models that capture key biological aspects of the process while remaining mathematically tractable. The most commonly used model is named after two of the founders of population genetics, Ronald A. Fisher (Fisher, 1930) and Sewall Wright (Wright, 1931).

The Wright–Fisher Model

The Wright–Fisher (WF) model describes a population with discrete, nonoverlapping generations. In each generation the entire population is replaced by the offspring from the previous generation. Parents are chosen via random sampling with replacement. In a haploid population of constant size N, the probability that an allele present in i individuals will be present in j individuals in the next generation is then given by the binomial sampling probability

$$P_{ij} = \binom{N}{j} (i/N)^j (1 - i/N)^{N-j} \quad 0 \le i,j \le N \qquad [1]$$

The transition probabilities P_{ij} define a discrete-time Markov process on the state space of allele frequencies $x(t) = i(t)/N$. Expected allele frequencies remain constant across generations, whereas the variance per generation is $\mathrm{Var}[x] = x(1-x)/N$. The probability that an allele eventually becomes fixed is simply its initial frequency. In particular, the fixation probability of a new mutation present in a single copy is $1/N$. It is straightforward to extend the WF model to diploid systems by exchanging $N \to 2N$, as well as to nonconstant population sizes by taking larger or smaller samples in each generation.

Several alternatives to the WF model have been proposed for describing random genetic drift, notably the Moran and the Cannings models (Ewens, 2004). The Cannings model is a generalization of the WF model that can incorporate arbitrary variance in allele frequency between generations, which can span a wide range in biological populations. The Moran model is a continuous-time model with overlapping generations. Additional levels of biological complexity that one may wish to incorporate include different sexes, age structure, demography, and population substructure. The study of drift in such models is often facilitated by the concept of an effective population size, which specifies the population size of an idealized WF model that approximates evolutionary patterns in the real scenario (Charlesworth, 2009).

Loss of Diversity in the WF Model

We can easily quantify the rate at which genetic diversity is lost in the WF model. Consider an allele present at frequency $x(t)$ in generation t. When drawing two random individuals from the population, the probability that only one of the individuals carries the allele is $H(t) = x(t)[1 - x(t)]$. This probability is called heterozygosity and provides a useful measure for the level of diversity in a population. In generation $t+1$, two possibilities need to be distinguished: We might pick two individuals that have different parents in generation t, in which case the probability that only one will carry the allele, by definition, is $H(t)$. However, since parents are drawn with replacement, we can also pick two individuals that happen to have the same parent in generation t. In this case, the two individuals will either both have the allele or neither will have it. The probability of picking two individuals that originate from the same parent is $1/N$ in a haploid population of size N, and so the probability that they have different parents is $1 - 1/N$. This yields the recursion $H(t+1) = 1/N \times 0 + (1 - 1/N) \times H(t)$. Defining $H_0 = H(t=0)$, we obtain

$$H(t) = \left(1 - \frac{1}{N}\right)^t H_0 \approx \mathrm{e}^{-t/N} H_0 \qquad [2]$$

Genetic diversity is thus lost at an exponential rate in the WF model. For example, after approximately $N\log(2)$ generations, half of the initial variation will be lost.

The Diffusion Approximation

The WF model specifies the drift process by a discrete-time Markov process over a finite state space, defined by the transition probabilities $P[x(t+1)|x(t)]$ between generations. In principle, we can calculate transition probabilities over longer time intervals through iterative summation over all possible intermediate states. However, these calculations have proven extremely difficult in practice.

An elegant solution to this problem was provided by Motoo Kimura in the 1950s. Kimura showed that in large populations the discrete WF process can be closely approximated by a continuous time, continuous space diffusion process (Kimura, 1955). Specifically, the time evolution of the probability density $\phi(x,t)$ that an allele is at frequency x at time t can be modeled by the partial differential equation

$$\frac{\partial}{\partial t}\phi(x,t) = \frac{1}{2}\frac{\partial^2}{\partial x^2}[b(x)\phi(x,t)] \quad [3]$$

where $b(x) = \mathrm{Var}[dx]/dt$. In a haploid WF population of size N, we have $b(x) = x(1-x)/N$. Equation [3] is also known as the Kolmogorov forward equation and together with its close analogue, the Kolmogorov backward equation, it enabled the derivation of analytical expressions for various evolutionary quantities. For example, the expected time that an allele initially present at frequency x will remain polymorphic in the population until it either becomes fixed or lost is $-2N[x\log(x) + (1-x)\log(1-x)]$ generations, which is the same as the average time that an allele present at frequency x has already spent in the population if it originally arose in a single copy (Kimura and Ohta, 1973). For those alleles at frequency x that do eventually fix, the average time to fixation is $2N[(1-x)\log(1-x)]/x$; for those that become lost, the average time to loss is $2Nx\log(x)/(1-x)$ (Kimura and Ohta, 1973).

Kimura's solution to the diffusion approximation provides the full distribution of transition probabilities over arbitrary time intervals, which allows for the calculation of a wide range of summary statistics such as allele-frequency distributions, fixation probabilities, and sojourn times. The profound impact of the diffusion approximation on modern population genetics has been greatly facilitated by the fact that it also offers a straightforward approach for incorporating selection into its calculations (Kimura, 1964).

Mutation Models

In natural populations, mutational processes constantly generate new genetic variants upon which drift can act. As with drift, we again rely on idealized models to study the consequences of such mutational processes. Three basic models are widely used:

K-Alleles Model

The K-alleles model assumes that there exist only a finite number of possible alleles $\{A_1,\ldots,A_k\}$ at a locus – for instance the four different nucleotide states A,C,G,T. Mutations convert allele A_i into allele A_j at rate μ_{ij}. This defines a rate-matrix, $\mathbf{U} = \{\mu_{ij}\}$, with diagonal elements given by $\mu_{ii} = 1 - \sum_{i\neq j}\mu_{ij}$. We can incorporate this model into the WF framework by combining allele frequencies into a vector $x(t) = (x_1,\ldots,x_k)$. After each generation, allele frequencies then simply need to be adjusted according to $x'(t) = x(t)\mathbf{U}$. If we were to neglect drift, this process will converge toward an equilibrium distribution of allele frequencies specified by the condition $x(t) = x(t)\mathbf{U}$. For example, in a model with only two alleles, these equilibrium frequencies will be given by $\bar{x}_1 = \mu_{21}/(\mu_{12}+\mu_{21})$ and $\bar{x}_2 = \mu_{12}/(\mu_{12}+\mu_{21})$.

Infinite Alleles Model

In contrast to the K-alleles model, the infinite alleles model assumes that there is no limit to the number of possible alleles at a locus and that each mutation produces a new allele that has not previously existed in the population. We can describe this model in terms of a Markov process for allelic states with an infinite state space: $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow \ldots$. Mutations are assumed to occur at a uniform rate in this model. The infinite alleles model is commonly used for describing haplotypes in a sufficiently large genomic region, if one can assume that every mutation or recombination event creates a novel haplotype.

Infinite Sites Model

The infinite sites model is an extension of the infinite alleles model that preserves information on the mutational history of each allele. We can construct this model from the infinite alleles model by assuming that our locus contains an infinite number of individual sites. Each mutation event produces a point mutation at a new site. This allows for the definition of an evolutionary distance between two alleles in terms of the number of point mutations by which they differ. The infinite sites model is often used for describing genomic regions in which the rate of point mutation per site is very low, such that double hits and back mutations can be ignored.

Patterns of Neutral Genetic Variation Under Mutation and Drift

In a WF population, drift systematically removes diversity from the population. This loss is counterbalanced in natural populations by the continuous generation of new mutations. The study of the interplay between these two processes has led to important insights into how genetic diversity can be maintained within populations even in the absence of selective forces.

The Coalescent Process

When studying patterns of diversity in real systems it is typically unrealistic to obtain information for every individual in

the population. Instead, observations will often rely on samples taken from the population. One of the most fruitful insights in modern population genetics was the realization that for understanding evolutionary patterns it often suffices to focus on the genealogical history of such population samples, rather than the population as a whole.

The mathematical framework that captures this approach, coalescent theory, was developed in the 1980s by Kingman (1982) and others. It has since come to be the workhorse of population genetics, especially for scenarios in which selective forces can be ignored. Due to its focus on population samples, results from coalescent theory are often directly relatable to empirical observations and tend to provide a very intuitive understanding for the interplay between patterns and processes. In addition, the coalescent process offers a very efficient framework for numerical simulations (Hudson, 2002).

Before we address how mutations can be incorporated into the coalescent framework, let us quickly review some key properties of the standard coalescent in the absence of mutation. The coalescent is a continuous-time Markov process for modeling the genealogy of a population sample under random genetic drift. Note that the coalescent is not a framework for inferring the actual genealogy of a given population sample, which may be very difficult in practice. Rather, it provides a stochastic model that allows us to study the patterns we would expect to observe in such a sample under a given evolutionary scenario. The key event of the coalescent process is that two lineages in the sample can coalesce when going backward in time because they share a common ancestor, reducing the number of remaining lineages by one. Consider a haploid WF population of size N, composed of genetically identical individuals. In this population, two lineages coalesce in the previous generation with probability $1/N$. In the continuous-time coalescent framework, we describe this event by a Poisson process with instantaneous coalescence rate dt/N. The expected time until a given pair of lineages coalesces is then $E[t_c]=N$ generations, and the probability that two lineages have not yet coalesced after t generations is $P[t_c>t]=(1-1/N)^t\approx e^{-t/N}$.

In samples with more than two individuals, the coalescent process is slightly more complicated, as coalescence can occur between any possible pair of lineages in the genealogy. In a sample of size n, there are initially $n(n-1)/2$ possible pairs. The average time until the first two lineages coalesce is thus $2N/[n(n-1)]$ generations. Each coalescence event reduces the number of remaining lineages by one until all lineages in the sample have reached a single common ancestor. The expected time to reach this most recent common ancestor is $2N(1-1/n)$ generations and the expected total length of all individual branches in the coalescent tree is $E[L_n]=2N\sum_{i=1}^{n-1}(1/i)$ generations. For a more comprehensive review of coalescent theory, we encourage the reader to consult Wakeley (2008).

Adding Mutations to the Coalescent

Neutral mutations do not interfere with the genealogy of a population sample because they have no effect on the reproductive success of the individuals that carry them. In a neutral scenario, we can therefore treat coalescence and mutation by two independent Poisson processes. In particular, we can generate the genealogy first and then add mutations to the given genealogy afterwards ('mutation-dropping'). Mutations that occur in individuals outside of the genealogy can be ignored, as those mutations will not be observed in the sample. Along the branches of the coalescent tree, mutations are dropped at the same rate at which they occur in individuals and this can be done using any of the mutation models specified above. Figure 1 illustrates how this approach relates to a WF model with neutral mutations and how the resulting patterns will differ between the infinite sites and infinite alleles models.

We can immediately derive the expected level of heterozygosity in this framework. The probability that two individuals differ by at least one mutation is simply the probability that a mutation has occurred along their genealogy prior to coalescence. Mutation and coalescence are independent Poisson processes in the model, coalescence occurring at rate $1/N$ and mutation occurring at rate 2μ (the factor 2 arises

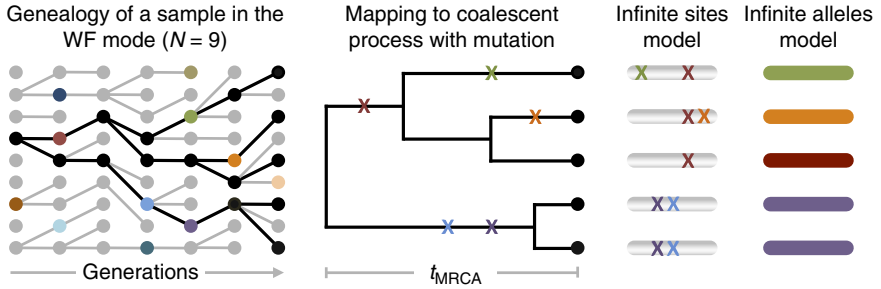

Figure 1 Correspondence between the WF model and the coalescence process. The left panel shows the genealogical relationships over six generations in a WF population with nine individuals. The black lines depict the genealogy of a sample of five individuals back to their most recent common ancestor. Colored circles indicate the occurrence of new mutations. The coalescent tree corresponding to this sample genealogy is shown in the middle panel. Note that in the coalescent we need to include only those mutations that occurred in individuals that are part of the sample genealogy. The right panel shows how patterns differ between the infinite sites and infinite alleles models. In the infinite sites model, every mutation occurs at a new site and each individual still carries all mutations that have occurred previously in its lineage. In the infinite alleles model, on the other hand, every mutation produces a new allele and the state of an individual is solely specified by the most recent mutation in its lineage.

because mutation can occur in either of the two lineages). The probability that a mutation occurs prior to coalescence is then

$$H = \frac{2\mu}{2\mu + 1/N} = \frac{\Theta}{1+\Theta} \quad [4]$$

with $\Theta = 2N\mu$. If $\Theta \ll 1$, we have $H \approx \Theta$, and the expected level of heterozygosity is thus simply twice the product of the population size and the mutation rate. Note that our derivation of eqn [4] applies to both the infinite sites and infinite alleles models. In contrast to a WF model without mutation, where heterozygosity decays exponentially, the addition of mutation therefore leads to the maintenance of an equilibrium level of heterozygosity that is proportional to the product of population size and mutation rate.

Site-Frequency Spectrum in an Infinite Sites Model

In the mutation-dropping approach we place mutations along all branches of the coalescent tree by a Poisson process with rate μ. The expected total number of mutations on the tree is then simply the product of the mutation rate and the expected total length of all tree branches (L_n). For a sample of size n, this yields

$$E[S] = \mu E[L_n] = \Theta \sum_{i=1}^{n-1} \frac{1}{i} \quad [5]$$

Under an infinite sites model, each of these mutations will have created a new polymorphic site in the sample. $E[S]$ therefore specifies the expected overall number of segregating sites in a sample of size n. Specifically, in a sample of size two, we expect to find $E[S] = \Theta$ segregating sites. For a derivation of the full probability distributions, $P[S = k]$, see Wakeley (2008).

In addition to the overall number of segregating sites in the sample, one might be specifically interested in the number of mutations, s_i, that are present in exactly i individuals in the sample. The vector $(s_1, s_2, \ldots, s_n)$ is called the site-frequency spectrum of the sample. In the infinite sites model, the expected value of s_i in a sample of size n is given by

$$E[s_i] = \frac{\Theta}{i}, \quad i = 1, \ldots, n \quad [6]$$

This famous equation has been derived in a number of ways, including the WF model, diffusion theory, and the coalescent framework, see, for example, Ewens (2004) for a collection of proofs.

Site-frequency spectra can be directly measured from population samples, making them a widely used statistic for evolutionary inferences. Importantly, we know that a number of processes, including demography and selection, are expected to produce characteristic deviations in the shapes of observed spectra from the theoretical prediction of eqn [6]. Comparison of empirical spectra with theoretical predictions has therefore become a popular approach for inferring the presence of such processes.

Ewen's Sampling Formula for the Infinite Alleles Model

Under the infinite alleles model every mutation event creates a new allele that maintains no information on its mutational history. In a sense, this makes the coalescent process simpler, as large parts of the genealogy will have no influence on the allele-patterns observed in a sample. Each lineage needs to be followed only until the most recent mutation, whereas mutations and coalescence events further back in time can be ignored. The mathematical process for describing this scenario is the so-called coalescent with 'killings,' in which coalescence and mutation are modeled simultaneously and each lineage is terminated once a mutation is encountered. The process stops when only a single lineage remains.

Since there is no information in the comparison of two alleles other than whether they are different or the same, the primary quantities of interest in this model are how many different alleles there are in the sample, and how many of those alleles are present in 1,2,… individuals, the counts of which we denote by a vector $(a_1, a_2, \ldots)$. The probability of observing a given set of counts $(a_1, a_2, \ldots, a_n)$ in a random sample of size n, drawn from a constant-sized population evolving under neutral mutation and drift, is (Ewens, 1972)

$$P[(a_1, a_2, \ldots a_n)] = \frac{n!}{\Theta_{(n)}} \prod_{j=1}^{n} \frac{(\Theta/j)^{a_j}}{a_j!} \quad [7]$$

where $\Theta_{(n)} = \Theta(\Theta+1)\ldots(\Theta+n-1)$. Note that the number of distinct alleles in the sample is simply $k = \sum_{i=1}^{n} a_i$. Equation [7] is known as Ewen's sampling formula. As already noted above, the infinite alleles model is frequently used for describing the expected haplotype frequencies at a neutral locus evolving under mutation, recombination, and drift.

The Neutral Theory of Molecular Evolution

Neutral alleles present in a single copy in a haploid WF population of size N eventually become fixed with probability $1/N$, assuming that mutational processes are not affecting allele frequencies. This important result holds true more generally for models in which all individuals in the population have equal, nonzero probability of ultimately becoming the common ancestor to all individuals present at a future point. In the infinite sites model with neutral mutations arising at rate μ per individual, the overall rate at which substitutions accumulate in the population is therefore

$$u = N\mu \times \frac{1}{N} = \mu \quad [8]$$

Hence, the substitution rate simply equals the mutation rate per individual and is independent of population size. Figure 2 provides an illustration for how neutral mutations in an infinite sites model can occasionally drift to fixation in the population.

There has been a long-standing debate about whether random genetic drift and neutral mutation suffice to describe the majority of patterns of molecular variation observed in real biological systems. While selectionists argue that natural selection plays an important role in driving molecular divergence between species, as well as maintaining genetic diversity within populations, the neutral theory of molecular evolution posits that the vast majority of observed genetic variation is in fact selectively neutral and that molecular divergence between species is primarily the result of drift (Kimura, 1983). One key prediction of neutral theory is that

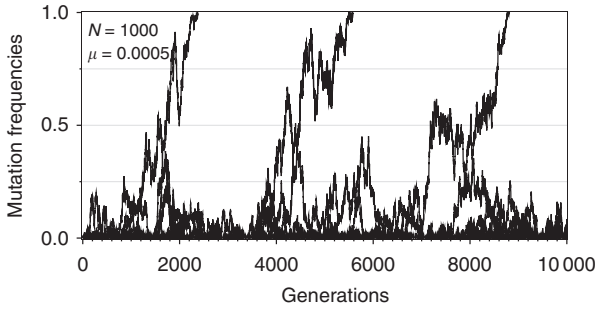

Figure 2 Trajectories of mutation frequencies in a simulated WF population of size $N=10^3$, evolving under an infinite sites model with mutation rate $\mu=0.0005$. A large number of mutations can be seen to arise over the course of the simulation, but the vast majority are quickly lost due to drift. Some mutations, however, succeed in drifting to higher frequencies and three of those mutations actually become fixed in the population, well within range of the $\mu \times 10^4=5$ substitutions we expect to observe over the duration of 10^4 generations.

substitution rates should then be approximately constant over time, and that we can therefore use the observed numbers of substitutions between species as a 'molecular clock' for dating phylogenetic events (Zuckerkandl, 1987).

The neutral theory of molecular evolution has been tremendously influential in helping us understand how stochastic processes can contribute to evolutionary dynamics. However, with the advent of population genomic data, it has become increasingly clear that natural selection does often play an important role in molecular evolution. Moreover, it is no longer clear whether the second pillar of neutral theory, random genetic drift, is always the dominant stochastic process, as it could be outrivaled by other stochastic processes such as genetic draft, which describes the fluctuations in allele frequency due to linkage with nearby selected mutations (Gillespie, 2000). Nevertheless, neutral theory has firmly established itself as the standard null model of population genetics and will likely continue to play an important role as the baseline scenario for the development of improved evolutionary theories (Kreitman, 1996).

See also: Adaptive Molecular Evolution: Detection Methods. Mutation and Genome Evolution. Neutral Evolution, Population Genetic Tests of

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Noncoding DNA Evolution: Junk DNA Revisited

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Nomenclature

bp base pair
CRE *cis*-regulatory element
eve S2E even-skipped stripe 2 enhancer
ncDNA noncoding DNA

Glossary

***Cis*-regulatory elements (CREs)** CREs are regions of noncoding DNA which regulate the transcription of nearby genes.
Enhancer The smallest fragment of DNA that, when linked to a reporter gene and transferred into an appropriate cell, executes a regulatory function in a fashion consistent with that of the native gene in its proper context.
Junk DNA Initial definition for nongenic DNA that had no known biological function.
Noncoding DNA (ncDNA) ncDNA sequences are components of an organism's DNA that do not encode protein sequences.
Position weight matrices (PWM) Quantitative representations of DNA-binding specificity for a transcription factor protein.
Transcription factor Sometimes called a sequence-specific DNA-binding factor, transcription factor is a protein that binds to specific DNA sequences, thereby controlling the rate of transcription.
Transcription factor binding sites Short DNA sequences (typically 4–30 base pairs long) that are the targets for binding by transcription factors.

Introduction

Numbers of proteins-coding genes do not change across the metazoan as a whole, and certainly not in relation to large differences in organismal complexity. The lack of correspondence between genome size (amount of DNA), morphological complexity, and coding proteins gene number is a prominent topic of discussion that started more then 40 years ago. Eukaryotic genomes mostly consist of DNA that is not translated into protein sequence (Alexander *et al.*, 2010). However, noncoding DNA (ncDNA) has been little studied relative to proteins. The lack of knowledge about its functional significance has led to hypotheses that much nongenic DNA is useless 'junk' (Ohno, 1972) or that it exists only to replicate itself (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). A large fraction of many eukaryotic genomes consists of middle-repetitive DNAs and transposable elements that may constitute 'selfish' DNA. Variety and patterns of their interspersion with coding DNA make no phenotypically functional sense. Perhaps, this excess of DNA that does not affect the organismal fitness is carried passively from generation to generation, because of its physical linkage to functional genes. Indeed, there are indications that removal of certain ncDNA can be done without deleterious effects on phenotype.

The ncDNA, we now know, performs many essential functions, especially in the regulation of gene expression, but it is unclear yet how much of it is necessary. Functional components of ncDNA regulatory sequences are called *cis*-regulatory elements (CREs), including enhancers, core promoters, matrix or scaffold attachment regions, insulators, and silencers (Levine and Tjian, 2003). These regulatory sequences are distributed among selfish transposons and middle or short repetitive DNAs. The genome is an extremely complex machine; functionally as well as structurally it is generally not possible to disentangle the regulatory function from the junk selfish activity. The idea of junk DNA needs to be revised.

Mutations in ncDNA that affect regulation of gene expression can be a major source of evolutionary change (Carroll *et al.*, 2001). To understand the mechanisms by which CREs contribute to evolution, several fundamental questions need to be addressed, such as how conservative are the function and the sequence of CREs on an evolutionary time scale, and how CREs evolve a new function.

Regulation of gene expression involves an interaction between transcription factors and CREs. Eukaryotic genes contain highly structured regulatory DNAs that direct complex patterns of expression in many different cell types during development. Classes of regulatory elements to which the transcription factors bind include core promoters, enhancers, silencers, and insulators (**Figure 1**).

This article highlights evolutionary forces that can shape structure/function of CREs as a portion of ncDNA in eukaryotes. The major emphasis will be to characterize the evolution of enhancers.

CRE Structure/Function

Unfortunately, there is no general definition of a CRE. The best working definition follows one given by Arnone and

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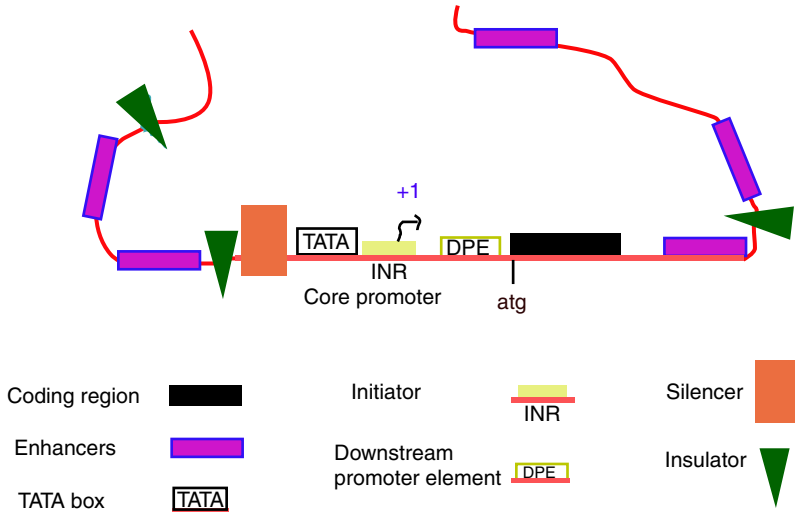

Figure 1 CREs in a eukaryotic transcription unit. TATA, TATA box; INR, initiator sequence; DPE, downstream core promoter element.

Davidson (1997) to enhancer: a *cis*-element such as an enhancer is defined as the smallest fragment of DNA that, when linked to a reporter gene and transferred into an appropriate cell, executes a regulatory function in a fashion consistent with that of the native gene in its proper context. This definition evidently simplifies natural relations between CRE structure and its function by paring down *cis*-neighboring sequences. It is not possible to define precise borders (up to one nucleotide) for any class of CREs. This fact evidently creates serious problems for evolutionary analyses.

Enhancers are perhaps the best understood type of CRE. The first eukaryotic enhancer was initially identified and characterized in mammalian virus called SV40 (Banerji *et al.*, 1981). A virus noncoding sequence of 72 bp could turn on expression in a manner relatively independent of its position and orientation with respect to a nearby rabbit β-globin reporter gene. Enhancers are typically 100–1000 bp long, and contain multiple short binding site sequences (6–12 bases in length) for targeting several different activators and repressors of transcription. Binding site motifs for a given transcription factor are often degenerate, and are better characterized as a probability (position weight matrix) than as a consensus sequence. Bound transcription factors can interact in many different contexts, and they can function as either activator or repressor of transcription. The spacing of binding sites modulates transcription factor interactions; mechanisms of interaction include direct competition for overlapping binding sites, quenching over short distances (<150 bp), and long-range (>150 bp) interactions (Ondek *et al.*, 1988; Arnosti *et al.*, 1996; Gray and Levine, 1996; Barolo and Levine, 1997).

A typical animal gene can have many enhancers as well as other CREs in order to ensure appropriate regulation in response to different temporal or spatial (surrounding cellular environment) cues (**Figure 2**).

A key property of enhancers is that they can act independently of each another. This independence is perhaps achieved by their physical separation along the ncDNA. Insulators are specific sequences that also contribute to specificity of gene regulation by restricting the action of one enhancer from activity of another enhancer. An enhancer can be located anywhere within a span of 100 kb upstream or downstream of a gene whose expression it regulates, including introns.

CREs and Length Variation of ncDNA

Insertions and deletions (collectively indels) obviously have impact on distance between interacting CREs as well on total length of ncDNA. There are several known mechanisms that generate indels: genome duplication, complete and partial chromosomal duplication, proliferation of transposable elements, replication errors, and unequal crossover. All of these diverse mutational mechanisms of indel production contribute to single locus as well as total ncDNA size variation in the genome.

It appears that the parameters of indel evolution such as mutation rate, insertions to deletions ratio, and mean indel size differ between species. Nevertheless, some general rules exist: deletion mutations are always more frequent than insertion mutations, the genomic prevalence of indels declines with length, and this decline is faster for insertions than for deletions (Lynch, 2007).

Small length changes, generally involving tandem repeats ranging in size from one to eight bases, are a common form of mutation in the noncoding regions. There is, for example, abundant length variation in the *Drosophila even-skipped* stripe 2 enhancer (S2E), both polymorphic and fixed differences between closely related species. The abundance of length differences in the S2E is surprising since spacing of transcription factors along the enhancer should be important for its function (Ludwig and Kreitman, 1995).

Analysis of short indel polymorphism and divergence in different compartments of the *D. melanogaster* genome (exons, introns of different lengths, and intergenic regions) in whole-genome dataset on 158 genotypes demonstrates the excess of short deletion over short insertion mutations. However, polymorphism and divergence data show that this deletion bias is almost completely compensated by selection: negative

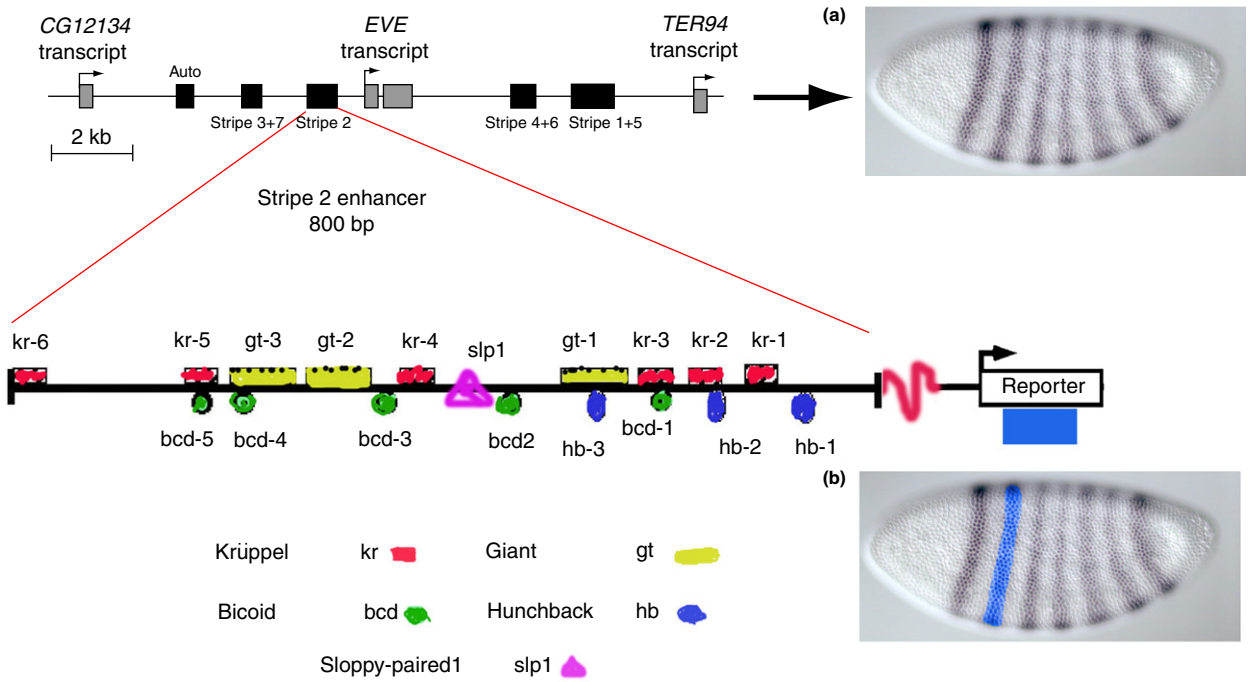

Figure 2 The *melanogaster even-skipped* locus of 16.4 kb is neighboring by two loci: *CG12134* and *TER94*. (a) The CREs-enhancers (black rectangles) regulate the *eve* seven stripes expression pattern at early embryogenesis stages. The *eve-skipped* protein; Stage 5. (b) Detailed view of the eve stripe 2 enhancer that contains many transcription factors binding sites (shown only experimentally validated binding sites for five transcription factors). This ~800 bp DNA fragment drives expression of reporter gene in stripe 2 (shown in blue).

selection is stronger against deletions, whereas insertions are more likely to be favored by positive selection. This implies a deletion bias, which needs to be compensated by opposing forces to prevent the genome from unrestrained contraction (Petrov, 2002; Leushkin *et al.*, 2013).

Preservation by Functional Constraint

Comparative genomics studies of ncDNA sequences show conserved regions interspersed among rapidly diverged segments, and conservation can be evident even after 300–450 million years of evolution (Muller *et al.*, 2002). Both the density and the block-lengths of highly conserved regions decrease as evolutionary distances increase. Interpretation of the punctate pattern of conservation in ncDNA has been guided by rules of molecular evolution first elucidated by Kimura (1983): "Functionally less important molecules or parts of molecules evolve (in terms of mutant substitutions) faster than more important ones." In other words, sequence-specific conservation of ncDNA implies functional constraint on these sequences and slower rates of molecular evolution.

Nearly 99% of the human genome does not encode proteins. A further 7% of the DNA has a functional regulatory gene expression role according to comparative genomics analysis of human genome with the pan-mammalian conserved sequence, ranging from dogs and rats to pandas and horses (Rands *et al.*, 2014). Thus, just to follow only comparative methods, there is no evidence for functional conservation of sequences for 92% of the human genome.

However, there are some serious caveats to the interpretation of quantitative comparative genomic data. First, conservative components of ncDNA in comparative genomics studies contain many different non-related classes of sequences, including *cis*-regulatory sequences, short and long functional noncoding RNAs, and cryptic genes. Additionally, comparative genomics has found noncoding elements that are conserved to varying degrees across mammalian or vertebrate genomes, which suggests some function conserved by natural selection. Lack of experimentally validated function for some ultra conserved elements, that can be >100 base pairs long and 100% identical across human, mouse, and rat genomes, shows that the extent of sequence conservation is not a good predictor of the functional importance of a sequence. Furthermore, the values obtained are also dependent on alignment methods and can vary substantially between different regions of a genome.

Crude comparative genomics analysis numbers alone provide little insight into evolutionary mechanisms acting in the noncoding portion of genome. Andolfatto (2005) has overcome the limitations described above by combining comparative genomic analysis with population-level variability data. To assess the mode of selection acting on ncDNA, he has analyzed polymorphism data for gene coding fragments and noncoding fragments scattered across the X chromosome of *D. melanogaster*. To estimate levels of between-species divergence, he compared *D. melanogaster* with its closely related sibling species, *D. simulans*. His analysis demonstrated that a large fraction of the non-translated genome is functionally important and subject to both purifying selection and

adaptive evolution. These results imply that, although positive selection is clearly an important facet of protein evolution, adaptive changes to ncDNA might have been considerably more common in the evolution of *D. melanogaster*.

Preservation of Function and Evolutionary Changes in CREs

Many functionally important noncoding sequences in genomes are not evolutionary conserved. Some of these have lineage-specific functions and others are simply missing by current comparative genomics methods. There are many examples of extensively diverged regulatory sequences that have retained expression specificity.

What can we say about the evolutionary processes governing the changes in these functionally conserved elements?

From experimental studies of evolutionary changes in functionally conserved regulatory elements in different species, the following observations can be gleaned: there is a lack of complete conservation of functional binding sites; there is rapid turnover of spacer sequences between binding sites; conservation is apparent; turnover of binding site architecture occurs; and there is co-evolution of sites within an enhancer.

The S2E from segmentation gene *even-skipped* in *Drosophila* illustrates these points. This *cis*-element drives expression of the gene in a transverse stripe approximately 3 to 5 cells in width in early blastula embryo in many different dipteran species (Stanojevic *et al.*, 1991; Small *et al.*, 1992). This enhancer has been extremely well characterized in the fruit fly, *D. melanogaster*. For this reason, this CRE has been intensively studied from an evolutionary perspective among other species, including sepsid fly, *Themira putris* (Hare *et al.*, 2008). Enhancer from these two species, which diverged about 100 millions years ago, are unalignable at the DNA sequence level. Predicted locations of the functionally important transcription binding sites are absolutely different (**Figure 3**). However, in transgenic experiments a sepsid S2E sequence can be identified that drives expression of reporter gene in an embryonic transverse stripe in nearly the same location as observed with the endogenous *melanogaster* S2E enhancer.

Models of Enhancer Evolution

A number of evolutionary models have been proposed to explain the observations concerning evolutionary changes in *cis*-elements such as enhancers. One proposal has been to model enhancer structure/function as a quantitative character. Many studies have shown that modular multibinding site architecture is required for eukaryotic enhancer function. The presence of multiple binding sites, each with many 'fuzzy'

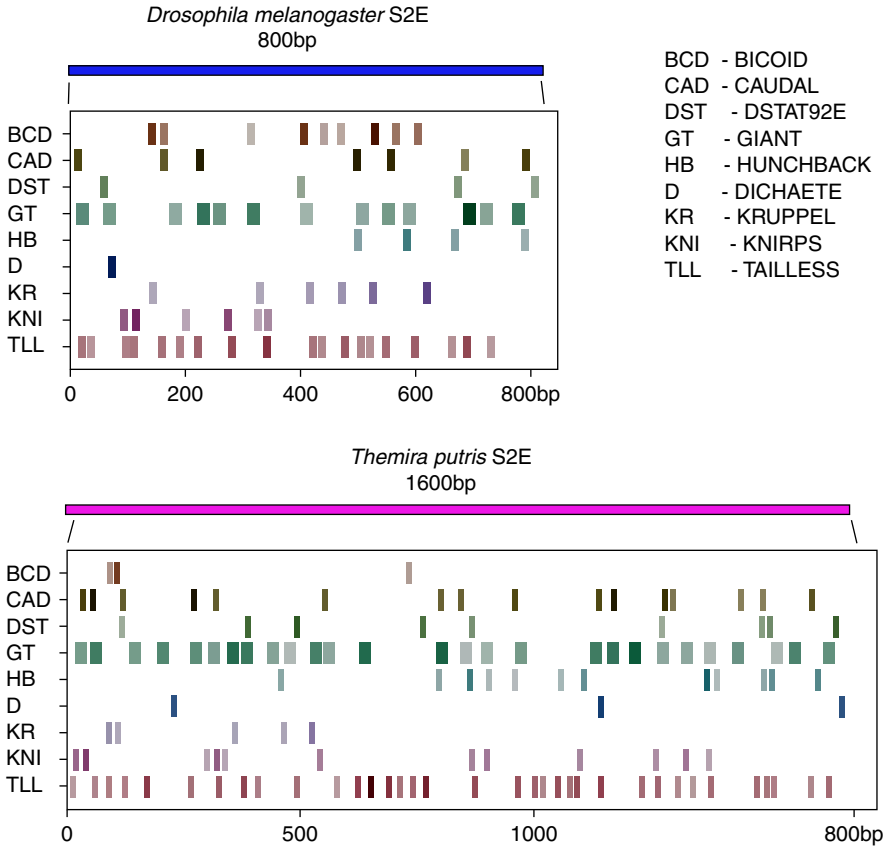

Figure 3 Strength and positions of predicted binding sites for transcription factors BCD (*bicoid*), CAD (*caudal*), DST (*Distat92E*), GT (giant), HB (*hunchback*), D (*Dichaete*), KR (*Kruppel*), KNI (*knirps*), and TLL (*tailless*) in the *even-skipped* stripe 2 enhancer of *Drosophila melanogaster* (~800 bp) and *Themira putris* (~1600 bp). Intensities of the color inside of the rectangles are proportional to the strength of binding site.

sites, and the possibility that subtle changes in spacing can also influence enhancer function, is compatible with the idea that many independent mutations will contribute to variation in gene expression. The spatio-temporal requirements for gene expression can then be viewed as a continuous character that can shift forwards and backwards subtly with mutations that affect transcription factor binding or interaction.

The above view naturally leads to the idea that the 'model of stabilizing selection' can be considered to be the major mode of enhancer evolution. Kimura (1981) investigated the rate of substitution assuming a quantitative character subject to stabilizing selection. If a large number of segregating sites (or loci) are involved, the average selection coefficient per mutant under stabilizing selection will be small. These weak mutations can then be controlled by genetic drift rather than selection, and the rate of substitution can be quite high. Applied to enhancer evolution, stabilizing selection can accommodate binding site turnover without disruption of primary enhancer function. The results of studies of the *eve* S2E evolution from *Drosophila* are consistent with the stabilizing selection model (Ludwig *et al.*, 1998; Ludwig *et al.*, 2000).

The evolution of some regulatory elements might also be consistent with a 'model of compensatory selection.' A pair of mutations at different sites (loci) that are singly deleterious but restore normal fitness in combination may be called compensatory neutral mutations. Kimura (1985) demonstrated that these mutations could easily become fixed in the population by genetic drift when the genes are tightly linked. Carter and Wagner (2002) modeled this process as it might apply to regulatory sequences. They found that large population size accelerates compensatory evolution, whereas small population sizes inhibit this form of drift from occurring.

Some theoretical and empirical studies have challenged the feasibility of compensatory evolution for transcription factor binding sites gain and loss (turnover) in CREs. A completely neutral model cannot achieve a high enough level of the turnover to explain observed rates for enhancer evolution in *Drosophila* (Durrett and Schmidt, 2008). The observed patterns of polymorphism and divergence in the transcription factor binding sites of well-characterized enhancers in two closely related *Drosophila* species are consistent with contributions of 'positive selection' to the transcription factor binding sites turnover as well as purifying selection in its maintenance (He *et al.*, 2011).

Origin of *Cis*-Elements with Novel Function

The major challenge for modeling the evolution of a novel regulatory element is to allow for the stepwise evolutionary progression of an element from one that initially contains by chance only a small number of functional transcription factor binding sites.

Moreover, the mechanisms of any CREs action always assume an interaction with their core promoter to achieve transcription of regulated gene. *Cis*-elements, including enhancers, cannot work alone without core promoters. Functional relatedness between the core promoter and enhancers, supporting the hypothesis that enhancers could have evolved from preexisting core promoter domains that bind essential transcription factors (Pikaard, 1994).

Three hypotheses have been proposed for how CREs evolve a new function through the modification and divergence of preexisting ones: first, duplication and DNA rearrangements involving either all or part of existing functional elements; second, modification of existing elements – for example, through gain and loss of binding sites, or acquisition of binding sites for additional transcription factors; and third, co-option of an existing element and expansion of its developmental function. These three scenarios adhere to generally accepted principles of developmental biology and population genetics; they involve the evolutionary modification of preexisting elements and they allow for a novel function to be gained without breaking the primary function of CREs.

Conclusion

Most of our current knowledge about evolution of ncDNA comes from direct experimental functional studies and/or indirect comparative genomics approaches.

Interspecific sequence comparisons of noncoding regions reveal conserved features, many of which are likely to be functional CREs. But despite obvious indications of selective constraint, the structure and sequences of *cis*-elements change over time, sometimes dramatically so – even in cases where expression patterns are conserved. Functional conservation of gene expression is not sufficient, therefore, to assure the evolutionary preservation of corresponding CREs.

It should be evident that a variety of evolutionary processes shape the structure of ncDNA. Functionally important regulatory sequences will tend to be conserved as a result of negative selection against deleterious mutations and positive selection for better-canalized performance. This need not be the case, however; some forms of stabilizing selection can maintain functional conservation of CREs for long periods of evolutionary time despite structural architecture turnover. In addition, compensatory evolution can even accelerate the substitution process in large populations to a level greater than the neutral rate of substitution. Finally, insertions and deletions have major impact on ncDNA evolution. This type mutation affects the transcription factor interactions that modulated by the spacing of binding sites in CREs. Negative selection is stronger against deletions, whereas insertions are favored by positive selection.

Eukaryotic CREs contain regulatory sequences that provide robustness of gene expression to genetic and environmental perturbation. Their function is not merely to turn genes 'on' or 'off' but to do so under a range of genetic and environmental (for example: gene dosage and temperature) conditions experienced by cells in a developing organism. Our work with *eve* S2E suggests that the architecture of enhancers (numbers and composition of the transcription binding sites) is adjusted by natural selection to ensure robust gene expression. Such adaptive fine-tuning may explain how CREs experience rapid sequence divergence between closely related species while exhibiting functional conservation (Ludwig *et al.*, 2011; Manu *et al.*, 2013).

Interactions between different CREs and multiple transcription factors could be seen as being parts of epistatic networks.

Under this view, all elements of this genetic system somehow should coevolve in both sequence-specific and location-specific ways to achieve the best possible functional performance. It will be difficult to elucidate how natural selection operates across such a complex system by examining either one gene at a time or the entire system of interacting genes, the two most common approaches employed in current research.

One of the main goals of fundamental biology is to identify CREs in ncDNA, understand their properties, and decipher how these elements regulate cell function by interacting with one another. The ultimate goal is to reveal the most basic laws of biology, which would allow us to understand functional aspects of evolutionary processes acting on ncDNA: preservation and variation of *cis*-elements structure/function and origin of *cis*-elements with novel function.

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See also: Compensatory Evolution. Genome Organization, Evolution of. Regulatory and Coding Changes in Developmental Evolution, Roles of

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Noncoding RNAs, Origin and Evolution of

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Glossary

Co-option Reuse of preexisting genes, proteins, or systems with different functions from original ones, which sometimes enables organisms to establish new phenotypes without generating new genes, proteins, or systems from scratch.

Horizontal gene transfer Transfer of genes or any other genetic elements between different organisms.

Small RNA One of the major classes of noncoding RNAs, normally less than 50 nucleotides in length. Most of them are involved in regulation of genes and genomes.

General Introduction

Noncoding RNAs (ncRNAs) include all RNAs that do not encode any proteins but function as RNA molecules. Ribosomal RNAs and transfer RNAs, which play central roles in protein synthesis, have been recognized as representatives of ncRNAs for a long time. However, several different ncRNAs have recently been identified with the advancement of molecular biology and sequencing technologies (Table 1).

Discoveries of small RNAs including microRNAs (miRNAs), short-interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs) have attracted much attention in recent years. Many of these small RNAs associate with several common proteins to regulate genes, transposons, and even exogenous viruses. Because the regulation of genes and genomes is one of the key factors in organismal evolution, we should understand the origin and evolution of not only protein-coding genes but also ncRNA genes as well as other regulatory elements.

In this article, we review the origin and evolution of ncRNAs. We will mainly focus on eukaryotic small RNAs, i.e., miRNAs, siRNAs, and piRNAs, which have been relatively well characterized, and will illustrate the interconnection among them, particularly regarding their evolutionary processes. We will also briefly mention prokaryotic small RNAs to describe the commonality in the evolutionary processes of small RNA-based regulatory systems between eukaryotes and prokaryotes. Readers who are interested in other ncRNAs may refer to the following review articles: Shabalina and Koonin, 2008; Fox, 2010; Cech and Steitz, 2014.

microRNAs

Among eukaryotic small RNAs, miRNAs were first discovered in 1993 in *Caenorhabditis elegans* (Lee *et al.*, 1993). miRNAs are 21–24 nucleotides (nt) in length and regulate a variety of protein-coding genes at the posttranscriptional stage. Many eukaryotes, such as animals and plants, have hundreds to thousands of miRNA loci in their genomes. Many of these loci are independently located throughout genomes but some are clustered and function cooperatively through polycistronic transcription. When a miRNA gene is transcribed, the transcript immediately forms a hairpin (palindromic) structure. After several processing steps mediated by RNases such as Dicer (and Drosha in animals), a mature miRNA/miRNA* duplex is assembled into a RNA-induced silencing complex (RISC) including Argonaute. In general, only the antisense strand (or mature miRNA), which is complementary to the target RNA, is selected in this process, and the sense strand (or miRNA*) is degraded. Finally, the RISC binds to the target mRNA and prevents its translation (Carthew and Sontheimer, 2009).

These processes are largely shared in animals and plants but some key differences should be noted. For example, in plants a miRNA in RISC generally binds to a target mRNA with nearly perfect base complementarity and cleaves it with endonuclease activities, whereas an animal miRNA imperfectly binds to its target and typically represses translation without cleavage. The relationship between miRNAs and their targets is thus not one-to-one but many-to-many, particularly in animals (Grun *et al.*, 2005). This indicates that a considerable number of protein-coding genes can be regulated by miRNAs. Indeed, a bioinformatic prediction suggested that as many as ~30% of human genes are likely to be targets of miRNAs (Lewis *et al.*, 2005).

Origin of microRNAs

As mentioned above, miRNAs are present in eukaryotes including animals and plants (Figure 1). There are two possible explanations for the origin of miRNAs. One possibility is that miRNAs originated independently in animals and plants. This idea can be supported by the fact that biogenesis and target suppression differ between plant and animal miRNAs (Shabalina and Koonin, 2008). In addition, miRNAs have not been identified in some eukaryotes, such as fungi and choanoflagellates, whose phylogenetic positions are between animals and plants (Axtell and Bowman, 2008). Moreover, miRNAs have not been identified in ctenophores, the most basal animals (Maxwell *et al.*, 2012). Therefore, miRNAs may have originated independently in the lineage ancestral to plants and green algae and the other lineage ancestral to cnidarians and bilaterians. However, a recent study supports a common origin of the miRNA system in animals and plants. Moran *et al.* (2014) reported that several cnidarian species such as hydras, a sister group of bilaterians, have cleavage-type miRNAs in the genomes, which is analogous to plants. The cnidarian miRNA

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Table 1 Characteristics of major noncoding RNAs (ncRNAs)

ncRNA	*Organism*	*Size (nt)*	*Source*	*Associated protein*	*Function*
Small RNA					
miRNA (microRNA)	Eukaryotes	21–24	Genomic loci (miRNA loci, introns)	Argonaute, Dicer, (Drosha[a])	Translational repression of mRNA, mRNA cleavage
siRNA (short-interfering RNA)					
Exo-siRNA (exogenous siRNA)	Eukaryotes	21–24	Viral or other exogenous RNAs	Argonaute, Dicer	Cleavage of exogenous RNA
Endo-siRNA (endogenous siRNA)	Eukaryotes	21–24	Genomic loci (mRNAs, Transposons, repeats), miRNA-cleaved RNAs, bidirectional transcripts	Argonaute, Dicer, RdRP	RNA cleavage
piRNA (Piwi-interacting RNA)	Animals	24–30	Genomic loci (transposons, repeats, piRNA loci)	Piwi, RdRP, Zucchini	Transcriptional repression, heterochromatin formation (DNA methylation), RNA cleavage
scnRNA (scan RNA)	Ciliates	25–32	Micronuclear	Dicer, Piwi	DNA elimination in developing macronuclei
crRNA (CRISPR RNA)	Prokaryotes	24–48	Genomic loci (CRISPR loci)	Cas	Cleavage of exogenous DNA/RNA
DsrA RNA	Eubacteria	~85	Genomic loci (DsrA loci)	Hfq	Translational repression and activation
Other ncRNA					
rRNA (ribosomal RNA)	Prokaryotes, Eukaryotes	100–5000	Genomic loci (rRNA loci in nuclei and mitochondria)	RNase III, RNase E, RNase G, ribosomal proteins	Protein translation
tRNA (transfer RNA)	Prokaryotes, Eukaryotes	73–93	Genomic loci (tRNA loci in nuclei and mitochondria)	RNase III, RNase P, RNase Z	Protein translation
snRNA (small nuclear RNA)	Eukaryotes	70–250	Genomic loci (snRNA loci)	RNase III, USB1	Modification, processing, and splicing of mRNA
snoRNA (small nucleolar RNA)	Eukaryotes	70–250	Genomic loci (introns of ribosomal RNAs)	RNase III, Rrp6p	rRNA processing, rRNA modification
lncRNA (long ncRNA)	Eukaryotes	>200	Genomic loci (*cis*-elements, introns, UTRs, pseudogenes)	Poly(A) Polymerase, Spliceosome	Transcriptional regulation

[a]Only in animals.

system may therefore be a mixture of the ancestral cleavage-type (plant-type) and the derived non-cleavage-type (animal-type). In other words, a transition from the cleavage-type to the non-cleavage-type may have occurred around the time of the cnidarian–bilaterian split. Future studies in different animal lineages will reveal more detailed aspects regarding the transitions.

Evolution of microRNAs

New miRNA genes have been generated by several mechanisms (Berezikov, 2011) that differ between animals and plants. In plants, transposons and duplicates of preexisting miRNA genes are major sources of new miRNA genes (Nozawa *et al.*, 2012). In animals or at least in flies, in contrast, many miRNA genes appear to have originated from introns (so-called 'mirtrons') or hairpin structures in intergenic regions (Nozawa *et al.*, 2010).

Many newly arisen miRNA genes, however, appear to have been inactivated and eliminated because of random mutations before acquiring solid functions. In other words, most of these new genes are not really authentic but seem just genomic regions having transcription activity without any solid roles. Indeed, young miRNA genes appear to evolve almost neutrally (Nozawa *et al.*, 2010), and the turn-over rate of these young miRNA genes is quite high (Lu *et al.*, 2008). Only a few young miRNA genes acquire solid functions and are integrated into a preexisting gene regulatory network. Then, these miRNA genes are maintained in genomes for a long time with very low substitution rates. It has been reported that mature sequences of these established miRNA genes show a very low nucleotide substitution rate that is comparable to those of *EF1α* and histone *H3* (Sempere *et al.*, 2006).

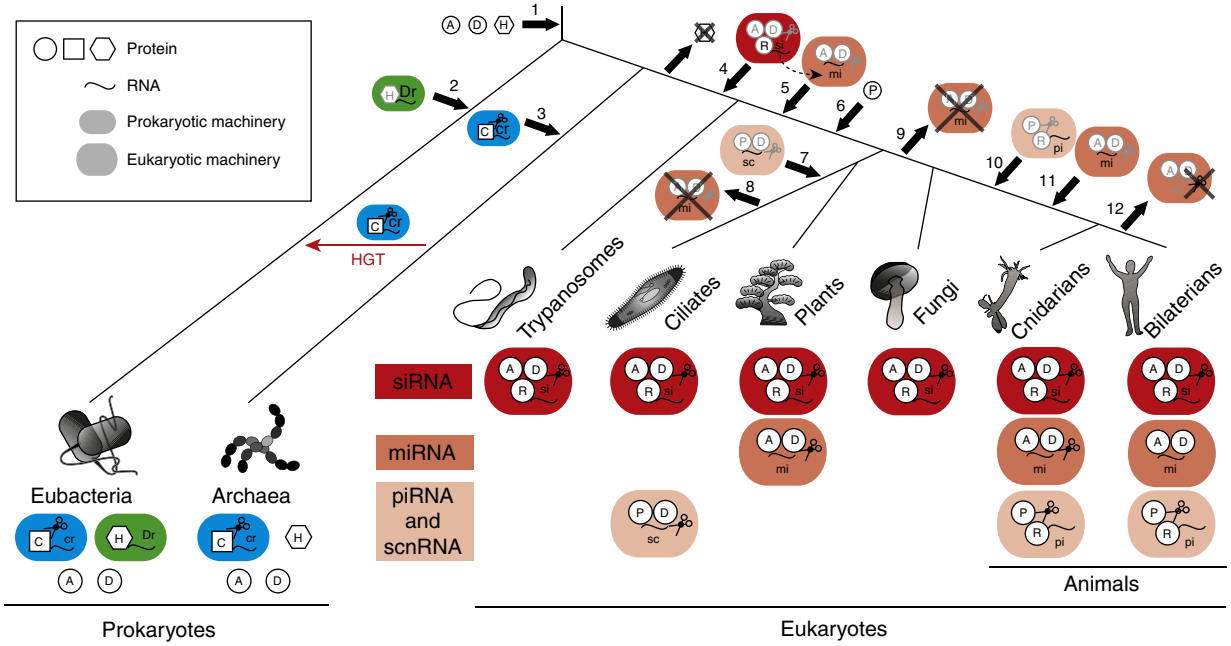

Figure 1 Phylogenetic distribution of small RNAs and their related proteins. Possible evolutionary events (gains and losses) regarding the small RNAs and related proteins are also given along the branches. Numbers along arrows correspond to the evolutionary events that are discussed in the main text. Gray objects are not involved in gains and losses. A red arrow represents horizontal gene transfer, whereas a broken arrow means a plausible origin of miRNAs from siRNAs. A, Argonaute; C, Cas; cr, crRNA; D, Dicer; Dr, DsrA RNA; H, Hfq; mi, miRNA; P, Piwi; pi, piRNA; R, RdRP; sc, scnRNA; and si, siRNA. Scissors indicate that the suppression of targets is generally accomplished by target cleavage. The phylogenetic relationship among species used is based on the timetree (Hedges *et al.*, 2006).

How is a new miRNA integrated into the gene regulatory network during evolution? Chen and Rajewsky (2007) proposed that a young miRNA is expressed only weakly in a specific tissue at a restricted developmental stage and that the effect of the miRNA on an individual would be weak or negligible. This model also predicts that natural selection continuously eliminates deleterious targets during evolution; therefore, the number of targets for a miRNA gradually decreases with time. This model has not been proven conclusively yet, and further studies are necessary to test this model.

Short-Interfering RNAs

siRNAs are also 21–24 nt long, like miRNAs, and generally work as guardians of genomes against viruses and other parasites. Unlike miRNAs, some siRNAs (exogenous siRNAs or exo-siRNAs) are directly generated from exogenous RNAs, whereas other siRNAs (endogenous siRNAs or endo-siRNAs) are derived from endogenous transcripts of genomic regions such as transposons, heterochromatic sequences, intergenic regions, and pseudogenes (Ghildiyal and Zamore, 2009).

Several sources of siRNAs such as viruses and bidirectional transcripts are double-stranded RNAs, but other precursors are single-stranded RNAs. These single-stranded RNAs are first converted into double-stranded RNAs by RNA-dependent RNA polymerases (RdRPs). All resultant precursors are double-stranded molecules with perfect complementarity. They are further processed by Dicer to mature duplex siRNAs which are then integrated into a RISC containing Argonaute. During this process, the sense (or passenger) strand of the duplex is degraded, and the RISC with the antisense (or guide) strand binds to its target RNA normally with perfect sequence complementarity and cleaves it (Carthew and Sontheimer, 2009).

Origin of Short-Interfering RNAs

Because siRNAs are the most widely distributed among the known eukaryotic small RNAs (**Figure 1**), a siRNA-like system may be the ancestral type of RNA-based regulation in eukaryotes (Shabalina and Koonin, 2008). This hypothesis is supported by shared features between siRNAs and miRNAs as well as between siRNAs and some other features with piRNAs. For example, both siRNAs and miRNAs associate with Dicer and Argonaute, whereas some siRNAs and piRNAs share functions to suppress transposons as mentioned in later sections.

To understand the diversification of siRNAs after origination, let us consider exo- and endo-siRNAs separately. Ancestral siRNAs are likely to have been exo-siRNAs derived from viruses and other parasitic RNAs, and that the first siRNA machinery in early eukaryotes was established to fight against these 'genomic predators.' Endo-siRNAs later came into existence as the functions of siRNAs began to diversify. Endo-siRNAs that are generated from transposons and heterochromatic sequences are apparently involved in the silencing of transposons and repetitive elements, whereas those from pseudogenes likely regulate their functional homologs. In some cases, endo-siRNAs are even produced from mRNAs. Because a subset of these endo-siRNAs can make long hairpins, they could be the ancestral state of miRNAs (broken arrow in **Figure 1**).

Evolution of Short-Interfering RNAs

Although siRNAs have a variety of functions, their major functions are defensive. The genes involved in siRNA pathways may have therefore evolved fast to trace the changes in exogenous RNAs. Indeed, the Argonaute (*Ago2*) and Dicer (*Dcr2*) genes, which are required for siRNAs, evolve much faster than their counterparts (*Ago1* and *Dcr1*, respectively) that are necessary for miRNAs (Obbard *et al.*, 2006). One possibility is that the proteins involved in siRNA pathways evolved under host–pathogen arms races similar to immune-related genes. Another possibility is that these proteins may have evolved faster due to under less functional constraints rather than the arms races. Because the molecules that directly interact with pathogenic RNAs are not these proteins but siRNAs, host–pathogen arms races on these proteins may not be necessary.

Piwi-Interacting RNAs

Compared with miRNAs and siRNAs, piRNAs are a bit longer in length, ranging from 24 nt to 30 nt, and they mainly function to suppress the activity of transposons. They are generated from long RNA transcripts that contain multiple piRNAs in tandem. There are hundreds of piRNA loci, each of which makes a large cluster (Ghildiyal and Zamore, 2009). During biogenesis, piRNAs, unlike miRNAs and siRNAs, do not require Dicer. Instead, long single-stranded primary piRNAs are cut into each unit of piRNAs by different nucleases (Kim *et al.*, 2009; Ipsaro *et al.*, 2012). The resultant piRNAs then associate with a specific member of the Argonaute family, Piwi, to form a RISC. The RISC binds to its target RNA based on a 10-nt complementarity with overhang between the piRNA and the target and cleaves the target RNA.

piRNAs are predominantly expressed in germline cells to suppress transposons at both transcriptional and post-transcriptional stages. It is interesting to note that piRNAs can also be generated by cleaved transcripts that are derived from transposons through the so-called 'ping-pong' cycle of piRNA amplification (Ghildiyal and Zamore, 2009). Through this process, a small amount of initial product can efficiently produce a large amount of piRNAs to suppress transposons.

Origin of Piwi-Interacting RNAs

piRNAs are confined to animals and presumably originated in the ancestor of animals (**Figure 1**). Piwi proteins, however, are found in ciliates, nonanimal eukaryotes. Indeed, a ciliate, *Tetrahymena thermophila*, has scan RNAs (scnRNAs) that are analogous to the animal piRNAs with respect to their length and their association with Piwi (Mochizuki and Gorovsky, 2004). Moreover, scnRNAs exist in another ciliate, *Paramecium tetraurelia*, where they function to determine mating types (Singh *et al.*, 2014). However, the biogenesis of scnRNAs resembles that of siRNAs but not that of piRNAs. In addition, they function mainly in DNA elimination during conjugation rather than binding to RNAs (Malone and Hannon, 2009). Therefore, the canonical piRNA machinery appears to have emerged in the ancestor of animals. In contrast, Piwi itself was generated before the divergence of ciliates and animals by duplication of genes that encode Argonaute and has functioned differently among lineages.

Evolution of Piwi-Interacting RNAs

As mentioned above, piRNAs are normally transcribed from piRNA gene clusters that predominantly consist of immobilized transposon remnants. Therefore, it is quite reasonable to hypothesize that piRNA clusters originated from transposons. piRNA clusters appear to have continuously increased at least in mammals (Assis and Kondrashov, 2009). Genomic locations of many clusters are conserved between species, but sequence contents in each piRNA cluster are quite different even between closely related species (Malone and Hannon, 2009). This expansion of clusters and rapid evolution of piRNA sequences could have been driven by positive selection to silence expanding and diverging repertoires of mammalian transposons, but the evidence is not convincing and further studies are necessary in a variety of organisms.

Small RNAs in Prokaryotes

miRNAs, siRNAs, and piRNAs have not been identified to date in prokaryotes. However, this does not mean that there is no RNA-based regulatory system in prokaryotes. Indeed, *Escherichia coli* has at least 60 small RNA genes in its genome (Majdalani *et al.*, 2005). Among them, the largest class of small RNAs, DsrA RNA, is analogous to miRNAs in eukaryotes with respect to its functions. This small RNA works cooperatively with a RNA chaperone protein, Hfq, and suppresses (or activates) the expression of target mRNAs via sequence complementarity (Aiba, 2007). Homologs of DsrA RNAs have been identified in many other eubacterial species. In addition, Hfq-like proteins have also been identified in archaea (Nielsen *et al.*, 2007).

A siRNA-like silencing system also exists in both eubacteria and archaea. In this system, small RNAs are transcribed from clustered regularly interspaced short palindromic repeats (CRISPRs) in genomes and bind to viral DNAs or RNAs for degradation in cooperation with Cas proteins. The RNAs derived from CRISPRs are called CRISPR RNAs (crRNAs). Because CRISPRs have been generated by repeated integrations of viral sequences (Barrangou *et al.*, 2007), the source of crRNAs is analogous to that of endo-siRNAs or piRNAs. In other words, this system is a type of 'memorization' of viral infections and can be regarded as a prokaryotic adaptive immune system. At present, 83% of archaeal and 45% of eubacterial species with sequenced genomes are known to have CRISPRs in their genomes (Grissa *et al.*, 2007). This machinery has recently been used to artificially modify the genomes of a variety of organisms including eukaryotes as one of the genome-editing technologies.

Interconnected Evolution of Small RNA Machinery

Based on the above information about small RNAs, we constructed a plausible scenario regarding the origins and

evolutionary processes of small RNA machinery (Figure 1). Here, small RNAs and related proteins are separately considered.

For prokaryotic machinery, Cas and CRISPRs are widely distributed in eubacteria and archaea. However, species with or without CRISPRs/Cas are highly intermingled, suggesting that none of them existed in the progenitor of all living organisms and that they were later generated and have spread into both eubacteria and archaea through horizontal gene transfers (HGTs) (Grissa *et al.*, 2007). Note that CRISPRs and Cas proteins are located adjacently in genomes; therefore simultaneous HGTs are quite possible. Regarding their origin, Makarova *et al.* (2011) compared the domains of Cas proteins among species and concluded that Cas as well as CRISPRs first originated in thermophilic archaea (evolutionary event 3 in Figure 1). This is consistent with the above statement that the proportion of species with CRISPRs is higher in archaea than in eubacteria. Therefore, the crRNA machinery as well as CRISPRs and Cas appear to have first originated in archaea and spread into many other species of archaea as well as eubacteria via HGTs (red arrow in Figure 1). Regarding the DsrA RNA machinery, the progenitor is likely to have possessed the Hfq protein (evolutionary event 1), because a wide range of eubacterial and archaeal species have the homologs. Yet, the functions of Hfq in the progenitor would have been different from the current ones, and the DsrA RNA machinery may have been established in the lineage of eubacteria by the generation of the DsrA RNA in combination with the co-option of Hfq (evolutionary event 2).

Regarding eukaryotic machinery, it is clear that several proteins such as Argonaute and Dicer already existed in the ancestor of all living organisms, because the homologs of these proteins are present even in prokaryotes (evolutionary event 1). The emergence of these proteins could be explained by aggregations of domains of preexisting proteins (Shabalina and Koonin, 2008). Yet, functions of Argonaute and Dicer in the ancestor appear to have been different from the current ones. Indeed, a function of Argonaute in eubacteria seems to acquire and maintain new plasmids (Wei *et al.*, 2012; Vogel, 2014). After the divergence from eubacteria and archaea, these proteins as well as novel proteins like RdRP started to function cooperatively with the small RNAs and established the first small RNA-based machinery in the early evolution of eukaryotes. Because siRNAs are most widely distributed in eukaryotes and defensive roles against predators are quite essential, the first machinery in eukaryotes may have been exo-siRNA-like machinery (evolutionary event 4).

After the divergence from trypanosomes, the miRNA-based regulatory system was established as the second machinery by generating miRNAs and co-opting the siRNA machinery (evolutionary event 5). Later, the system was independently lost in the lineages ancestral to fungi and animals (evolutionary event 9) as well as to ciliates (evolutionary event 8). miRNAs have not been identified in these 'in-between' lineages, but under this scenario, several proteins such as Argonaute and Dicer were maintained even in these species possibly for other functions, for example, siRNA machinery. Then, miRNAs were regenerated in the lineage ancestral to cnidarians and bilaterians (evolutionary event 11). Note that, at the time of this regeneration, the miRNA machinery in animals probably had a cleavage activity like the plant type. Yet, the machinery lost the cleavage activity in the bilaterian lineage after the split from cnidarians (evolutionary event 12).

Similar to the miRNA machinery, Piwi also came into existence in the lineage ancestral to ciliates and animals after splitting from trypanosomes (evolutionary event 6). However, the ancestral functions of Piwi appear to have been different from the current ones. Later, the piRNA machinery was established in the animal lineage by generating piRNAs in combination with the co-option of Piwi and RdRP (evolutionary event 10). Similarly, the scnRNA machinery was generated in ciliates by creating scnRNAs as well as the co-option of Piwi and Dicer (evolutionary event 7).

Conclusion

In conclusion, the RNA-based regulatory systems appear to have diversified mainly by repeated generations of ncRNAs in combination with recurrent co-options of preexisting proteins. A majority of ncRNAs share a basic aspect that they guide associated proteins to targets through sequence complementarity. This ncRNA–protein relationship has given rise to a variety of regulatory systems. Yet, the evolutionary processes of ncRNA-based regulatory systems largely remain mysterious. One of the challenges stems from the limited sizes of many ncRNAs. Because small RNAs are mostly less than 30 nt in their mature form, there may be insufficient information to distinguish evolutionary relationship (orthology) from independent origins. To understand the origins and evolutionary processes of the systems, we also need to consider both proteins that associate with ncRNAs and the targets of each of the ncRNAs. Given sufficient functional information, we will be able to take an integrative approach to understanding the evolutionary aspects of ncRNA-based regulation.

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See also: Noncoding DNA Evolution: Junk DNA Revisited. Systems in Evolutionary Systems Biology

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Novel Structures in Animals, Developmental Evolution of

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Glossary

Anlagen The cell or tissue primordia in an embryo that form the basis for a particular organ or other structure.
Co-option The acquisition of new roles or characters from ancestral ones, especially for genes.
Developmental capacitance The ability of developmental factors to buffer against potential changes due to genetic or environmental variation.
Developmental plasticity The ability of an organism to modify its phenotype in response to external cues.
Evolutionary novelty Derived body parts that usually lack homologous relations to structures in ancestral lineages and often possess the potential for new functionality.
Exaptation A trait either originally selected for another purpose or a by-product of a different trait's formation that now has a new function.
Extended Evolutionary Synthesis A broader theoretical framework for evolution that claims to incorporate recent empirical and theoretical advances (e.g., developmental plasticity or niche construction).
Gene regulatory network (GRN) A collection of hierarchically organized gene interactions that modulate gene expression.
Heterochrony Developmental changes in rate or timing.
Heterotopy Developmental changes in spatial location.
Homologous Biological structures that share common ancestry.
Hypertrophic zone Area of increasing cell size.
Imaginal discs Undifferentiated cells in insects that develop into specific adult structures.
Modern Synthesis The standard theoretical framework for evolution that developed in the twentieth century and emphasized natural selection as the explanation of organismal adaptation and species diversification.
Module Semiautonomous elements loosely connected to one another within a larger system.
Sexually dimorphic Phenotypic differences separating opposite sexes.

Overview

Historical Perspective

In an often misquoted passage from *On the Origin of Species*, Darwin claimed that, "How a nerve comes to be sensitive to light hardly concerns us more than how life itself first originated" (Darwin, 1859, p. 187). The origin of new structures was not a question on his research agenda: "Darwin explicitly disavowed theorizing … about the origins of the most primitive eyes" (Lustig, 2009, p. 113). It is not that he thought it an unanswerable question ("several facts make me suspect that any sensitive nerve may be rendered sensitive to light"). Instead, his aim was to show how natural selection increased complexity: "Natural selection has converted the simple apparatus of an optic nerve merely coated with pigment and invested by transparent membrane, into an optical instrument as perfect as is possessed by any member of the great Articulate class" (Darwin, 1859, p. 188). However, many evolutionary biologists have been concerned with explaining the origin of new structures, usually with special attention to how novel variation originates in and through developmental processes. For example, morphologists and paleontologists routinely attempted to account for the origins of higher taxa, which were characterized by distinct structures (e.g., chordates), with reference to developmental transformations, such as the dramatic morphological effects of precocious sexual maturation in a larval form (Love, 2007).

On the landscape of contemporary biology, the problem of explaining the origin of novelties emerged as a locus for criticizing the population genetic framework of evolutionary theory found in the Modern Synthesis: "It does not help much to say that there were one or two mutations that created eyespots and that these alleles were selected" (Wagner, 2000, p. 97). More positively, the problem represented a signature feature of research in evolutionary developmental biology (Evo-devo): "finding answers to what constitutes an evolutionary innovation … and how developmental mechanisms have changed in order to produce these innovations are major issues in contemporary [Evo-devo]" (Olsson and Hall, 1999, p. 612). This feature distinguished Evo-devo from other evolutionary research. "Evolutionary innovations are outside the scope of any current research program … we see in the problem of innovation and the evolution of body plans a unique opportunity for [Evo-devo] to develop its own independent identity as a research program" (Wagner *et al.*, 2000, p. 822). These themes are echoed in subsequent surveys of research on the origin of novelty (e.g., Moczek, 2008) and have constituted a hallmark in calls for an Extended Evolutionary Synthesis (Pigliucci and Müller, 2010). Apart from these broader questions about the disciplinary autonomy of Evo-devo and the adequacy of particular conceptions of evolutionary theory, when earlier discussions of the origins of higher taxa (e.g., Garstang, 1928) are set alongside recent Evo-devo studies (e.g., Lowe *et al.*, 2015), current investigations can be described aptly as tackling old evolutionary problems with new experimental and phylogenetic tools (Love and Raff, 2003).

Despite a consensus that explaining the origins of novelty requires understanding development ("it is essential to include

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developmental mechanisms in the explanation of evolutionary innovations" (Wagner, 2000, p. 97)), there are ongoing debates about what counts as an evolutionary innovation or novelty and what causal factors, developmental or otherwise, best explain their origin. In addition to controversies surrounding the relative significance of gene regulatory network (GRN) changes in comparison to other factors (e.g., developmental plasticity), there remains a question about whether natural selection plays any explanatory role. After reviewing aspects of these debates, we outline three case studies that are representative of contemporary research into the developmental evolution of novel structures and conclude with reflections on prospects for future inquiry.

What Is an Evolutionary Novelty?

Ernst Mayr defined an evolutionary novelty as "any newly acquired structure or property which permits the assumption of a new function," which fit within the explanatory framework of the Modern Synthesis: "The problem of the emergence of evolutionary novelties then consists in having to explain how a sufficient number of small gene mutations can be accumulated until the new structure has become sufficiently large to have selective value" (Mayr, 1960, p. 357). In contrast, a different definition has been in view for most work in Evo-devo on the origin of novel structures. "A morphological novelty is a structure that is neither homologous to any structure in the ancestral species or [serially homologous] to any other structure in the same organism" (Müller and Wagner, 1991, p. 243). Instead of functional properties ('selective value'), morphology is accented in a phylogenetic context ('nonhomologous structures'); instead of mutations entering a gene pool, the developmental generation of qualitatively new variation is the focus. The growth of cladistic methods for phylogenetic reconstruction and emergence of molecular developmental genetics gave this structure-oriented definition operational traction in the context of Evo-devo. And yet the definition relies on the concept of homology, which has exhibited its own share of semantic disagreements across biological disciplines (Brigandt, 2003).

For some researchers, these difficulties suggest that defining evolutionary novelty is a fruitless endeavor. Although the emphasis has been on novelty as a qualitative departure from an ancestral condition ("quantitative change is only part of the story of evolution, for it does not address the question of the origin of discrete (qualitatively different) novelties" (West-Eberhard, 2003, p. 6)), the existence of a continuum between a qualitative difference and a mere quantitative variant makes it difficult to distinguish novelty from nonnovelty in absolute terms. Biologists draw the line on this continuum differently (Brigandt and Love, 2010; Palmer, 2012), and there are always precursors or homologous features at lower levels (e.g., gene expression, cells, or tissues) for those structures deemed qualitatively novel (Shubin *et al.*, 2009; Hall and Kerney, 2012). However, serious biologists never assumed that evolutionary novelties lacked precursors (Wagner, 2014). How then should we interpret this diversity of definitions?

Rather than trying to identify a single, correct definition of evolutionary novelty, one way forward is to shift attention from defining the concept – delineating the set of entities a term classifies ('categorization') – to characterizing the explanatory agenda associated with the concept (Brigandt and Love, 2012). The meaning of the term 'innovation' or 'novelty' serves to indicate explanatory expectations for the study of diverse morphological features (**Figure 1**). For example, the criterion of nonhomology makes a trait's novelty a matter of what was historically present in a lineage. Forward-looking definitions characterize novelty in terms of the developmental potential for future morphological variation and diversification (Wagner and Zhang, 2011) or the capacity to transform

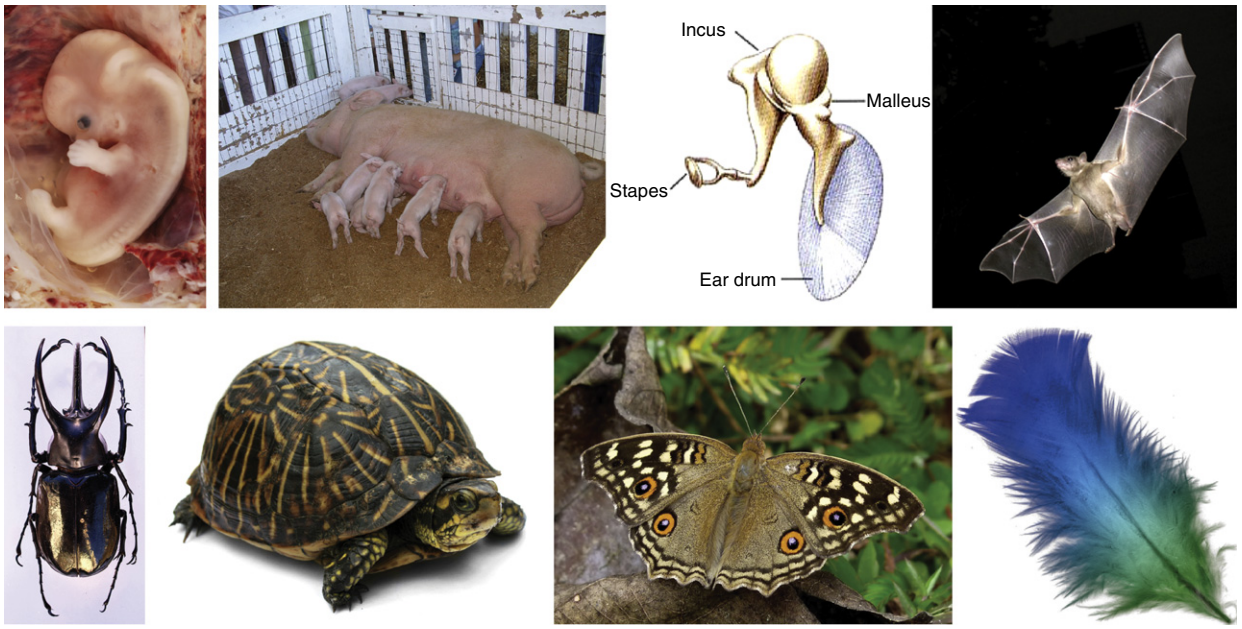

Figure 1 Examples of phenotypes commonly referred to as novel structures. Clockwise from the top-left: tetrapod autopod, mammary glands, mammalian middle ear, bat wings, feathers, butterfly eyespots, turtle shell, and beetle horns (open source images).

an ecosystem's carrying capacity (Erwin, 2012). The explanatory relevance of different causal factors can vary. Thus, focusing on characterizing the explanatory agenda highlights questions about adequate explanations for particular novelties, whether in terms of developmental mechanisms or adaptive advantages in ecological contexts.

On this construal, we can refer to evolutionary novelties as derived body parts that usually lack homologous relations to structures in ancestral lineages and often possess the potential for new functionality. However, to understand explanatory expectations in different disciplinary contexts, we should observe how the concept is structuring the problem space. This is often indicated by allied concepts used in conjunction with a specific definition of novelty. Discussions of novelty as nonhomology have stressed the importance of distinguishing character identity (e.g., the forewing of insects) from character states (e.g., wing blade vs. protective cover (elytra)), clarifying a particular sense of homology relevant to studying the developmental genetic origin of new structures (Wagner, 2014). Emphasizing the hierarchical level at which homology and novelty apply plays a key role in dissecting how mechanisms of gene regulation changed evolutionarily to produce novel anatomical structure (Shubin *et al.*, 2009). Linking novelty to evolvability accents the mapping relations between genotype and phenotype (Pavličev and Widder, 2015). Investigating the innovative ecological impact of a structural novelty makes natural selection germane to the explanation (Shirai *et al.*, 2012). (This strategy can be extended to include functional properties, such as behavior (Brown, 2014).) Additionally, typologies of evolutionary novelty help to carve out explanatory expectations. Müller's (2010) threefold typology of morphological novelty focuses attention on the epigenetic nature of developmental processes: type 1 – the primary anatomical architecture of a metazoan body plan; type 2 – discrete new elements added to an existing body plan; and type 3 – major changes to an existing body plan character. In alignment with this typology, Müller and colleagues have offered a physical explanation for types 1 and 2 novelties that is different from standard Evo-devo approaches (Newman *et al.*, 2006; Newman and Müller, 2005; see below).

How Do Novel Structures Arise?

Once the concept of evolutionary novelty is seen as an indicator of an explanatory agenda, it facilitates making explicit the expectations or criteria of adequacy related to the problem space. These include the need to specify the relevant level of structural organization, the degree of generality that can be derived from studying particular novelties, and the necessary disciplinary contributions for an adequate explanation (Brigandt and Love, 2012). The most prominent strategy of research in Evo-devo involves investigating how developmental genetic changes contribute to the construction of novel morphological structures, especially the formation of new GRNs. "Evolutionary change in animal form cannot be explained except in terms of change in [GRN] architecture" (Davidson, 2006, p. 29); "the evolution of development and form is due to changes within GRNs" (Carroll, 2008, p. 30); "Novelty requires the evolution of a new [GRN]" (Wagner and Lynch, 2010, p. R50). These changes can involve duplications followed by the differentiation of paralogous genes (Gompel and Prud'homme, 2009), modifications of regulatory interactions (Bloom *et al.*, 2013; Shirai *et al.*, 2012), and the cooption of gene expression from one time or context to another (Saenko *et al.*, 2008; True and Carroll, 2002). For example, a shift in the spatial location of gene expression (heterotopy), rather than a shift in the timing of gene expression (heterochrony), was responsible in part for the origin of the vertebrate jaw (Shigetani *et al.*, 2005). Thus, novel structures at one level of organization can arise from changes in homologous GRNs (Shubin *et al.*, 2009); they are explained by the recombination and redeployment of preexisting ancestral variation, rather than as a consequence of novel genes.

Although there is widespread agreement that evolutionary novelties arise from altered expression patterns due to GRN changes, some have argued for the importance of evolving protein–protein interactions based on overlooked functional divergence in conserved transcription factors (Lynch and Wagner, 2011). Günter Wagner has offered one of the most comprehensive theoretical perspectives on the origin of novelty with his genetic theory of homology (Wagner, 2014). Since there have been GRN changes without corresponding changes in animal form, a decoupling of developmental mechanisms and homologous characters (Abouheif *et al.*, 1997), Wagner isolates a subset of these network relations: character identity networks or ChINs (Wagner, 2007). These highly conserved GRNs can be activated by diverse inductive signals, but their activity determines which fate a cell adopts from among its intrinsic possibilities through specific regimes of gene expression. The developmental evolution of novel structures then consists in the origination of new ChINs.

Other Evo-devo practitioners do not focus exclusively on developmental genetics and view other mechanisms as pertinent to explaining novelty. For example, novel traits may begin as conditional structures that occur due to developmental plasticity (Moczek *et al.*, 2011; Palmer, 2012; West-Eberhard, 2003). Regulatory modules are often reused and recombined in response to different environmental conditions, potentially generating novel phenotypic responses. Subsequent mutations in GRNs can permanently establish these structures. A variation on this approach has stressed the significance of physical forces (e.g, fluid flow or differential adhesion) in generating basic morphological motifs (e.g., segmentation or tissue layering) under conditions of developmental plasticity early in metazoan evolution (Newman *et al.*, 2006; Newman, 2012). This explanation stresses the significance of epigenetic interactions at aggregate scales during development with evolutionary novelties arising from combinations of physical patterning processes (e.g., biochemical oscillation) and cell properties (e.g., polarity) under different environmental circumstances. Although this approach remains highly contested (Love and Lugar, 2013), work on the genomic complement available at the origins of multicellularity is clarifying to what degree this hypothesis is feasible (Suga *et al.*, 2013; Tweedt and Erwin, 2015).

From an ecological vantage point, it has been proposed that there are only two distinct possibilities for the origination of a structural novelty: exaptation or developmental capacitance (Moczek, 2008). Exaptation, a trait either originally

selected for another purpose or a by-product of a different trait's formation that is now exposed to a novel selective environment, presents the opportunity for a new adaptive function. Developmental capacitance refers to processes that buffer against genetic or environmental variation until a threshold for restraint is reached and breached. The potential was always there, but remained cryptic until exposed. For both possibilities, the emphasis is on what conditions make it possible for natural selection to act and thereby solidify the new trait via its functionality. But population genetic approaches relevant to explaining the origin of novel structures are available also, such as work on canalization and robustness (Hansen, 2006; Wagner, 2005). Although these do not replace or compete with explanations that invoke the rearrangement of ancestral variation through GRN changes, they concentrate on variational properties of phenotypes in order to ascertain their evolutionary potential, which can inform both why a trait is stable and how new structures become individuated evolutionarily (Pavličev and Widder, 2015).

Case Studies

Turtle Shells

The turtle shell represents the evolutionary addition of new parts to the standard vertebrate body plan: a dorsal carapace and ventral plastron connected by lateral bridges – more than 50 dermal bones not found in any other vertebrate (Gilbert *et al.*, 2001). Instead of the vertebrae and ribs encompassing the entirety of the neural and costal bones, they act as initiation centers for the ossifying dermal elements. The ribs grow in a lateral rather than ventral direction, and appear to become trapped within the ossifying dermis. A carapacial ridge, exclusive to turtles, appears on the flanks of embryos in early development and later extends anteriorly and posteriorly. This ridge may direct later development of the ribs, akin to the apical ectodermal ridge (AER) in the vertebrate limb bud, as its removal results in ribs growing ventrally in a manner more consistent with other amniotes (Burke, 1991).

Both ribs and plastron begin ossification prior to hatching; dermal bones of the carapace develop after hatching (Gilbert *et al.*, 2001). The ribs and neural arches undergo three successive stages of ossification to form the carapace: perichondral, endochondral, and membranous (Kuratani *et al.*, 2011). Recent paleontological finds have illuminated intermediate forms relevant to the origin of the shell. *Odontochelys* ($\sim$220 mya) exhibits a complete plastron but only a few neural plates for the carapace (Li *et al.*, 2008). This fossil appears to mirror the developmental origins of the plastron prior to the carapace. However, the evolutionary origin of the plastron remains enigmatic, not least because phylogenetic relationships among turtle species are contested (Lyson and Gilbert, 2009).

A peculiar feature of the skeletal arrangement in turtles is the ventral location of the pectoral girdle in relation to the ribcage, completely encapsulated within the shell, in contradistinction to all other vertebrates (**Figure 2**). Using *Sox9* to mark skeletal precursors, scapula 'anlagen' in turtles were observed to develop more anteriorly compared to mouse and chicken (Nagashima *et al.*, 2009). Given that turtle ribs are comparatively shorter initially, this in combination with the anterior positioning of the scapulae results in an overhang of the first rib (which does not participate in carapace formation) and eventual coverage of the scapulae by the remaining ribs as they grow laterally and anteriorly. Simultaneously, as the ribs fold the lateral body inward, the serratus anterior muscle rotates in a ventral medial direction and pulls the scapula to its final position underneath the developing carapace (Nagashima *et al.*, 2009). Here again, modern turtle development is suggestively consistent with the earliest known fossil (*Odontochelys*), which had its scapulae anterior to the ribs. Additionally, the latissimus dorsi and pectoralis muscles display connectivities that lack an equivalent outside of turtles (Kuratani *et al.*, 2011). The nuchal scute, at the anterior-most portion of the carapace, has been considered a novel structure, but it has been reported recently to derive from the cleithra: paired dorsal elements originally associated with the ancestral tetrapod pectoral girdle that have been lost in other tetrapods except frogs (Lyson *et al.*, 2013). Lyson *et al.* propose that these elements underwent a dorsomedial migration and eventually fused to form the nuchal scute.

Studies of the turtle shell illustrate that an indisputably novel structure might arise through developmental changes that may be less radical than originally thought. However, these minor tweaks along the evolutionary path have led to a profoundly different structure. Identifying the unique changes that resulted in the formation of the shell (e.g., rib outgrowth arrest followed by lateral infolding, serratus anterior muscle rotation, or dorsomedial cleithra migration) is necessary to account for its developmental evolutionary origination.

Bat Wings

Bats are the only mammals capable of powered flight. This advantage has led to an unprecedented adaptive radiation – bats account for over 20% of all mammalian species. The novel structure of bat wings results from a combination of components: greatly elongated digits, retention of interdigital tissues, and specialized wing membranes. In pterosaurs, the only other vertebrates to use a membranous wing for powered flight (Lingham-Soliar, 2015), the membrane was completely supported by the fourth digit; bats use all digits, excluding the thumb, to support the membrane. The distinctive morphology of the bat wing is a direct result of its development, beginning with a unique mechanism for interdigital membrane maintenance.

In bats, only hind limbs experience apoptosis of interdigital tissue (Weatherbee *et al.*, 2006). The retention of interdigital tissue in the forelimb is a result of increased *Fgf8* and *Gremlin* expression, which combine to inhibit cell death and decrease *Bmp* expression. Similar expression is presumed to be responsible for inhibiting cell death in the webbed feet of ducks (Merino *et al.*, 1999). *Fgf8* is normally expressed in the AER of other tetrapods, though in bats it is expressed at earlier stages, remains active later, is expressed in the forelimb interdigital area, and has an AER expression domain that is up to three times wider than mice (Cretekos *et al.*, 2007; Weatherbee *et al.*, 2006). These factors combine to preserve tissue in the bat's wing via a unique mechanism.

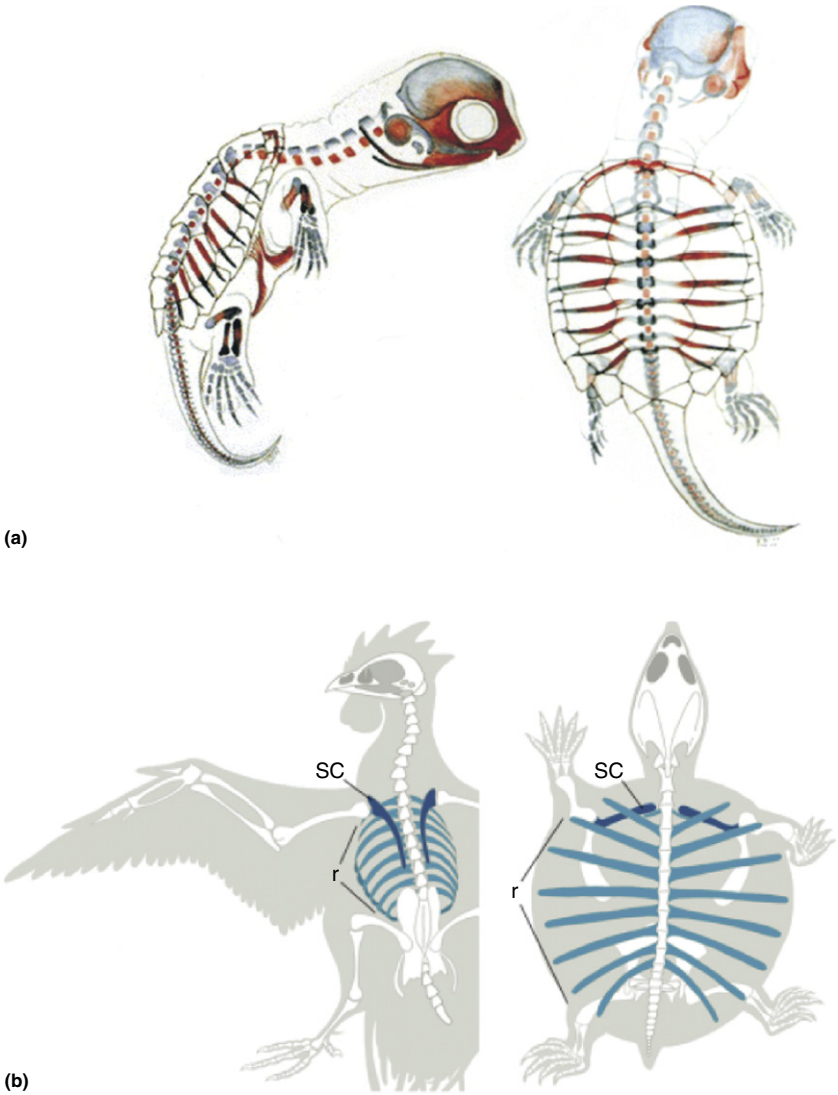

Figure 2 (a) Drawing of cleared and stained turtle embryo with cartilaginous (blue) and calcified (red) structures. Note the development of ribs prior to the formation of the carapace. (b) Drawing of avian scapulae (sc) external to the ribcage (r) compared to turtle scapulae inside of the ribcage. Modified from Gilbert, S.F., Loredo, G.A., Brukman, A., Burke, A.C., 2001. Morphogenesis of the turtle shell: The development of a novel structure in tetrapod evolution. Evolution & Development, 3, 47–58; Kuratani, S., Kuraku, S., Nagashima, H., 2011. Evolutionary developmental perspective for the origin of turtles: The folding theory for the shell based on the developmental nature of the carapacial ridge. Evolution & Development, 13, 1–14.

The earliest known fossil bat dates to ~50 mya. It already possesses the distinct morphology of modern bat wings and has proportionally similar lengths for metacarpals and phalanges (Sears *et al.*, 2006). A prominent hypothesis for the origin of bat flight is that it arose via gliding (Dudley *et al.*, 2007), facilitated by lateral wing membranes (plagiopatagium), similar to that seen in extant gliding animals (squirrels, lemurs, and some marsupials). Plagiopatagium outgrowth starts at stage 14 in bat development (Cretekos *et al.*, 2005) and by stage 17 it begins connecting to digit V of the forelimb. Early cartilage condensations of the limb are similar in size for both mice and bats, but bat limbs begin to drastically elongate later in development. Coincident with this elongation is a pronounced increase in the size of the hypertrophic zone of the metacarpals, accounting for over 30% of the growth plate (Sears *et al.*, 2006); hypertrophic zones in mice never exceed 12%. Bat and mice digits cultured in *Bmp2* show increased elongation, while those cultured in *Noggin* (a *Bmp* antagonist), exhibit stunted growth. *Bmp2* expression is more intense and continuous in bat forelimbs than in their own hind limbs or in mouse forelimbs (Sears *et al.*, 2006).

Shh is expressed in a wider domain within bat forelimbs than in other tetrapods, which produces a loss of symmetry across the anterior–posterior axis. Furthermore, unlike mice at comparable stages, a second round of *Shh* expression is initiated later in bat development and leads to expression of *Ptc1* (Hockman *et al.*, 2008). A novel patch of *Fgf8* expression in bat limbs immediately precedes the initiation of *Shh* and *Ptc1*. The

renewed *Shh* expression site corresponds to the location of *Bmp2* identified in earlier studies. *Bmp2* activates *Gremlin*, which in turn promotes *Fgf* expression. This suggests a *Shh–Fgf* feedback loop (*Fgf8* → *Shh* → *Bmp2* → *Gremlin* → repeat) that contributes to both forelimb elongation and survival of the interdigital membrane (Hockman *et al.*, 2008).

In addition to elongated digits and membrane retention, bats have unique wing musculature. The occipito-pollicalis muscle complex extends along the anterior edge of the wing and supports the anterior wing membrane (propatagium) and sheet-like muscles that run through the plagiopatagium, all of which influence the shape of the wing. The occipito-pollicalis originates from cranial mesoderm, as illustrated via its innervation by cranial nerve VII (facial nerve). Furthermore, *Fgf10* is expressed anterior and posterior to the forelimb and along the flanks, persisting after similar signaling in the limbs of other tetrapods has ceased (Tokita *et al.*, 2012; **Figure 3**).

Within the bat limb there are multiple examples of temporal and spatial gene regulatory changes (heterochrony and heterotopy) that contribute to its unique structure. This amalgam of changes in the expression of a few key genes has transformed the adult phenotype (e.g., *Bmp* reduction between digits and increased activity within digits). When combined with forelimb bones that have enlarged medullary cavities, providing uniquely low bone mass for a mammal (Cooper *et al.*, 2012), and highly compliant and elastic bones (Cooper and Sears, 2013), these results show that many of the developmental steps required in the origin of the bat's skeletal architecture have been elucidated.

Beetle Horns

Beetle horns are among the world's most dramatic sexually selected traits (Emlen, 2000). These rigid extensions

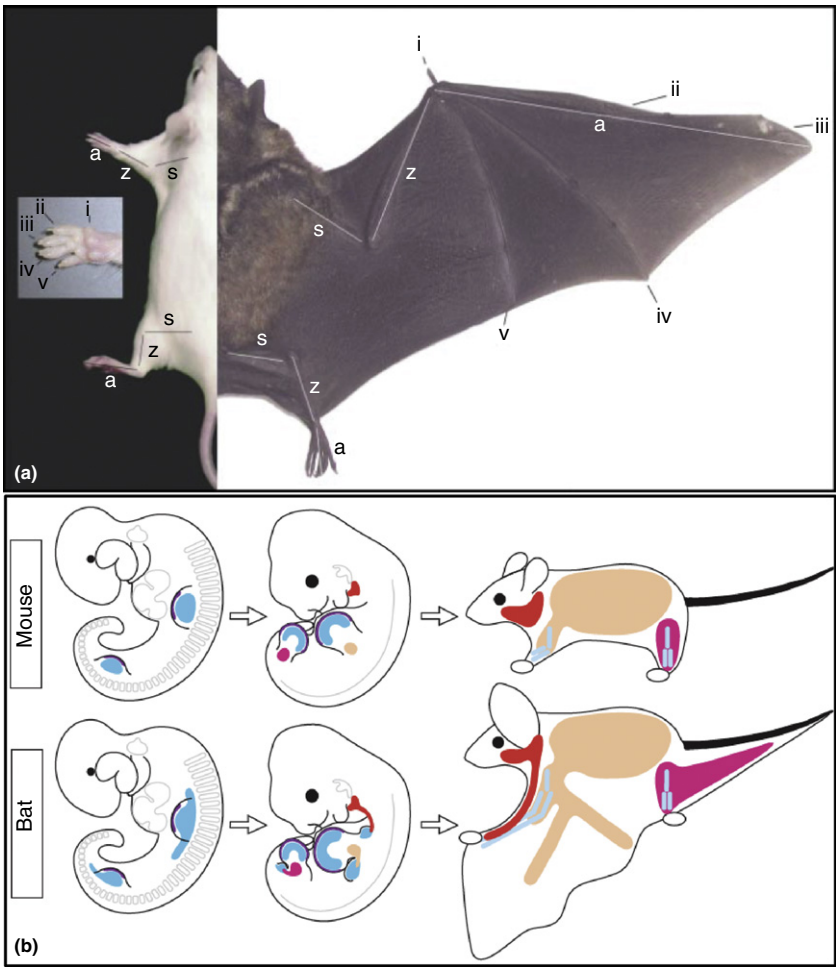

Figure 3 (a) Comparison of limb morphology between mouse (left) and bat (right), digits labeled i–v (anteriorly to posteriorly), lines drawn for stylopod (s), zeugopod (z), and autopod (a). Note that propatagium is anterior to zeugopod, and pluropatagium is connecting the forelimb and hind limb. (b) Illustration comparing muscle and membrane development in mouse and bat. Fgf10 expression (blue) is expanded in bats, where the future wing membrane will develop; Fgf8 expression (purple) in the AER; stylopod, zeugopod, and autopod (light blue); developing muscle (tan) expanding into the wing membrane in bats. Modified from Cretekos, C.J., Rasweiler IV, J.J., Behringer, R.R., 2001. Comparative studies on limb morphogenesis in mice and bats: A functional genetic approach towards a molecular understanding of diversity in organ formation. Reproduction, Fertility and Development 13(8), 691–695; Tokita, M., Abe, T., Suzuki, K., 2012. The developmental basis of bat wing muscle. Nature Communications 3, 1302.

of the exoskeleton have originated repeatedly in evolution and are present in thousands of species (Emlen *et al.*, 2007). Beetle horns display an astounding variation in shape, structure, and location, including the front of the head, base of the head, thorax, or some combination thereof (**Figure 4**). They range from tiny knobs to more than 10% of a beetle's body mass, and horn length scales with body size so that bigger males have longer horns (Moczek and Emlen, 1999). Horn expression is sexually dimorphic, predominantly found in males, where larger horns often equate to disproportionate access to females (Moczek and Emlen, 2000).

Nutritional conditions during larval stages determines both adult body size and horn length (Moczek, 1998). Horns develop at the same time and manner as many other paired appendages (eyes, legs, wings) via imaginal discs. These invaginations detach from the epidermis and undergo rapid cellular division, mediated by juvenile hormone (JH), to quickly increase in size (Emlen and Nijhout, 1999). A better nutritional environment leads to higher concentrations of JH, which in turn results in larger, horned adults. Any mutation that affects JH expression and signaling has the potential to impact horn evolution (Emlen, 2000). Horn development also affects nearby morphological traits. The size of cephalic horns can be negatively correlated with eye size, and thoracic horns can be negatively correlated with wing size (Nijhout and Emlen, 1998). These tradeoffs vary between species.

Beetle horns lack obvious homology to other insect traits, but share similar developmental aspects with .appendages (Moczek and Nagy, 2005). *Distal-less* (*Dll*) is expressed in the mediodistal portions of legs and horns, and *homothorax* (*hth*) is expressed in the proximal and medial portions of legs and horns (Moczek *et al.*, 2006). Both expression patterns have been co-opted and conserved. However, *dachshund* (*dac*) expression is found in the proximal and medial portions of legs, but is expressed throughout the entire horn (Moczek *et al.*, 2006). Although *dac* expression is not required for horn development, Moczek and Rose (2009) found that *Dll* affects head and thoracic horns, whereas *hth* affects thoracic horns only. The differential co-option of genes that function to pattern appendages plays a central role in the developmental evolution of horns.

Expression patterns co-opted for horn development not only diverge from what is observed for appendages but also differ within and across beetle species. *Doublesex* (*dsx*) is consistently elevated in male horns but not found in legs (Kijimoto *et al.*, 2009). In some species, *dsx* knockdown reduces the size of male head horns, but induces the formation of horns in females (Kijimoto *et al.*, 2012). Thus, *dsx* typically promotes male horn development and suppresses female horn development, conditional on nutritional intake in larvae. In other species, *dsx* knockdown caused large branching horns in previously hornless males, while resulting in a single horn for females (Kijimoto *et al.*, 2012). This illustrates a dramatic reversal in what is normally a sexually dimorphic trait and provides an example of how labile regulatory inputs (e.g., nutritional conditions or sex specific variables) can differentially interact with highly conserved gene expression to produce novel morphologies.

Insights

Prospects for Advancing Inquiry

The turtle shell, bat wing, and beetle horns capture only a small subset of novel structures currently under investigation. These case studies illustrate the emphasis in Evo-devo on changes in gene expression during development as the primary explanatory factor for novelties. At the same time, they demonstrate how explanations require multiple disciplinary contributions, such as the morphology and paleontology that helps to delineate the character state of the ancestral lineage to establish a phylogenetic context for explaining the turtle shell or bat wing, or the role of developmental ecology that assists in accounting for the growth dynamics of beetle horns. In conclusion, we describe three dimensions of inquiry – conceptual, empirical, and theoretical – that hold promise for future inquiry into the origins of evolutionary novelty.

Inquiry related to the conceptual dimension can advance our understanding of the origin of novelty by intentionally exploring different definitions of novelty. Many researchers operate with a single preferred conception of novel structures. If we think of these conceptions as different models (Wagner, 2014), then switching between them focuses our attention on different properties of biological systems and encourages analyzing the evolutionary significance of different causal factors. The key is to recognize that distinct standards or criteria of explanatory adequacy accompany these different models and foreground some factors while relegating others to the background. Research on the origin of beetle horns is an exemplar in this regard, moving back and forth between developmental genetics and evolutionary ecology. Additionally, new concepts can suggest new explanatory expectations, such as character 'swarms' and 'variational modalities' (Wagner, 2014). Explanatory accounts derived from different definitions may be more complementary than competing, especially those that appeal to different kinds of developmental mechanisms or adaptive benefit. Progress with respect to explaining the developmental evolution of novel structures is nurtured when explanatory aims are articulated precisely, their scientific significance is widely comprehended, and the standards that determine the elements and structure of an adequate and integrated explanatory framework are as explicit as possible (Brigandt and Love, 2012).

Inquiry related to the empirical dimension can advance our understanding of the origin of novelty by increasing the manipulative capacity of experimentation and augmenting our inferential capacities. The ability to precisely change genetic variables and ascertain their phenotypic effects has long played a key role in explaining novelty, but new technologies, such as CRISPR-Cas genome editing (Sander and Joung, 2014), will provide more discriminating tests of hypotheses for putative novelties in particular species. Continued sequencing efforts, both to catalog genes and characterize their expression profiles (e.g., via RNA-seq; Wang *et al.*, 2009), will be critical as well. However, as noted, explaining the origin of novel structures requires a combination of disciplinary approaches and therefore empirical advances are needed on multiple fronts. Morphological and paleontological investigations have illustrated this dramatically in the case of the tetrapod limb

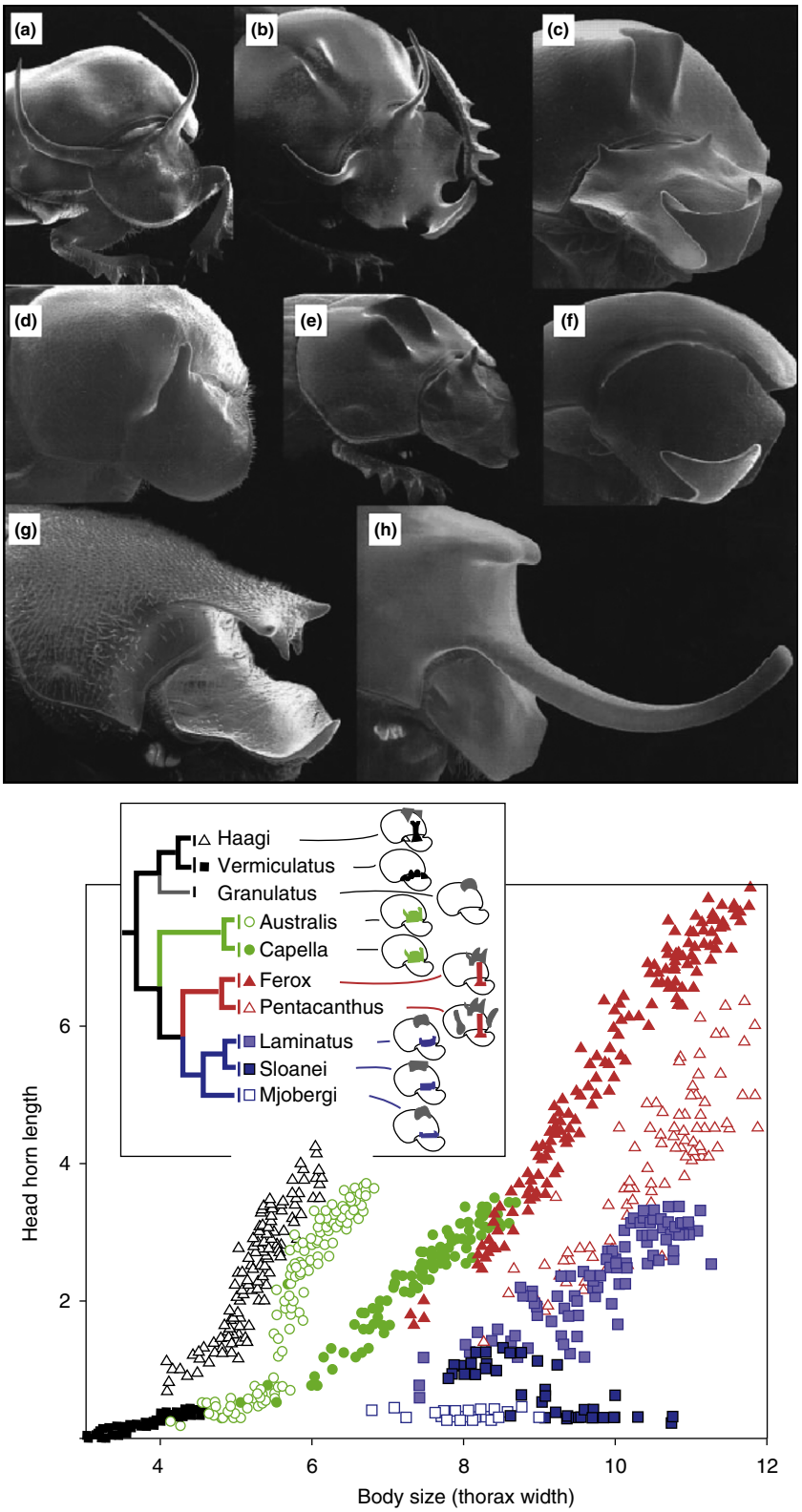

Figure 4 Top. Photographs (a–h) showing the diverse shapes, sizes, and locations of beetle horns within the genus *Onthophagus*. Bottom. Scaling relationships between horn length and body size for nine species of *Onthophagus* beetles. Modified from Emlen, D.J., 2000. Integrating development with evolution: A case study with beetle horns results from studies of the mechanisms of horn development shed new light on our understanding of beetle horn evolution. Bioscience 50, 403–418; Emlen, D.J., Lavine, L.C., Ewen-Campen, B., 2007. On the origin and evolutionary diversification of beetle horns. Proceedings of the National Academy of Sciences of the United States of America 104, 8661–8668.

(Shubin *et al.*, 2006). Integrating developmental genetic and physico-chemical mechanisms can augment our understanding of how new traits originate (e.g., Sheth *et al.*, 2012). Finally, increasing phylogenetic resolution can change how existing empirical data from various approaches bear on explanations of novelty (e.g., Kocot *et al.*, 2011). Investigating evolutionary novelties that represent the best available juxtaposition of these empirical tools is likely to generate the deepest insights, though it may mean choosing new and unexpected model systems (Wagner *et al.*, 2014).

Inquiry related to the theoretical dimension can advance our understanding of the origin of novelty by probing more quantitative dimensions of the genotype–phenotype map and drawing out abstract generalizations across disparate systems. The former provides a bridge to the rich tradition of population genetic theorizing that has been perceived as antagonistic to Evo-devo's developmental explanations of novelties (Nunes *et al.*, 2013). It also introduces different concepts into explanatory accounts (e.g., pleiotropy or epistasis) that expose new angles to understand the origin of qualitatively distinct variation at particular phylogenetic junctures (e.g., Pavličev and Wagner, 2012; Rice, 2012). More abstract generalizations can be derived from particular forms of theorizing by identifying commonalities across taxa and levels of organization, such as shared network architecture in metabolism and gene regulation (Wagner, 2011). Furthermore, this theorizing need not be concerned only with population level modeling. While the jury is still out on Wagner's genetic theory of homology (Wagner, 2014), it has distinct advantages in suggesting specific kinds of manipulative experiments in different taxa. Overall, it will be innovative combinations of these different types of advances in conceptual, empirical, and theoretical dimensions that harbor the most promise for increasing our understanding and providing deeper explanations of the developmental evolution of novel structures in animals.

See also: Adaptation, History of. Developmental Plasticity and Phenotypic Evolution. Evolvability, Quantitative Genetics of. Gene Networks Driving Development, Conservation and Evolution of. Genotype to Phenotype: Insights from Evo-Devo

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Novel Structures in Plants, Developmental Evolution of

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Glossary

Abaxial The side of a laminar organ located further away from the axis.
Adaxial The side of a laminar organ located closer to the axis.
Centripetal The growth direction of organs from the outside to the inside.
Co-option The acquisition of new functional repertoires by a gene, different from the preexisting ancestral ones or the recruitment of an alternative gene to that commonly associated with a feature.
Ectopic The expression of a gene or occurrence of a feature outside of the common place or position.
Flower primordium (a) The earliest recognizable developmental stage of an organ, often formed exclusively by parenchymatic homogeneous tissue.
Gametogenesis The process by which haploid spores undergo division and differentiation to form mature gametes. In land plants the spore formation by meiosis is followed by mitosis generating a multicellular haploid phase in the life cycle.
Groundplan The phylogenetically fixed patterns of (mostly) floral structure referring to the organization of parts.
Heterochrony A shift in the timing of a developmental event.
Heterologous transformation The gene functional characterization experiments that commonly use overexpression of a target gene from a non-model species in a model organism.
Homeotic A shift in the identity of an organ or any developmental feature.
Homolog The biological features (genes, organs, structures) sharing common ancestry.
Horizontal gene transfer The transfer and acquisition of genes between different species using alternative mechanisms to reproduction and heritage.
Meristems The pluripotent cell groupings in the plant body.
Metatopy The placement of organs or structures out of their regularly expected positions resulting often from abnormal growth.
Orthologs The genes derived from speciation events that correspond to the same copy across related organisms.
Paralogs The genes derived from duplication events that correspond to gene copies.
Páramos High mountain Andean biomes found below the permanent snowline, with extreme temperature fluctuations from day to night, composed of grasses, unique rosette, and cushion plants.
Pluripotency The developmental potential in cells without a committed identity of differentiating into any cell type.
Radialization The loss of ab/adaxial identity in a laminar organ resulting in a cylindrical structure.
Regulatory module The interactions between a few transcription factors that have been phylogenetically and functionally conserved from the collection of hierarchical genes in a complete gene regulatory network.
Resupination The rotation or recurving of a structure during development.
Shoot apical meristem (SAM) The site of active cell division in the apex of the shoot that allows for the maintenance of pluripotent cells and open growth in plants above ground. It constantly nourishes three generating layers: the protodermis, ground tissue, and procambium.
Sporogenesis The process by which diploid cells undergo meiosis to form spores.
Subfunctionalization The partition of ancestral functions among gene copies from an ancestral single, often pleiotropic gene.
Synorganization The integration of floral organs by changes in symmetry and organ fusion.
Whole genome duplication (WGD) The event by which a cell or an organism retains two copies of the entire genome.

Introduction

Plants, unlike animals, grow continuously from embryonic cell groups called meristems, which are responsible for primary and secondary histogenesis, organogenesis, and ultimately, morphogenesis. In addition to the plant genetic and epigenetic machinery, these processes are driven also by a number of environmental conditions. Plant lifespan and indeterminate (sometimes called open) growth and organogenesis contrast with the lack of cell migration and the mechanical constraints of intercellular communication caused by the cell wall. All plant organs are formed from three fundamental tissues (protodermis, procambium, and ground meristem; Foster and Gifford, 1980; Steeves and Sussex, 1990), from which no more than 20–25 cell types are formed upon complete differentiation, only 10% of the estimated number of cell types in vertebrates. Additionally, their limited individual movements prevent plants from escaping predators, finding better sources of nutrients, or avoiding harsh environments (Steeves and Sussex, 1990; Cronk, 2009). Instead, plants have developed unique signaling systems that integrate their growth, physiology, and phase transitions with environmental conditions. Thus, postembryonic development in plants is often modular and cyclic, as the position and fate of

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meristems are highly predictable over time, including the development, growth and renewal of stems, leaves, and reproductive organs.

Indeterminate growth and morphogenesis have allowed plants to achieve an incredible array of modifications in form, both above and below ground that often result in convergent features in response to a particular environment, although root growth is less predictable and occurs at a more random fashion in comparison with the aerial shoots. Examples of convergent life forms are several mosses, ferns, and angiosperms from cold, high mountain areas, which grow as 'cushion plants' (**Figure 1(c)**) due to the reduced size, tightly congested branching, compact leaves, and clonal growth at the ground level. Other examples are leaf shape, starch storage organs, and flower elaboration for pollinator attraction, some of which are explained below.

As a result of the remarkable variation in plant form in response to the environment, a major challenge arises when attempting to recognize homologous structures. Comparative morphology takes into account key approaches, traced back to the morphological writings on plant metamorphosis by J. W. von Goethe, the German poet, philosopher, and one of the most outstanding members of the *Naturphilosophie* school. Goethe proposed that all plant organs are nothing but leaves, more or less contracted, or expanded during the individual but continuous metamorphosis (Goethe, 1790; Foster and Gifford, 1980). This explanation was the onset of the modern concept of serial homology, based on positional and developmental similarities of intraindividual structures (Foster and Gifford, 1980; Rutishauser and Sattler, 1985; Weberling, 1989; Endress, 1994). Thus, independently of how an organ looks, if is derived laterally from the shoot apical meristem it is a leaf or a leaf homolog, and if it occurs axillary to this leaf (i.e., an axillary meristem), it is a branch homolog. Following this same idea, during the reproductive phase of the plant, a lateral organ formed along the inflorescence axis is a bract homolog. In turn, a bract subtends a floral meristem, which is a branch homolog formed by floral organs. In flowers, axillary meristems are suppressed, and internodes along the floral axis are extremely shortened or lacking; thus, floral organs develop in a very limited domain called the floral receptacle, often in a centripetal sequence, as follows: sepals, petals, stamens, and carpels. Their order, position, and function allow their identification in most flowers, as they organize into the typical four whorls, calyx, corolla, androecium, and gynoecium, respectively. Sometimes extra whorls arise or there are extraordinary events of switch of functions, homeotic and heterochronic shifts, fusion of parts, or even inverted positions of whorls. Some of these processes will be discussed below in the section of floral morphology. These developmental and evolutionary traits challenge the interpretation of homology in flowers, but at the same time makes the study of floral morphology one of the most fascinating topics in contemporary plant biology (Weberling, 1989; Endress, 1994; González and Pabón-Mora, 2015).

Development is an additional criterion emphasized here that can be used to test plant homologous structures, as homologous structures should follow a common ontogenetic pathway that can be traced during early organogenesis and histogenesis (Hofmeister, 1862; Foster, 1928; Wardlaw, 1952; Gifford, 1954; Cronk, 2009). One of the most outstanding examples is the identification of structural homologies in fruits. Fruits undergo drastic morphogenetic, ontogenetic, and histogenetic modifications from the same floral organ, the carpel(s); they have been highly exposed to selective forces for at least 120 My, and have developed extremely efficient mechanisms for food storage and seed dispersal, which ultimately have allowed flowering plants to be dominant on Earth (Roth, 1977).

A third approach, key to comparative morphology is the study of developmental genetic mechanisms underlying organ position, identity, and polarity in plants (Steeves and Sussex, 1990; Cronk, 2009). It has been established that specific transcription factors alone or in combinations are responsible for the identity of the different lateral organs during vegetative and reproductive growth. In particular, molecular developmental biology studies in model plants, including *Antirrhinum majus* (Plantaginaceae), *Arabidopsis thaliana* (Brassicaceae), and *Oryza sativa* and *Zea mays* (Poaceae), have identified specific genetic modules that are turned on to control, for instance, leaf differentiation, polarity, and shape. Leaf morphogenesis is known to be regulated by genes that control differentiation of the adaxial or upper face versus the abaxial or lower face, like the HDZIP-III transcription factors, and other genes that control determinacy and leaf dissection, like the KNOX and the TCP genes (Kidner and Timmermans, 2010; Koenig and Sinha, 2010).

Similarly, floral organ identity has been attributed to specific combinations of transcription factors, in this case MADS-box and APETALA2/ERF proteins. They are turned on at specific time points after floral meristem initiation to control the identity of each primordium and to regulate downstream targets accordingly to the role of each organ. In stamens and carpels, downstream target genes of organ identity genes include those responsible for sporogenesis and gametogenesis, whereas in petals they often include epidermal changes such as papillae differentiation and pigment accumulation to attract pollinators (Coen and Meyerowitz, 1991; Weiss, 2000).

Homologous structures, besides sharing a common position, and the same ontogenetic trajectory, are also expected to be controlled by the same genes. However, expression, functional and evolutionary studies of genes involved in leaf and flower morphogenesis in non-model organisms have shown that gene lineages have functionally evolved independently, often resulting in the recruitment of gene homologs into different functions across taxa. Integrating gene lineage evolution studies into evolutionary and developmental questions has turned out to be extremely informative, as plant genomes are the result of cumulative rounds of whole genome duplication or WGD (Soltis *et al.*, 2010, 2014; Jiao *et al.*, 2011). Thus, plant genomes vary dramatically in terms of copy number from a particular gene family, their regulation and likely their interaction partners, thus, raising the possibilities that gene functional evolution has been affected by gene and genome duplications. Specific case-by-case scenarios have been described by Adams and Wendell (2005), Flagel and Wendell (2009), and Wendell (2015), among many others. Recently, numerous reviews have addressed comparative floral development and the understanding of floral evolution from an evo-devo approach, highlighting some case studies of plant

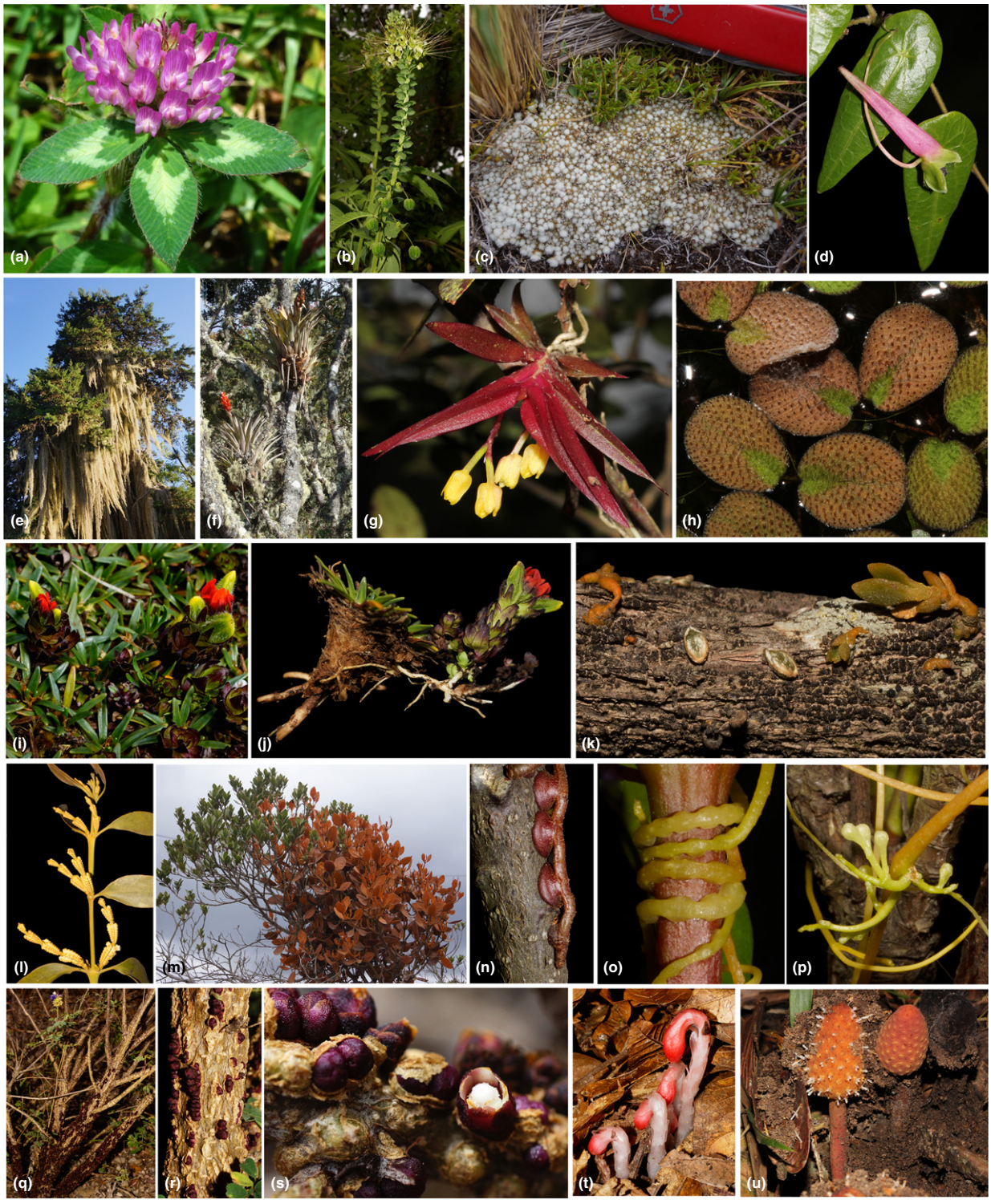

Figure 1 Plant life forms. (a) Terrestrial herb, *Trifolium pratense,* Fabaceae. (b) Shrub, *Cleome anomala,* Cleomaceae. (c) Páramo cushion plant, *Paepalanthus lodiculoides*, Eriocaulaceae. (d) Vine, *Tropaeolum lindenii*, Tropaeolaceae. (e) Hanging epiphyte, *Tillandsia usneoides,* Bromeliaceae, on a tree of *Cupressus lusitanica*, Cupressaceae. (f) Epiphytic tank plant, *Tillandsia turneri*, Bromeliaceae. (g). Epiphytic hanging orchid *Pterostemma antioquiense.* (h) Aquatic herbaceous fern, *Salvinia* sp., Salviniaceae. (i)–(j) Facultative hemiparasite, *Castilleja paramensis*, Orobanchaceae (to the right), and its host *Plantago rigida*, Plantaginaceae (to the left), in high Andean Paramos. (k)–(l) Green, obligate hemiparasite, *Phoradendron nervosum*, Viscaceae; seeds and seedlings (k) and a flowering branch with modular inflorescences (l). (m) Brown, obligate hemiparasite, *Dendrophtora avenia*, Viscaceae. (n) Epicortical root with three secondary cup-shaped haustoria of *Antidaphne viscoidea*, Eremolepidaceae on its host *Quercus humboldtii* (Fagaceae). (o)–(p) *Cuscuta* sp., Colvolvulaceae, haustoria (o) and floral buds (p). (q)–(s) Holoparasite *Pilostyles boyacensis*, Apodanthaceae, on its host *Dalea cuatrecasasii*, Fabaceae. (t)–(u) Micoheterotrophic *Monotropa uniflora,* Ericaceae (t), and *Helosis* sp. Balanophoraceae (u).

adaptation and innovation that have investigated the relationships between gene duplications, gene regulatory networks, and morphological diversification (Specht and Howarth, 2014; Glover *et al.*, 2015).

Here, we provide an overview of plant life forms and morphology, including leaf shape, inflorescence architecture, flower and fruit morphology, and briefly comment on the underlying genetic bases for organ identity. Finally, we discuss novel structures in two case studies of non-related plant lineages with parallel evolution of similar flower synorganization and fruit variation. The reader will notice that most of the examples are in tropical plants, as one of our goals is to find non-model organisms among the fascinating repertoire of shape variation in the tropics.

Habit or Life Form

Land plants or embryophytes dominate the Earth in terms of number of species and diversity of life forms, life histories, and niches they occupy. Their forms have evolved most likely from small aquatic plants that needed water most of the time as a substrate and a vector for reproduction, to large woody plants that can reach up to 80+ meters tall and 6+ meters in diameter. Embryonic tissues, called meristems, have provided land plants the great advantage of continuous primary growth (i.e., in length), as well as secondary growth (i.e., in width) and elaboration of an efficient vascular system for water and nutrient transport. With the acquisition of vascular tissue, every major lineage of land plants diversified more or less independently into extraordinary life forms, including herbs, either annual or perennial (**Figures 1(a)–1(c)**); shrubs, with more or less woody, highly branched individuals without a distinct trunk; climbers, either vines (herbaceous) or lianas (woody) (**Figure 1(d)**); and trees, which are woody plants usually 5+ meters tall, with a main trunk that clearly differs in growth from lateral or higher order branches (**Figure 1(e)**). Two life forms that are frequent in alpine and high mountain environments are the cushion plants, when one or more individual produces numerous branches that tightly grow just above the ground (**Figure 1(c)**); and the rosette plants, when the leaves are tightly arranged on the distal portion of elongated (simple or compound) stems that can reach up to 10 meters tall (**Figure 1(j)**).

Epiphytic plants, that is, those plants that grow on other plants without taking nutrients from them, appear also in every major lineage of land plants, including members in the bryophytes, ferns and allied, and flowering plants (**Figures 1(e)–1(g)**); only a few instances of epiphytes (e.g., *Zamia pseudoparasitica*, Zamiaceae) are known to occur among gymnosperms. Epiphytes are much more frequent in wet, tropical forests. Flowering plant families with a high number of epiphytes are bromeliads (**Figures 1(e)** and **1(f)**), orchids (**Figure 1(g)**) and aroids, among others. Notably, epiphytism and changes in metabolism have been linked with the orchid radiation, a family with notorious diversification of life forms and floral displays reaching over 25 000 species (Silvera *et al.*, 2009; Givnish *et al.*, 2015). Although epiphytes are more abundant among monocots, a few eudicot families also show a number of epiphytic species, including the Ericaceae, Gesneriaceae, Melastomataceae, and Rubiaceae (Benzig, 1989).

Parasitic plants are extraordinary life forms among several lineages of seed plants, including one gymnosperm and members of at least 12 non-related lineages of flowering plants (Heide-Jørgensen, 2008). They have developed structural and physiological mechanisms that allow them to take part or all of the nutrients they need from their hosts, usually other flowering plants. Four categories of parasitic plants are recognized, depending on how invasive and obligate such mechanisms are: (1) facultative hemiparasites are those that maintain its photosynthetic properties and can survive with or without the host; this is the case of the 'Indian paintbrushes,' the common name for several species of *Castilleja* (Orobanchaceae; **Figures 1(i)** and **1(j)**). (2) Obligate hemiparasites, which still keep their photosynthetic repertoire but cannot complete their own life cycle without a host; this is the case of many families of the order Santalales, three of which (Eremolepidaceae, Loranthaceae, and Viscaceae, commonly known as mistletoes) exhibit conspicuous parasitism and some of the most penetrating organs, called haustoria (**Figures 1(k)** and **1(n)**), which can even break through hard, tropical woods, such as those of Fagaceae, Rosaceae, or Salicaceae, among many others; some can even develop epicortical roots and secondary disk-like haustoria (**Figure 1(n)**). Common hemiparasites in the tropics also include the 'dodders,' *Cuscuta* (Convolvulaceae), which develops series of discoid holdfasts directly from the soft but strangler coiled stems (**Figures 1(o)** and **1(p)**); unlike vascular systems in haustoria of most parasitic plants, which are mainly formed by xylem, those in *Cuscuta* are also formed by phloem. (3) Holoparasites are those that lost all the photosynthetic properties, as well as all or most of its vegetative organs, as the Apodanthaceae (**Figures 1(q)** and **1(s)**), the Hydnoraceae and the Rafflesiaceae. (4) Myco-heterothrophic plants, when the host–parasite is mediated by underground fungi forming a tripartite system that also causes complete suppression of photosynthesis and extreme modifications in the vegetative body of the parasite; examples are *Monotropa* (Ericaceae; **Figure 1(t)**) and all members of the Balanophoraceae (**Figure 1(u)**).

Current research topics in the study of plant parasitism are: (1) Morphological and physiological changes in both the parasitic and the host plant with emphasis in the molecules that can be exchanged both ways (e.g., Vaughn, 2003; Birschwilks *et al.*, 2006). (2) Genetic changes in both parasite and host, in particular the occurrence of horizontal gene transfer (HGT) and the profound genomic rearrangements and reduction particularly in the parasite mitogenomes (e.g., Skippington *et al.*, 2015); these mechanisms are of great interest in the understanding of processes downstream of the first infection, as well as the maintenance of the parasitic strategy not only during ontogeny but also during evolution (e.g., Mower *et al.*, 2010; Yoshida *et al.*, 2010; Xi *et al.*, 2012, 2013). (3) Comparative transcriptomic analyses of the unique modified root system into a haustorium, the highly modified intrusive organ responsible for host penetration and nutrient taking. Recent studies of different parasitic plants during early stages of haustorial establishment have identified a number of key 'parasitism' genes that include proteases, cell wall modifying enzymes, and extracellular secretion proteins (Yang *et al.*, 2014).

Leaves

Leaf Morphology Variation

Leaves are lateral organs that usually perform photosynthesis. Leaves are extremely diverse in architecture, size, form, and function. A major trend in leaf evolution is the switch from leaves with one midvein (in lycophytes such as Selaginellaceae and Lycopodiaceae, and some fern allies such as Equisetaceae; Figures 2(a) and 2(b)) to leaves with dichotomous venation (as in most ferns and the gymnosperm *Ginkgo*; Figures 2(c) and 2(d)) to leaves with complex vein systems along a midvein in other gymnosperms (such as Cycadales and some Gnetales, Figure 2(e)), and most flowering plants (Figures 2(f)–2(p)).

Leaf position along the stem is one of the most useful taxonomic traits, especially in seed plants. Leaves can be opposite, if they arise at the same node (Figures 2(d)–2(h)); or alternate, when only one leaf per node is formed (Figures 2(i)–2(k)). A special case occurs when leaves are whorled, that is when more than two leaves are formed per node (Figure 2(l)). Sometimes, chaotic leaf position is due to the formation of aerial tubers, such as in *Anredera baselloides* (Basellaceae). A metatopic phenomenon called anaphysis can drastically displace a leaf to an ectopic, supranodal position, giving rise to pseudo-opposite (such as in several Solanaceae), pseudo-whorled or pseudo-alternate leaves.

Leaves have a marked dorsiventral polarity, and very often the adaxial surface is oriented upwards. In some taxa such orientation is inverted due to resupination, that is, the torsion of the petiole. Inverted orientation can affect leaves from one side in branches with two-ranked leaf arrangement, as in *Retrophyllum* (Podocarpaceae Figure 2(i)), or all leaves, as in *Bomarea* (Alstroemeriaceae Figure 2(k)).

Leaf shape and size often varies drastically in the same individual. Variation includes changes in width and length, color, texture, and dissection. This variation occurs via three main mechanisms: (1) heterophylly, if juvenile leaves are drastically different from the adult leaves (Figure 2(n)); (2) heteroblasty, if leaves of the main branches are different from those of the higher order branches; this is evident in *Berberis* (Berberidaceae; Figures 2(o)); or (3) anisophylly, when the two (rarely more) leaves of the same node are unequal in size; this occurs in taxa with opposite (rarely whorled) leaves, such as some Asteraceae (Figure 2(p)), Melastomataceae, Rubiaceae, and Solanaceae.

Early leaf development begins with the differentiation of two portions, the hypophyll, that later will form the leaf base and the stipules (Figure 2(o)); and the epiphyll, which later forms the petiole and the lamina (Figure 2(o)).

The most conspicuous structural modification of leaves lies in the dissection (Figure 3(e)) or division (Figures 3(f)–3(i)) of the lamina into several portions (Figure 3(e)–3(i)). If the portions become completely separate from each other, the leaf is considered compound (as opposed to simple; Figures 3(f)–3(i)), and each portion is called a pinna (in ferns) or a leaflet in seed plants (e.g., *Cycas*; Figure 3(i)). Programmed cell death (PCD) can heavily dissect simple entire leaves resulting in compound or perforated leaves.

Stipules are lateral outgrowths of the leaf base in a vast number of flowering plants. They are taxonomically important and functionally diverse, although in most taxa they are vestigial and do not have any apparent function. Three of the most noticeable functions of stipules are secretion through extrafloral nectaries (e.g., *Passiflora*, Passifloraceae; Figure 3(a)), bud protection (e.g., *Trifolium*, Fabaceae; Figure 3(b)), or mechanical support as tendrils (e.g., *Smilax;* Figure 3(c)). In the case of *Cecropia* (Urticaceae; Figure 3(d)), *Ficus* (Moraceae), and *Magnolia* (Magnoliaceae) their protective role is evident as they overtop and tightly enclose the shoot apical meristem; this is why they are sometimes considered (erroneously) as terminal.

Leaf variegation occurs when some areas of the lamina are white, yellowish, gray, or other colors but green (e.g., some Commelinaceae, Figure 3(m); some Solanaceae, Figure 3(n)). Leaf structure and function changes over time occur in many seed plants that grow in temperate or cold conditions. One of the most common modifications in leaf structure and function occurs in many seed plants that grow in temperate or cold conditions. In these plants it is common that the first series of leaves on each branch are modified into scale leaves or cataphylls (Figures 3(i)–3(k)). They are extremely important for protections of the vegetative and reproductive meristems during dormancy. Other leaf modifications include: spines in cactae (Figure 3(l)); tendrils (e.g., in some Asteraceae such as *Mutisia*, in which the terminal leaflets of a compound leaf are transformed into tendrils: Figure 3(h)); trap-leaves, either by sticky, glandular hairs (such as in *Drosera*, Droseraceae), tactism such as the *Dionaea* (Droseraceae); or pitcher-like modifications such as those in Nepenthaceae or Sarraceniaceae.

Genetic Basis of Leaf Development

The genetic basis of leaf development is a major theme in developmental biology. Leaves are thought to develop in the following phases: (1) establishment of the dorsiventral, proximodistal, and mediolateral domains; (2) expansion of the lamina; and (3) differentiation of tissue-specific domains (Tsukaya, 2006; Efroni *et al.*, 2010; Nakata *et al.*, 2012). The abaxial–adaxial polarity results in functionally and structurally important differences between the upper and the lower surfaces in adult leaves. In *Arabidopsis* several transcription factors have been identified as controlling this process, including *ASYMMETRIC LEAVES1* (*AS1*), *AS2*, *CLASS III HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIPIII*), *FILAMENTOUS FLOWER* (*FIL*)/*YABBY*, and *KANADI* (*KAN*), among others (McConnell and Barton, 1998; Siegfried *et al.*, 1999; McConnell *et al.*, 2001; Emery *et al.*, 2003; Eshed *et al.*, 2004; Zgurski *et al.*, 2005; Kidner and Timmermans, 2010). The orthologs *PHANTASTICA* (*PHAN*) in snapdragon and *LePHAN* in tomato determine the adaxial domain in leaves; thus, their mutants exhibit radialized (that is, loss of dorsiventrality) needle-like leaves, with abaxial identity surrounding only xylem tissue (Kim *et al.*, 2003; Waites and Hudson, 1995; Kidner and Timmermans, 2010). Collectively these genes are known as the *ARP* (after *AS1*, ROUGH *SHEATH2* (*RS2*, in maize) and *PHAN*). They are expressed at the site of leaf initiation and in young leaf primordia, and antagonize *WUSCHEL* and Class 1 *KNOTTED1-LIKE HOMEOBOX* (*KNOX1*) genes (Waites *et al.*, 1998; Timmermans *et al.*, 1999; Ori *et al.*, 2000;

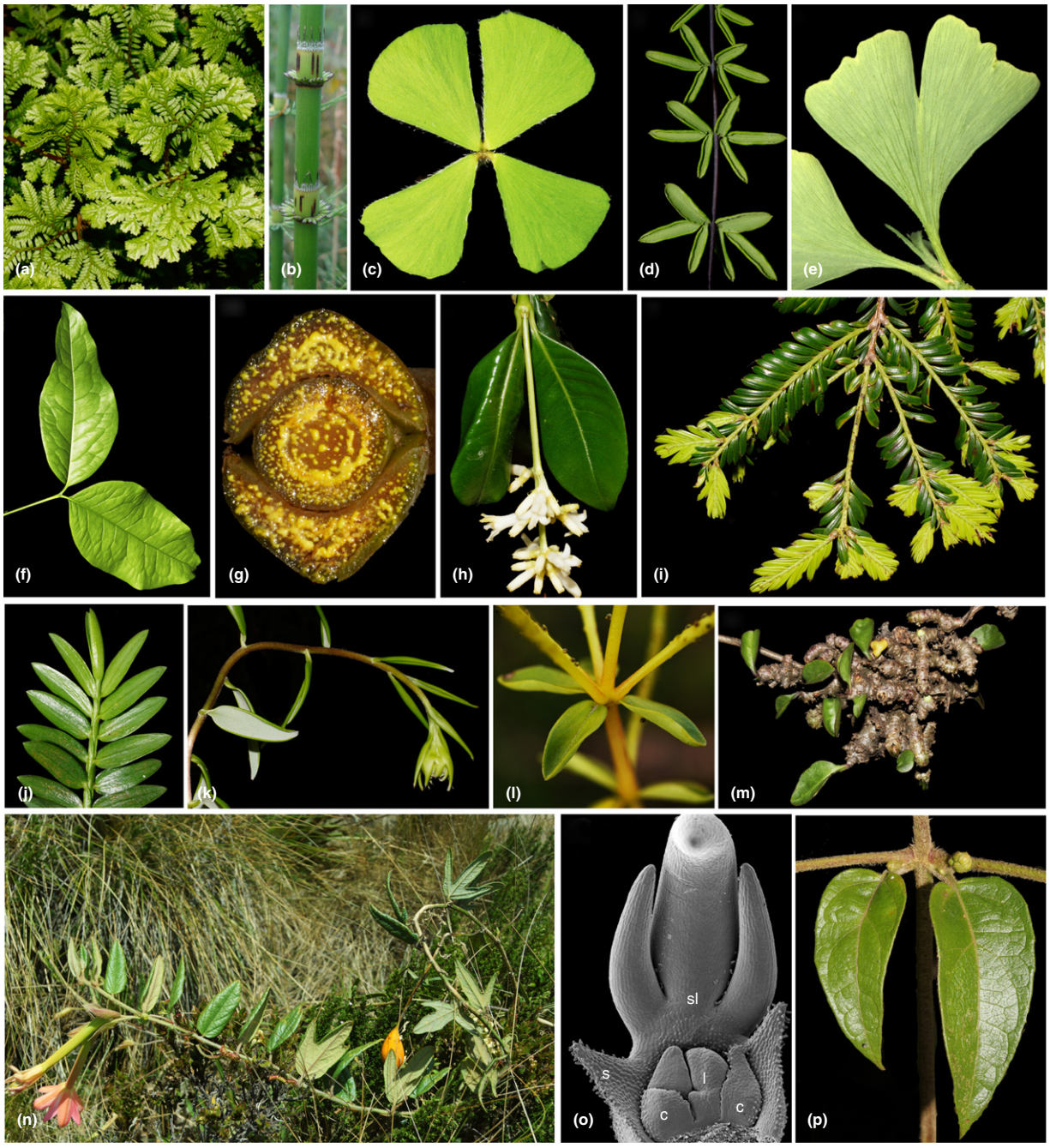

Figure 2 Leaf organization. (a)–(b) Microphylls. (a) Four-ranked branches with microphylls of *Selaginella diffusa*, Selaginellaceae. (b) Whorled microphylls of *Equisetum gigateum*, Equisetaceae. (c) Paired leaflets of the aquatic fern *Marsilea mollis*, Marsileaceae. (d) Paired three-segmented leaflets of the fern *Pellaea ternifolia*, Pteridaceae. (e) Fan-shaped, bilobed leaf of the gymnosperm *Ginkgo biloba*, Ginkgoaceae. (f)–(h) Opposite leaves of the gymnosperm *Gnetum* sp., Gnetaceae (f), and the angiosperms *Clusia multiflora*, Clusiaceae (g) and *Psychotria boqueronensis*, Rubiaceae (h). (i)–(k) Alternate leaves in the gymnosperms *Prumnopitys montana* (i) and *Retrophyllum rospigliosii*, Podocarpaceae (j), and the angiosperm *Bomarea* sp., Alstroemeriaceae (k). (l) Whorled leaves in *Peperomia* sp., Piperaceae. (m) Chaotic leaf position due to aerial tubers in *Anredera baselloides*, Basellaceae. (n) Heterophylly in *Passiflora adulterina*, Passifloraceae. (o) Heteroblasty in *Berberis glauca*, Berberidaceae. (p) Anisophylly in *Mikania* sp., Asteraceae. c, cataphyll; l, leaf; s, stipule; sl, spiny leaf.

Katayama *et al.*, 2010), which promote meristem indeterminacy (Byrne *et al.*, 2000, 2002). The ARP-KNOX gene regulatory module also antagonizes proximal (close to the axis) and distal (afar away from the axis) cell division in formed leaves. While *ARP* genes promote distal identity, *KNOX1* genes maintain proximal cell division in leaf development after this has ceased in more distal areas (Schneeberger *et al.*, 1998; Tsiantis *et al.*, 1999; Byrne *et al.*, 2000).

Leaf adaxial identity is also controlled by *HD-ZIPIII* class genes. In *phb*, *phv*, and *rev* mutants, leaves are abaxialized and a

Figure 3 Leaf modifications. (a)–(d) Stipules in *Passiflora vitifolia,* Passifloraceae (a), *Trifolium pratense,* Fabaceae (b), *Smilax kunthii,* Smilacaceae (c) and *Cecropia* sp., Urticaceae (d). (e) Palmately lobed leaf of *Ricinus communis* (Euphorbiaceae). (f), (g) Pinnately compound leaves in the fern *Jamesonia* sp., Pteridaceae (f), the basal eudicot *Mahonia* sp., Berberidaceae (g). (h) Compound leaf of *Mutisia clematis*, with distal leaflets modified into tendrils. (i)–(k) Scale leaves (c) in the gymnosperm *Cycas revoluta*, Cycadaceae (i), and the monocot *Cyperus papyrus,* Cyperaceae ((j), (k)). (l) Modified leaves into spines of *Opuntia ficus-indica*, Cactaceae. (m) Variegated leaf of *Tradescantia zebrina*, Commelinaceae. (n) Variegated, spiny leaf of *Solanum quitoense,* Solanaceae.

loss of meristematic functions occur (McConnell and Barton, 1998; McConnell *et al.*, 2001; Prigge *et al.*, 2005). These genes are strongly regulated, on one hand by two microRNAs, *miR165* and *miR166* that accumulate abaxially and degrade abaxial transcripts of *HDZIPIII* genes, thereby displacing all mRNAs to the adaxial region (Tang *et al.*, 2003). On the other hand, negative regulation of *HDZIPIII* genes also occurs by the dimerization with the *LITTLE ZIPPER* (*ZPR1–4*) proteins. This interaction prevents HDZIPIII proteins from binding target DNA sequences (Wenkel *et al.*, 2007).

Promoters of abaxial identity, and thus antagonistic to the *HDZIPIII* genes, include *KANADI* and *YABBY* genes. The *kan* and *fil yab2–5* mutants in *Arabidopsis* have adaxialized leaves with altered vascular patterning, which instead of activating leaf programs, reinitiated shoot meristem genetic programs (Sawa *et al.*, 1999; Siegfried *et al.*, 1999; Eshed *et al.*, 2001, 2004; Sarojam *et al.*, 2010). It has been shown that *KANADI* homologs in rice and corn are also responsible for the specification of leaf polarity (Candela *et al.*, 2008; Zhang *et al.*, 2009). *YABBY* gene homologs on the other hand are responsible for polarity maintenance, lamina outgrowth and establishing of the leaf margin (Eshed *et al.*, 2004; Yamaguchi *et al.*, 2004; Ishikawa *et al.*, 2009; Sarojam *et al.*, 2010). Thus, *YABBY* homologs interact closely with genes that play roles in the expansion of the lamina, which include WOX transcription factors. *WOX* gene family members include the *Arabidopsis PRESSED FLOWERS (PRS)*, the maize *Narrow sheath1* and *2* (ns1, ns2) and the *Petunia MAEWEST* (*MAW*), all of which control lateral growth (Scanlon *et al.*, 1996; Nardmann *et al.*, 2004; Vandenbussche *et al.*, 2009). Interestingly, flattened unifacial leaves, which are a novel leaf form occurring in the monocot *Juncus* (Juncaceae), are the result of genetic changes

that induce abaxialization and include expression changes of *YABBY* orthologs that include the *Juncus DL* (named after the YABBY rice homolog *DROOPING LEAF*) (Yamaguchi *et al.*, 2004, 2010). Overall, data in *Arabidopsis* as well as in non-model species suggest a complex regulation of abaxial–adaxial identity, linked with marginal growth of lateral organs, proper vascular differentiation and even axillary meristem development (Champagne and Sinha, 2004; Zgurski *et al.*, 2005).

The establishment of the dorsiventral domains allows the induction of marginal growth and complexity. Genes controlling leaf complexity and leaf shape are transcription factors involved in the meristematic maintenance of the shoot apex, that often get recruited later in development in the leaf margins to regulate compound leaf development (reviewed in Champagne and Sinha, 2004; Efroni *et al.*, 2010). Leaf complexity is generally linked with the *KNOX1* genes *SHOOT MERISTEMLESS* (*STM*) in *Arabidopsis* and its ortholog *KNOTTED1* (*Kn1*) in maize, that are responsible of maintaining proper shoot apical meristem (Long *et al.*, 1996; Vollbrecht *et al.*, 2000). *KNOX1* genes are repressed by *ARP* genes during leaf initiation to acquire determinacy. However, after the differentiation of the leaf primordium, *KNOX1* genes either remain down-regulated in species with simple leaves (Nishimura *et al.*, 1998); or they can be turned on again in the leaf margin in species with compound leaves to generate new marginal outgrowth (Hareven *et al.*, 1996; Bharathan *et al.*, 2002). In the formation of a compound lamina, the antagonistic genes to *KNOX1* are the *NO APICAL MERISTEM* and *CUP SHAPE COTYLEDON3* (*NAM/CUC3*) genes, which are responsible for the formation of the leaf dissection zones (Blein *et al.*, 2008; Berger *et al.*, 2009). It is thought that this regulatory module has been maintained during angiosperm evolution to directly control leaf complexity and leaf shape (Blein *et al.*, 2008; Berger *et al.*, 2009). *KNOX1* genes regulate leaf complexity in most angiosperms studied (reviewed in Hay and Tsiantis, 2010), with the exception of pea (and close relatives) and columbines (*Aquilegia*), where other floral meristem identity genes have been co-opted to regulate leaflet formation and proper development (Hofer *et al.*, 1997; Champagne *et al.*, 2007; Pabón-Mora *et al.*, 2013).

Inflorescences

There are two main types of inflorescences, racemose and cymose. Racemose inflorescences are characterized by an apical meristem that grows indeterminately and never becomes a terminal flower (**Figure 4**). Conversely, cymose inflorescences are those in which the apical meristem sooner or later switches from an inflorescence meristem with indeterminate growth to a floral and determinate, as it ends up forming a terminal flower (**Figure 5**).

Racemose inflorescences are either simple, when all flowers are developed directly from the main axis (or rachis) of the inflorescence (**Figures 4(a)–4(j)**); or compound, when the inflorescence produces branches of second, third or more orders before forming the flowers (**Figures 4(k)**, **4(n)**, and **4(q)**). The most common types of racemose inflorescences are: (a) racemes, if flowers bear a peduncle that is subtended by a bract; racemes are often associated with the presence of flowers with bilateral symmetry, often pollinated by insects, birds, or bats (**Figures 4(a)–4(e)**). This is the case of *Castilleja* (Orobanchaceae; **Figure 4(a)**) and *Crotalaria* (Fabaceae; **Figure 4(c)**); however, racemose inflorescences can also bear radially symmetrical flowers, as in *Echeveria* (Crassulaceae; **Figure 4(b)**) or the monocot *Puya* (Bromeliaceae; **Figures 4(c)** and **4(d)**). (b) Spikes, if flowers are sessile and directly attached to the axis; in this case, bracts are often vestigial or lacking, and flowers are tightly congested and develop in spirals along the axis; spikes are often linked with the occurrence of variously reduced and small flowers, often wind pollinated, like in *Piper* (Piperaceae; **Figure 4(f)**) or *Hedyosmum* (Chloranthaceae; **Figure 4(g)**); in these two taxa, the modified spike is called amentum, as the main axis is thickened. In the latter genus, although inflorescences are unisexual, both staminate and carpellate inflorescences are indeterminate (Doria *et al.* 2012). (c) Catkins, if the hanging spikes are unisexual, as in, for example, members of the order Fagales (**Figure 4(h)**). (d) Spadices, if spikes subtended by one (rarely more) large and colorful modified leafs called the spathe, such as those found in *Houttuynia* (Saururaceae; **Figure 4(i)**) and *Anthurium* (Araceae; **Figure 4(j)**). (e) Indeterminate capitula or heads, if the internodes of the inflorescence become extremely shortened, and the flowers are congested and closely tightened on a common receptacle and usually surrounded by involucral bracts; capitula are typical in the Asteraceae (**Figures 4(k)–4(m)**), and often are formed by two different types of flowers, ligular (also known as the ray flowers) and tubular (also known as the disk flowers). (f) Umbels, if internodes are compressed giving the appearance of flowers born at the same point, as in most Apiaceae (**Figure 4(n)**). (g) Solitary flowers can be evolutionary derived from racemose inflorescences with elongate, leafy branches with indeterminate growth, and one flower per subtending leaf.

The main types of cymose inflorescences are (a) solitary flowers, if they are terminal and formed directly from the shoot apical meristem; thus, a single flower is formed at the end of a branch, like in magnolias (Magnoliaceae; **Figure 5(a)**) or at the end of the entire plant, like in some poppies (Papaveraceae; **Figure 5(b)**). (b) Monochasia, if only one branch (or second order flower) is formed on each node below the terminal flower, as in *Aristolochia maxima* (Aristolochiaceae, **Figure 5(c)**) and *Bomarea* (Alstroemeriaceae; **Figure 5(d)**). (c) Dichasia, if the development of the terminal flower is followed by the initiation of two opposite flowers below it, like in *Vismia* (Hypericaceae; **Figure 5(e)**), *Meriania* (Melastomataceae; **Figure 5(f)**) and *Lehmaniella* (Gentianaceae; **Figure 5(g)**). (e) Pleiochasia, if 4, 6, 8, or more opposite flowers are developed below the terminal flower, which is often the first to develop and reach maturity, like in *Berberis* (Berberidaceae; **Figure 5(h)**).

Cauliflory and Ramiflory

In woody plants mainly from tropical forests, it is frequent that blooming is drastically delayed with respect to the vegetative growth of the plant. Thus, inflorescences are located on lignified stems, a phenomenon called cauliflory, if it occurs on the main trunk; or ramiflory, if it occurs on higher order branches. In both cases, the inflorescence meristems remain

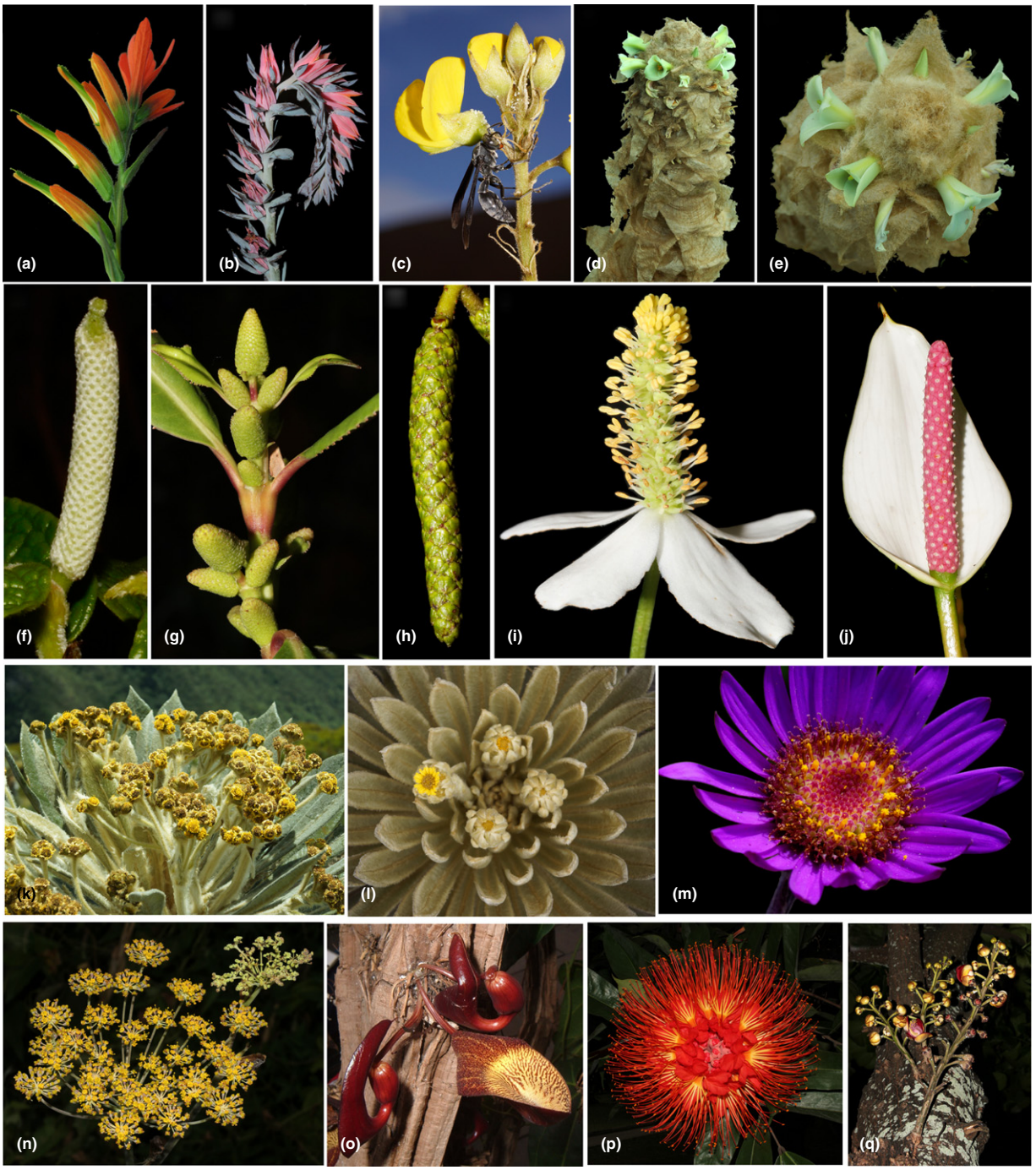

Figure 4 Racemose inflorescences. (a)–(e) Racemes of *Castilleja fissifolia*, Orobanchaceae (a), *Echeveria rosea*, Crassulaceae (b), *Crotalaria micans*, Fabaceae (c) and *Puya trianae*, Bromeliaceae ((d), (e)). (f)–(g) Amenta of *Piper artanthe*, Piperaceae (f) and *Hedyosmum racemosum*, Chloranthaceae (g). Staminate catkin of *Alnus acuminata*, Betulaceae. (i)–(j) Spadices of the magnoliid *Houttuynia cordata*, Saururaceae (i) and the monocot *Anthurium* sp., Araceae (j). (k)–(m) Heads or capitula of the Asteraceae *Espeletiopsis guacharaca* (k), *Espeletia* sp. (l) and *Senecio formosus* (m). (n) Compound umbella of *Foeniculum vulgare*, Apiaceae. (o)–(q) Cauliflorous racemose inflorescences of *Aristolochia leuconeura*, Aristolochiaceae (o), *Brownea* sp., Fabaceae (p) and compound raceme of *Couroupita* sp., Lecythidaceae (q).

dormant for months or years immediately beneath the cork meristem of the cortex, usually axillary to the leaf scars. Not all cauliflorous inflorescences have the same architecture. They may be racemose, like in *Brownea* (Fabaceae; Figure 4(p)) and *Couroupita* (Lecythidaceae; Figure 4(q)), or cymes, like in *Crescentia* (Bignoniaceae; Figure 5(j)). Even in the same genus (e.g., *Aristolochia*) cauliflorous inflorescences can be racemes (e.g., in *Aristolochia leuconeura*; Figure 4(o) or cymes (e.g., *Aristolochia arborea*, Figure 5(i), and *A. maxima*, Figure 5(c); González, 1999). Such positional convergence strongly

Figure 5 Cymose inflorescences. (a)–(b) Terminal flowers of *Magnolia* sp., Magnoliaceae (a), and *Argemone mexicana,* Papaveraceae (b). (c)–(d) Monochasia of *Aristolochia maxima*, Aristolochiaceae (c) and *Bomarea diffracta,* Alstroemeriaceae (d). (e)–(g) Dichasia of *Vismia* sp., Hypericaceae (e), *Meriania dimorphanthera*, Melastomataceae (f), and *Lehmanniella pulchra*, Gentianaceae (g). (h) Pleiochasia of *Berberis samacana,* Berberidaceae. (i)–(j) Cauliflorous cymes of *Aristolochia arborea*, Aristolochiaceae (i) and *Crescentia cujete,* Bignoniaceae (j). (k) Cyathium of *Euphorbia cyathophora,* Euphorbiaceae. (l) Peloric terminal flower of *Calceolaria* sp., Calceolariaceae. (m) Pseudoterminal flower of *Datura stramonium,* Solanaceae. (n) Giant terminal inflorescence of *Agave americana* (Agavaceae) with lateral units formed by syndesmic (fused) branches.

suggests that cauliflory or ramiflory is a heterochronic mechanism and a highly adaptive strategy for pollination rather than an acquisition via common ancestry.

Pseudanthia and Peloria

The following extreme modifications occur in inflorescence architecture: (a) cyathium, if a condensed inflorescence resembles and functions as a single flower, but is in fact formed by unisexual male flowers that surround female ones, with reduced or completely absent perianth (Prenner *et al.*, 2011); the cyathia are typically found in *Euphorbia* (Euphorbiaceae; **Figure 5(k)**). (b) Aberrant flowers, or peloria, are often formed in the terminal position of otherwise racemose inflorescences; common examples of peloria are known in *Digitalis* (Plantaginaceae) and *Calceolaria* (Scrophulariaceae; **Figure 5(l)**), where a radially symmetrical flower ectopically forms at the end of the inflorescence and it is surrounded by the typical bilateral flowers. Peloria also occur in inflorescences of the basal eudicot *Gunnera* which begins its development as racemose, but a terminal flower becomes evident during late ontogeny (Mora-Osejo *et al.*, 2011). (c) Unusual displacement of flowers by metatopies, when early fusion of two or more flowering axes, or an axis and one or more floral peduncles occur; this occurs, for example, in the monocot *Agave* (Agavaceae, **Figure 5(n)**) inflorescences, making very hard to interpret the resulting branching patterns in such cases; these metatopies are classified as recaulescence (fusion of the floral peduncle with the petiole of its subtending leaf), concaulescence (fusion of the floral peduncle with the shoot axis), anaphysis (displacement of a floral bud to an ectopic, extra-axillary position) or sindesmy, when two or more axes of an inflorescence fuse with the shoot; concaulescence may result in that a lateral flower appears as terminal (i.e., pseudoterminal) position, as in *Atropa* and *Datura* (Solanaceae; **Figure 5(m)**); on the other hand, recaulescence occurs when the floral axis grows fused to the leaf basis, resulting in epiphyllous inflorescences, that is one or more flowers ectopically growing on top of the leaf or bract, as in some orchids, *Tilia* (Malvaceae) and Phyllonomaceae.

Genetic Basis of Inflorescence Development

The genetic control of racemes versus cymes ultimately depends on the instructions received by the shoot apical meristem. If the instructions are those of maintaining pluripotency it will be a raceme; conversely, if instructions are those of forming a terminal floral meristem, it will become a cyme. In *Arabidopsis* the transition from vegetative to reproductive development is controlled by a number of pathways that include hormonal outputs, autonomous competence, cold temperature responses, and photoperiodic changes (Amasino and Michaels, 2010). A major finding of the past decades has been the identification of florigen that was thought to be the key signal for the transition to flowering. Florigen turned out to be a mobile transcription factor named *FLOWERING LOCUS T* (*FT*) that is produced in leaves but translocated to the shoot apex to promote flowering. FT acts together with *LEAFY* (*LFY*) to provide the identity of the floral meristem. *FT* and *LFY* antagonize the inflorescence meristem gene *TERMINAL FLOWER1* (*TFL1*) (also known as *CENTRORADIALIS -CEN*, the *Anthirrinum* ortholog) which is also a mobile signal; in racemes *TFL1* stays active at the distal point and flowers never form, whereas in cymes it is repressed by the floral meristem genes, and thus flowers do form (Amaya *et al.*, 1999; Conti and Bradley, 2007). The interactions between *FT*, *LFY*, and *TFL1*, first reported in *Arabidopsis*, are maintained in other phylogenetically distant plants, like tomato and grasses. However, the number of gene copies has changed over time in different lineages; as a result, grasses, for instance, possess redundant copies of *TFL1* homologs as well as *FT* homologs (Molinero-Rosales *et al.*, 1999; Lifschitz *et al.*, 2006; Périlleux *et al.*, 2014; Zhang and Yuan, 2014). Inflorescence variation is one of the most outstanding plant features that will provide ground for further genetic variation and novel players, as non-model species are included in evo-devo studies. Most levels of complexity in inflorescences have not yet been assessed in terms of their underlying genetic bases.

Flowers

Flowers are the most important evolutionary novelties of the dominant plants on Earth, the Angiosperms. They are formed by a succession of extremely modified leaves along an axis with compressed internodes, and lacking axillary meristems. These modified leaves of a flower are considered individual organs. Organs on each series can initiate either in a spiral fashion (**Figures 6(a)** and **6(b)**), or in whorls (**Figures 6(c)** and **6(d)**). These organs are grouped in four (sometimes three or less) series and occupy a fixed position in the flower (**Figures 6(a)–6(f)**), as follows: the outer organs are called sepals, and altogether form the calyx; next, the petals, which form the corolla; then, the stamens, which altogether form the androecium; and the carpels (rarely only one), which are in the center of the flower and form the gynoecium. Additional floral whorls include the presence of an epicalyx in members of the Loranthaceae (**Figure 6(m)** and Malvaceae (**Figure 6(n)**), among other families, and the unique corona, occurring in Passifloraceae (**Figure 6(o)**).

The outer floral organs (altogether called the perianth) are sterile, and the inner ones are fertile. The perianth is highly variable in terms of form and function, although they often times are laminar; frequently, sepals are green and protective, while petals are colorful and play important roles in pollination (**Figures 6(e)** and **6(f)**). When sepals and petals look alike they are called tepals (**Figures 6(g)** and **6(h)**). Stamens and carpels, responsible for the sporogenesis of pollen grains and ovules, respectively, have a 3D arrangement due to the formation of internal cavities where sporogenesis and gametogenesis occur (**Figures 6(i)–6(l)**). The gynoecium, the most important key innovation of flowering plants, is syncarpic if the two or more carpels are partially or totally fused, or apocarpic if the carpels remain as individual organs. The basal portion of the gynoecium forms the ovary, which encloses one or more ovules; the distal portion of the gynoecium is usually differentiated into a style and a stigma, and both structures play critical roles during pollination (**Figures 6(i)**, **6(j)**, and **6(l)–6(n)**).

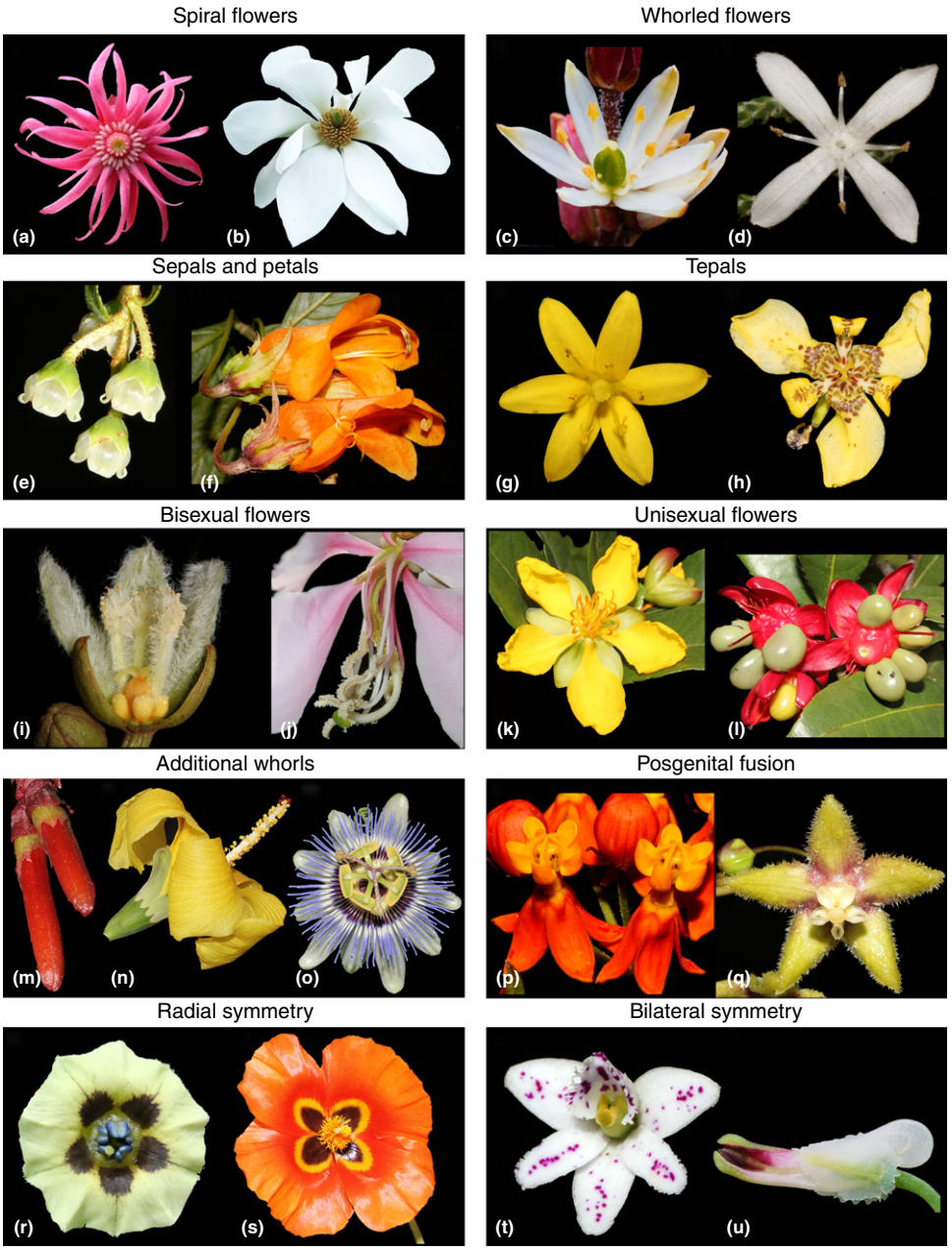

Figure 6 Variation in floral morphology. (a)–(b) Spirally flowers of *Illicum* sp., Illiciaceae (a) and *Magnolia* sp., Magnoliaceae (b). (c) Whorled flowers of *Isidrogalvia* sp. Tofieldiaceae (c) and *Aragoa cleefii,* Plantaginaceae (d). (e)–(f) Biseriate perianth in Ericaceae (e) and *Columnea strigosa*, Gesneriaceae (f). (g)–(h) Tepals of *Hypoxis humilis*, Hypoxidaceae (g) and *Tigridia* sp., Iridaceae (h). (i) Bisexual flowers of *Vismia sp.*, Hypericaceae (i) and. *Bauhinia aff. picta*, Fabaceae (j). (k)–(l) Unisexual, male (k) and female (l) flowers of *Ochna* sp., Ochnaceae. (m)–(n). Epicalyx of *Aetanthus mutisii,* Loranthaceae (dark purple in m), and Malvaceae (dark green in n). (o) Corona (light purple) of *Passiflora caerulea*, Passifloraceae. (p)–(q) Gynostegium of *Asclepias curassavica* (p) and *Blepharodon* sp. (q), Apocynaceae. (r)–(s) Radially symmetrical corolla of *Physalis peruviana*, Solanaceae (r) and *Glaucium corniculatum*, Papaveraceae (s). (t)–(u) Bilaterally symmetrical perianth of *Epidendrum fimbriatum*, Orchidaceae (t) and *Fumaria capreolata*, Papaveraceae (u).

In a number of taxa (e.g., papaya, *Cannabis*, cucumber, and mistletoes), flowers lack one of the two fertile series, or one of them is not functional or vestigial, giving rise to unisexual flowers (Figures 6(k)–6(l)). Unisexuality occurs primarily by arrested development of stamens or carpels; however, it does not occur following a common process across angiosperms, suggesting that unisexuality does not necessarily have homologous genetic bases in different taxa (reviewed in Diggle *et al.*, 2011).

Phylogenetic analyses of angiosperms define five major groups (Figure 7). Although early diverging angiosperms display greater variation in their floral ground plan, they are the

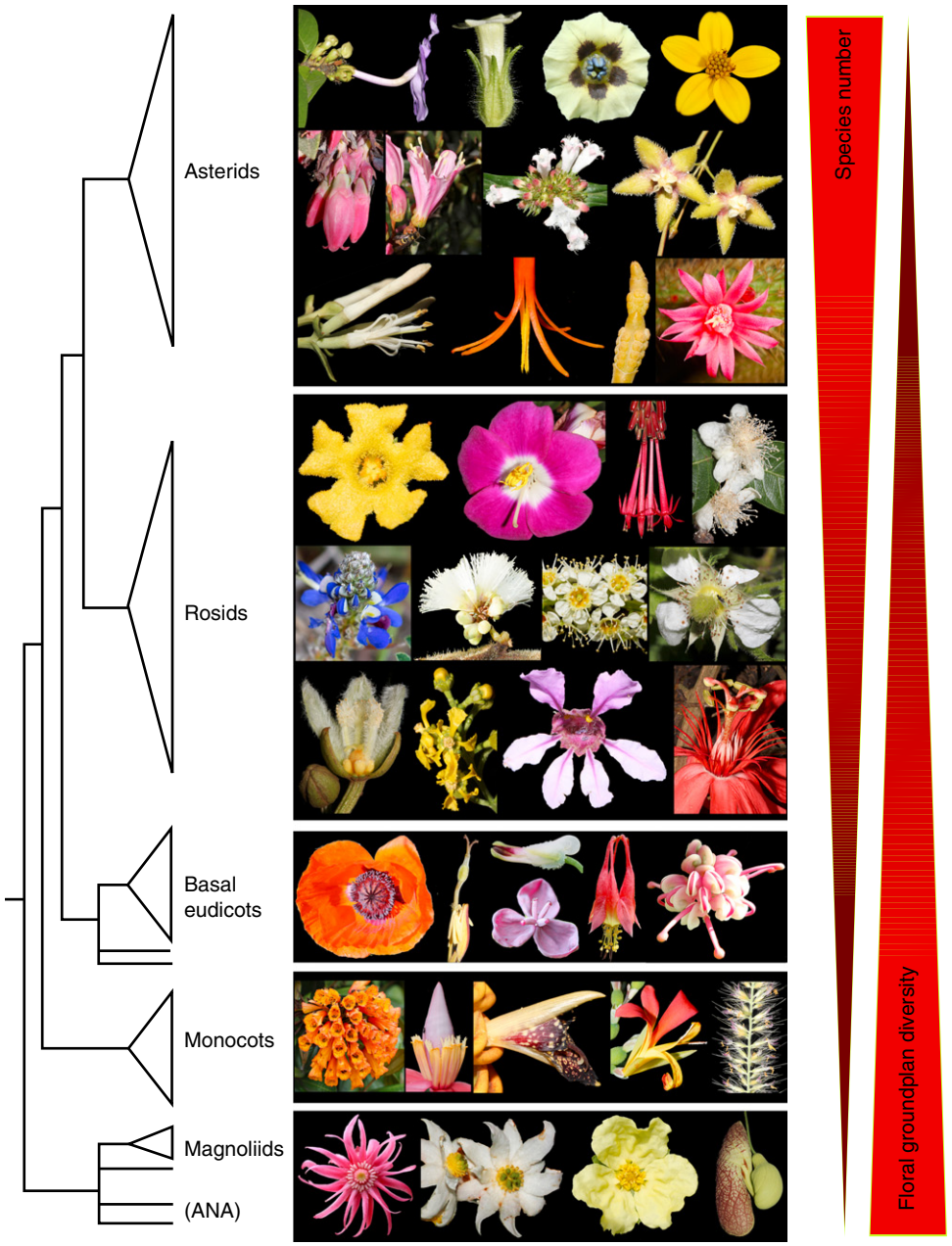

Figure 7 Trends in floral evolution. Summarized phylogenetic angiosperm tree showing the five main groups ANA, magnoliids, monocots, basal eudicots, and core eudicots (including rosids and asterids). Examples of floral variation are shown in front of each clade. Red triangles to the right indicate the inversely proportional relationship between increasing species number (e.g., core eudicots comprise approximately 75% of the total number of species) and decreasing floral groundplan variation (e.g., most flowers of core eudicots are 4–5 merous, whorled, with predominantly fused organs).

least diversified in terms of number of species (Figure 7). The early diverging angiosperms include *Amborella*, Nymphaeales, and Austrobaileyales (called the ANA grade), the monocots, and the magnoliids and Chloranthales. Whereas number of parts is often variable and high in magnoliids and basal angiosperms, most monocots flowers are trimerous (Figure 7). The remaining flowering plants are the eudicots (Wickett *et al.*, 2014); they are further divided into basal and core eudicots. Core eudicots are by far the most specious clade, as it comprises close to 75% of the flowering plant species; they comprise the rosids and the asterids (Figure 7). Flowers of core eudicots are predominantly bisexual, whorled, with 4 or 5 (rarely 6) sepals, petals, and stamens, a syncarpous gynoecium formed by 2–5 carpels. Fusion between organs is very common, and it is a determining factor driving pollination syndromes; for instance, long, tubular corollas are often hummingbird, moth, or butterfly-pollinated, while convoluted, trap-flowers are often linked with fly pollination (Proctor *et al.*, 1996). Fusion between different whorls is less frequent but, yet, it plays important roles in pollination;

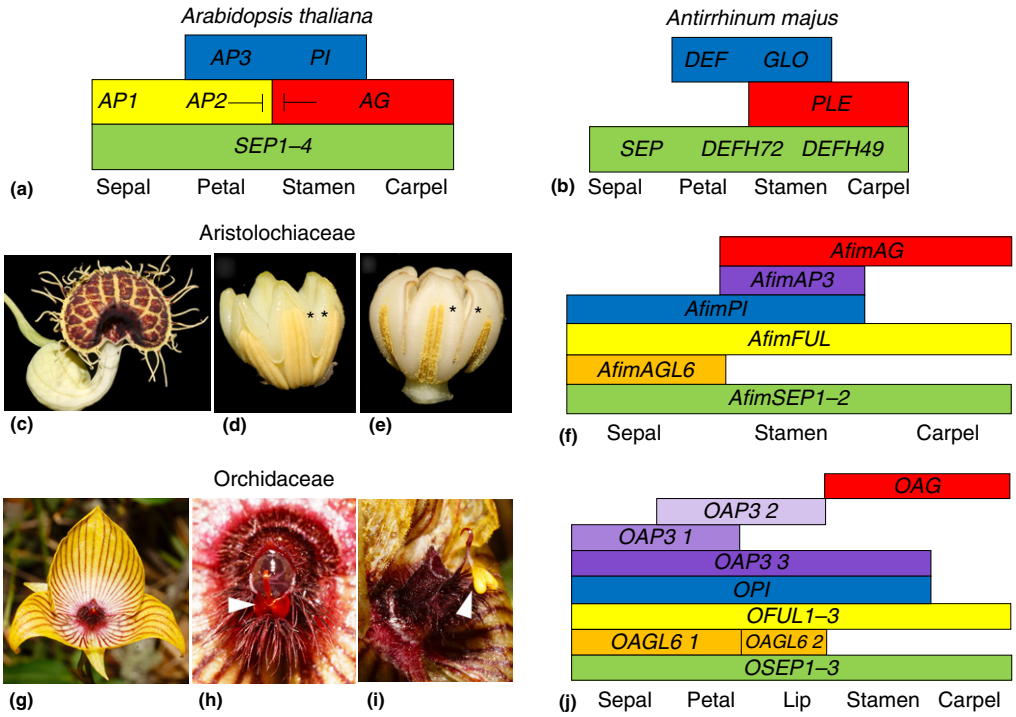

Figure 8 (a) Diagram of the ABCE model of flower development based on homeotic mutants of *Arabidopsis thaliana* (Brassicaceae); in yellow, A-class genes *APETALA1* (*AP1*) and *APETALA2* (*AP2*); in blue, B-class genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*); in red, C-class gene *AGAMOUS* (*AG*); in green, E-class genes *SEPALLATA 1–4* (*SEP1–4*). Bars represent the mutual repression between A and C-class genes resulting in unique interactions in the sterile *vs.* the fertile floral organs. (b) Diagram of the BC model, based on homeotic mutants of *Antirrhinum majus*; note the absence of A-class genes, as there are no homeotic sepal-to-petal mutants; according to this model the specification of the floral meristem is sufficient to provide sepal identity and only B and C-class genes are needed to form petals, stamens and carpels. (c)–(j) Diagrams of the models for synorganized flowers in Aristolochiaceae and Orchidaceae. (c)–(f) *Aristolochia fimbriata* flower (c), and gynostemium in its 'female' (d) and 'male' (e) phases. (f) Modified ABCE model in *Aristolochia fimbriata*, based on expression studies; note the broad expression of A-class genes, the lack of mutual repression with C-class genes and the inclusion of *AGL6* as a putative key player in perianth identity (Pabón-Mora *et al.*, 2015). (g)–(j) *Telipogon nervosus* flower (g), and gynostemium in its 'female' (h) and 'male' (i) phases; arrows point to the stamen before and after dehiscence. (j) Modified ABCE model in Orchidaceae, based mostly in data from *Oncidium* spp.; note an increase in the number of copies of A, B, and AGL6 genes; different combinations of *AGL6* and *AP3* paralogs specify sepal, petal and lip development. Redrawn based on Mondragón-Palomino, M., 2013. Perspectives on MADS-box expression during orchid flower evolution and development. Frontiers in Plant Sciences 4, 377; Tsai, W.-C., Pan, Z.-J., Hsiao, Y.-Y., Chen, L.-J., Liu, Z.-J., 2014. Evolution and function of MADS-box genes involved in orchid floral development. Journal of Systematics and Evolution 52, 397–410; and Hsu, H.-F., Hsu, W.-H., Lee, Y.-I., *et al.*, 2015. Model for perianth formation in orchids. Nature Plants 15046.

examples of such fusion between stamen(s) and carpel(s) into a gynostemium occur independently in Apocynaceae (**Figures 6(p)** and **6(q)**), Aristolochiaceae and Orchidaceae (**Figure 8**).

Several WGD events have been mapped to particular points of the phylogeny, as follows: one before the diversification of angiosperms (ε), two duplication events in the monocots (ρ, σ), one before the diversification of the eudicots (γ), and two restricted to the Brassicaceae (α, β) (Jiao *et al.*, 2011). These duplication events challenge the ongoing studies addressed to identify paralogous versus orthologous genes and their corresponding functions. Model flowering plants for genetic studies are the rosids *A. thaliana* (Brassicaceae), *A. majus* (snapdragon, Plantaginaceae), *Solanum lycopersicum* (tomato, Solanaceae), and the monocots *O. sativa* (rice, Poaceae) and *Z. mays* (corn, Poaceae). In addition, 37 plant genomes, the majority belonging to the core eudicots or the monocots, have been sequenced and are publicly available. The genetic basis of floral organ identity has been identified based on homeotic mutants of *A. thaliana* and *A. majus*. The resulting model illustrates how the expression patterns and interactions of three classes of MIKC MADS-box transcription factors and an *ERF/AP2* gene (collectively named A, B, and C-class genes) provide the identity to each floral whorl (Schwarz-Sommer *et al.*, 1990; Coen and Meyerowitz, 1991). According to the ABC model, A-class genes (*APETALA1* and *APETALA2*) establish the identity of floral meristem and provide sepal identity, A and B-class genes (*APETALA3* and *PISTILLATA*) control petal identity, B and C-class genes (*AGAMOUS*) regulate stamen identity, and C-class genes alone are responsible for carpel identity, as well as the termination of the floral meristem (Bowman *et al.*, 1989, 1991; Yanofsky *et al.*, 1990). This model was modified after the discovery of *SEPALLATA* transcription factors responsible for the floral transition and the initiation of all floral organs, thus an updated ABCE model includes E-class genes (the *Arabidopsis SEP1*, *SEP2*, *SEP3*, and *SEP4*) (Pelaz *et al.*, 2000). A caveat of the model is the lack of a mutant phenotype in *Antirrhinum* with homeotic changes in sepals and petals, challenging a universal A-function and raising an

alternative BC model where perianth is formed by default, after the specification of the floral meristem (Causier *et al.*, 2010).

One other trait that has been key for angiosperm diversification is floral symmetry, and specifically the independent evolution of a bilateral perianth (**Figures 6(y)** and **6(u)**) from a radially symmetrical condition (**Figures 6(r)** and **6(s)**). The genetic basis for floral symmetry in *A. majus* involves the asymmetric expression of four *TCP* transcription factors (acronym of *TEOSINTE BRANCHED1*, from *Z. mays*; *CYCLOIDEA*, from *A. majus* and *PROLIFERATION CELL FACTOR*, *O. sativa*): *CYCLOIDEA* (*CYC*), *DICHOTOMA* (*DICH*) and *RADIALIS* (*RAD*) controlling dorsal floral identity and an opposing gradient of *DIVARICATA* (*DIV*), controlling ventral identity (reviewed in Preston and Hileman, 2009; Hileman, 2014).

In the next section we will revisit organ identity and symmetry genes in flowers of two non-related families that show parallel evolution in synorganized perianth, stamens, and carpels.

Case Study – Floral Synorganization in Aristolochiaceae and Orchidaceae

Aristolochia (Aristolochiaceae)

The family Aristolochiaceae belongs to the order Piperales, a lineage that includes species with tiny flowers lacking perianth, like in *Peperomia* and *Piper* (Piperaceae), or with medium-sized flowers with a bipartite perianth, like in *Saruma* (Aristolochiaceae), and taxa with medium to large size, highly synorganized flowers with a single but elaborated perianth, like in *Aristolochia* (Aristolochiaceae). The bilaterally symmetrical perianth in *Aristolochia* is the result of the early fusion of three sepals that grow asymmetrically during development to form a kettle-trap mature flower with petaloid characteristics such as pigmentation and epidermal modifications including trichome formation to ensure fly pollination (González and Pabón-Mora, 2015). In addition, stamens and carpels in *Aristolochia* fuse congenitally forming a gynostemium with anthers on the outside and stigmatic lobes on the inside (González and Stevenson, 2000a, 2000b; González and Pabón-Mora, 2015; Pabón-Mora *et al.*, 2015).

Floral organ identity genes

Transcriptomic studies have shown that *Aristolochia fimbriata* possesses a single copy of each of the MADS box genes, except *SEPALLATA* (E-class genes), which has two paralogs. Given that the early diverging *Amborella trichopoda* already possesses two copies of these genes, it is likely that the *SEP* paralogs are likely a result of a duplication event prior to the diversification of flowering plants. *In situ* expression studies in *A. fimbriata* show the canonical expression patterns of B (*APETALA3* and *PISTILLATA*) and C-class (*AGAMOUS*) genes in stamens and carpels, despite the fact that these organs are fused. These studies also show expression of B-class genes only in the staminal portion of the gynostemium, and an ectopic expression of *PISTILLATA* at late developmental stages in the inner epidermis of the perianth (González and Pabón-Mora, 2015; Pabón-Mora *et al.*, 2015). Thus, B-class gene expression patterns support a sepal-derived perianth, and suggests the recruitment of *PI* in the acquisition of petal traits by the sepals in *Aristolochia* (Horn *et al.*, 2014; Pabón-Mora *et al.*, 2015).

The E-class (*SEPALLATA*) genes appear to be conserved with respect to the patterns established by the model, whereas expression of *APETALA1/FRUITFULL* homologs (A-class genes) is detected in all floral organs, leaves and fruits, suggesting pleiotropic and not perianth specific roles (Pabón-Mora *et al.*, 2012, 2015). Interestingly, the initiation of perianth primordia in *Aristolochia* is marked by the expression of *AGAMOUS-like 6* (*AfimAGL6*). *AGL6* is also turned on later on during development in the outer portion of the ovary and in the ovules, similarly to what has been observed in *Arabidopsis* (Schauer *et al.*, 2009). *AGL6* is the sister gene lineage to the *SEPALLATA* gene lineage and together, they are sister to the *APETALA1/FRUITFULL* gene lineage. Thus, it is likely that closely related genes have been recruited for perianth identity in *Aristolochia*.

Floral symmetry genes

So far, studies in all core eudicot species with bilateral flowers show asymmetrical expression of *CYC* homologs, in particular one of the three paralogs (*CYC2*) resulting after two consecutive duplication events in the gene lineage coincident with the radiation of the core eudicots. In noncore eudicots and monocots, differential expression of the preduplication *CYC/TB1* homologs seems to correlate with changes in symmetry, suggesting that at least in some taxa (e.g., Papaveraceae and Commelinaceae) they are also involved in establishing floral bilateral symmetry (Bartlett and Specht, 2011; Preston and Hileman, 2012; Hileman, 2014; Jabbour *et al.*, 2014). Nevertheless, the expression patterns of *CYC/TB1* homologs in *A. arborea* show a more or less homogeneous expression throughout the perianth in the overlapping domains with the B-class *PISTILLATA* genes, and there is no evidence of a dorsiventral gradient regulating changes in perianth growth (Horn *et al.*, 2014). Moreover, heterologous transformation studies demonstrated that *AarCYC* genes do not produce a phenotype similar to that caused by the overexpression of core eudicot *CYC2* genes, suggesting functional changes in TCP genes in *Aristolochia*. Altogether the data suggest that bilateral symmetry in *Aristolochia* flowers is not controlled by asymmetric expression of *CYC/TB1* genes; instead, they point at novel regulatory networks yet to be found controlling perianth curvature, asymmetric elongation of the median sepal and bilateral symmetry in the fused sepals in the magnoliids (Horn *et al.*, 2014).

Orchidaceae

Orchid flowers are also highly synorganized. The perianth is formed by three sepals, which often are petaloid or bilateral, two lateral petals and a medial petal (called the lip or labellum) that often is highly elaborated and distinct from the lateral petals. The androecium in most orchids consists of a single stamen, fused to the stigma into a gynostemium; pollen of most orchids aggregate into two or four sacs called pollinia, which carry the entire pollen load as a single structure. Sometimes (in the subfamilies Apostasioideae and Cypripedioideae) two or three stamens are still formed, and pollen grains do not group into pollinia (reviewed by Endress, 2015).

Floral organ identity genes

An increasing number and diversification of all MADS box genes involved in floral organ identity has occurred in Orchidaceae (Cai *et al.*, 2015). Two consecutive gene duplications in the *AP3* gene lineage have resulted in four clades, followed by the specialization of gene copies in a specific whorl (Mondragón-Palomin and Theißen, 2011; Tsai *et al.*, 2014). In particular, the lip has a unique combination of *AP3* gene copies compared to the lateral petals (Mondragón-Palomino, 2013; Tsai *et al.*, 2014). Recent studies have identified that *AGL6* genes are also key players in perianth development. Two paralogs are found from a duplication predating the evolution of orchids, one of them has specialized in providing sepal and petal identity, whereas its paralog contributes primarily to lip identity (Hsu *et al.*, 2015). Thus, gene duplication and subfunctionalization have been proposed as key drivers of perianth morphological diversification in orchids. The gynostemium identity in orchids seems to be controlled by *AG-like* genes, and a lack of expression of one *AG* paralog has been correlated with the occurrence of homeotic transformations from the gynostemium to tepals (Wang *et al.*, 2011).

The role of other MADS box genes is less clear. The *PI* genes do not seem to be restricted to the petals and stamens but have broad expression patterns. However, there are conflicting reports regarding their function, as some experiments show complementation of the *Arabidopsis pi* mutant (Xu *et al.*, 2006) and some do not, questioning their contribution to perianth and stamen identity (Tsai *et al.*, 2008). *FUL-like* genes have been found broadly expressed but have not been functionally characterized (Yu and Goh, 2000). *SEP* genes are found in all floral organs and functional analyses suggest that they play key roles in perianth identity, although their contribution to stamen and carpel identity is unclear (Pan *et al.*, 2014).

Floral symmetry genes

Floral symmetry *CYC/TB1* candidate genes have been identified in many monocots including most model species like rice and maize (Mondragón-Palomino and Trontin, 2011). Studies in Commelinaceae have shown that asymmetric expression of *CYC/TB1* genes, controlled by a dorsiventral *APETALA3* gradient, underlies shifts in symmetry, like their core eudicot homologs (Preston and Hileman, 2012). Interestingly, *CYC/TB1* genes are difficult to identify via directed cloning or by identity BLAST searches in the available transcriptomes of orchids. Only recently, one ortholog of *CYC/TB1* was isolated from *Orchis italica* (*OitaTB1*) and its expression is mostly restricted to leaves, suggesting that Orchidaceae, like *Aristolochia*, is also an exception to the acquisition of bilateral symmetry *via* the recruitment of *CYC/TB1* genes (DePaolo *et al.*, 2015). Closely related TCP genes, like *PCF-like* class I and *CIN-like* class II are good candidates for activation and repression of cell division during development, but their contribution to bilateral symmetry has not been addressed (Mondragón-Palomino and Trontin, 2011; DePaolo *et al.*, 2015). One aspect to keep in mind is that bilateral symmetry of orchid perianth may not be linked with that in the gynostemium, which strongly suggests that different candidate genes may independently control symmetry in these organs. Other candidate genes for establishing early floral bilateral symmetry, at least in Orchidaceae, may include those involved in organ fusion and abortion in the inner whorls.

Fruits

Fruits are the result of morpho-anatomical changes in the carpels after the fertilization of the ovules and represent an outstanding example of extreme ontogenetic transformation leading to a myriad of forms and seed dispersal strategies. Fruit classification takes into consideration number and fusion of carpels, tissue types, texture, and dehiscence to release the seeds (Roth, 1977). Three distinct layers on the fruit wall are often apparent: the exocarp, which includes the outermost layer, and only occasionally including hypodermal tissues; the mesocarp, often formed by multiple middle layers and the vascular tissue; and the endocarp, which consists of one to few layers in contact to the developing seeds, often inner to the vascular bundle (Richard, 1819; Sachs, 1874; Roth, 1977; Pabón-Mora and Litt, 2011; Thadeo *et al.*, 2015). Some trends can be noticed with respect to fruit evolution (**Figure 9**). Fruits that come from apocarpic gynoecia are less frequent and develop into characteristic follicles, like in the magnoliid *Illicium* (Illiciaceae) and the basal eudicot *Aquilegia* (Papaveraceae), or druplets, like in the blackberries (Rosaceae). Capsules (or dry dehiscent fruits), berries (or fleshy fruits), and drupes are by far the most recurrent fruit types, often derived from syncarpic gynoecia; however, they are evidently not homologous, as they have appeared reiteratively in many groups of flowering plants (**Figure 9**).

Dry dehiscent fruits are characterized by generating tension between a soft (parenchyma) and a hard (sclerenchyma) tissue and have limited periclinal cell division. In contrast, fleshy fruits have continuous cell division, both periclinal and anticlinal, and little or no hard tissue; thus, most edible fruits fall into this category (Pabón-Mora and Litt, 2011; Thadeo *et al.*, 2015). Drupes are unique in that they are rich in hard tissue in the inner layers, but it is deposited in a continuous layer (s), thereby avoiding tension and rupture and allowing fleshy layers to proliferate in the outer layers, like in peach and avocados. A single family, like the Solanaceae, may have many fruit types; for example, early diverging taxa such as the tobacco have capsules, whereas the remaining members of the family, including peppers, tomatoes and eggplants, have predominantly fleshy fruits (Pabón-Mora and Litt, 2011; **Figure 9**).

Novelties in fruits are common themes throughout angiosperm evolution. Morphological diversification may also include other organs besides the carpels, such as the receptacle, bracts, sepals or petals, like in the apple or the strawberry (Esau, 1967; Weberling, 1989). The structural identity of these accessory organs often require developmental studies; one such case is the fruit of the magnoliid *Hedyosmum* (Chloranthaceae; **Figure 9**), which looks like a fleshy fruit derived from an inferior ovary (Gustafsson and Albert, 1999), but it is instead a small, black drupe derived from superior ovary, and tightly enclosed by white fleshy bracts that remain until late stages of fruit development (Doria *et al.*, 2012). Corn is also a unique fruit, where the kernel or caryopsis (collectively

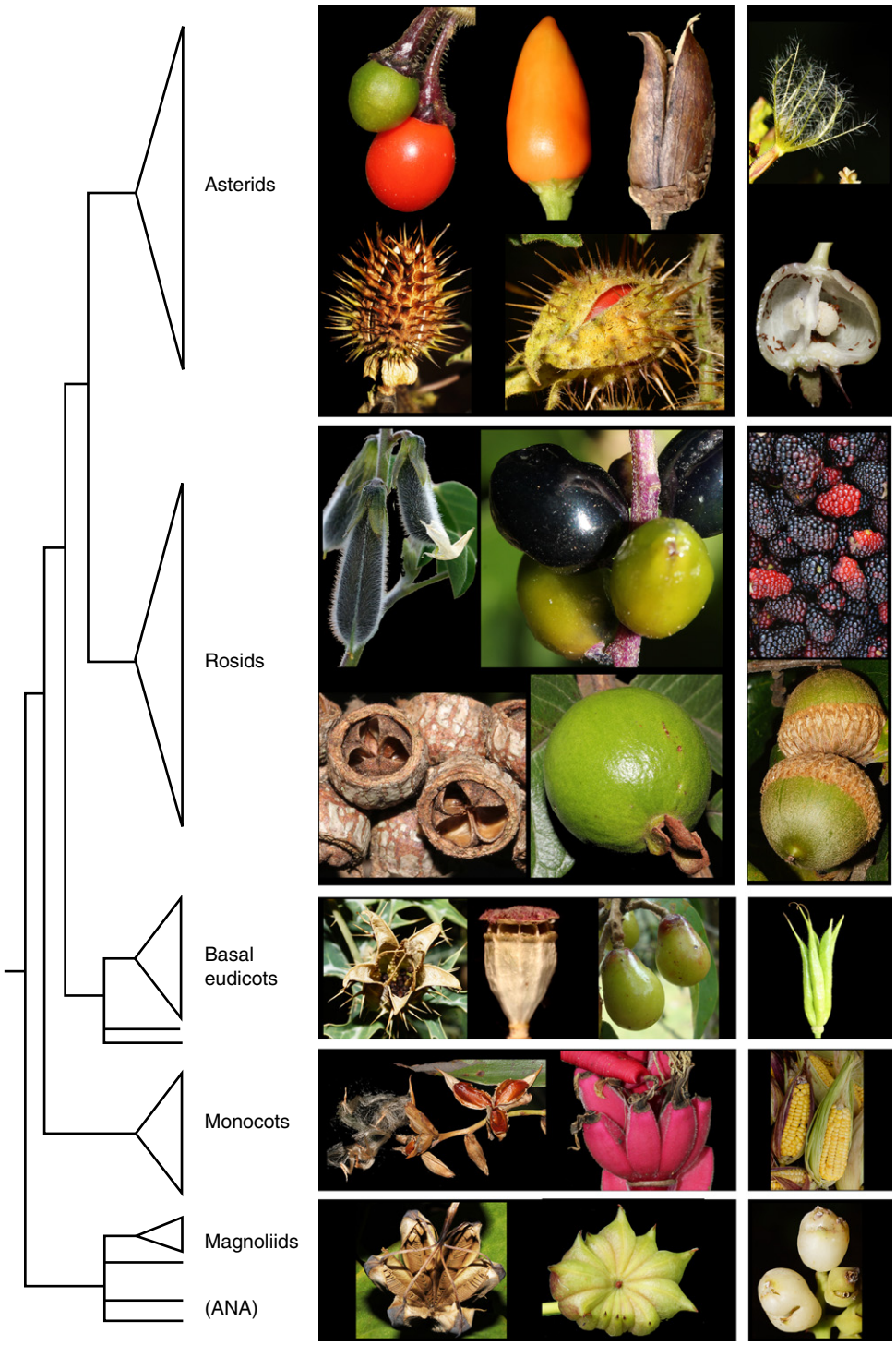

Figure 9 Trends in fruit evolution. Summarized phylogenetic angiosperm tree showing the five main groups ANA, magnoliids, monocots, basal eudicots, and core eudicots (rosids and asterids) with examples of fruit variation therein. Examples on the left and central columns correspond to dry dehiscent fruits and fleshy fruits, respectively; note that these two fruit types occur independently in all major groups. Examples on the right column correspond to particular variations of fruit types, on each major clade, from bottom to top; fleshy bracts of *Hedyosmum* (Chloranthaceae) enclosing dry drupes; corncob of *Zea mays*, Poaceae; multifollicles of *Aquilegia,* Ranunculaceae; acorns of *Quercus humboldtii*, Fagaceae; blackberry druplets of *Rubus* sp. Rosaceae; inflated berry of *Burmeistera caldasensis*, Campanulaceae; and cypsela of *Valeriana clematitis*, Valerianaceae.

grouped in the corncob, which is technically a multiple fruit) is the result of a strongly asymmetrical development of three carpels, two of which become vestigial. This phenomenon, called pseudomonomery, is also found in fruits of a number of non-related angiosperm families (reviewed in González and Rudall, 2010; **Figure 9**).

Unique novel fruits in the core eudicots include: (1) acorns, which is a dry, indehiscent nut that develops from one of the many flowers of the catkin, accompanied by a series of tightly appressed bracts. (2) Cypselas (common in Valerianaceae and Asteraceae), where an achene derived from an inferior ovary, is accompanied by a feathery calyx for wind dispersal, and (3) inflated berries (e.g., some *Burmeistera*, Campanulaceae), where a large air chamber is formed during fruit development (**Figure 9**).

The molecular basis of fruit diversity is largely unknown. However, the molecular genetic network during fruit histogenesis in *A. thaliana* is well characterized (reviewed in Ferrándiz, 2002; Roeder and Yanofsky, 2006; Seymour *et al.*, 2013). *Arabidopsis* fruits are specialized capsules called siliques; they develop from two fused carpels that dehisce longitudinally (Avino *et al.*, 2012). The silique has two valves separated by the replum, a thin persistent septum present only in the Brassicaceae. The replum and the valves are joined together by the valve margin, which is further composed of a separation layer closest to the replum, and a lignified tissue closer to the valve. The endocarp of the valves becomes lignified late in development and plays a role, along with the lignified layer and separation layer of the valve margin, in fruit dehiscence (Ferrándiz, 2002).

Developmental genetic studies have identified key players in the genetic network patterning the *Arabidopsis* fruit. FRUITFULL (FUL) is necessary for proper valve development and represses SHATTERPROOF 1/2 (SHP 1/2) (Gu *et al.*, 1998; Ferrándiz *et al.*, 2000). SHP1/2 are necessary for valve margin development (Liljegren *et al.*, 2000). REPLUMLESS (RPL) is necessary for replum development and represses SHP1/2 (Roeder *et al.*, 2003). The repression of SHP1/2 by FUL and RPL keeps valve margin identity to a small strip of cells. SHP1/2 activate INDEHISCENT (IND) and ALCATRAZ (ALC), both necessary for the differentiation of the dehiscence zone between the valves and replum (Girin *et al.*, 2011; Groszmann *et al.*, 2011). IND is important for cell lignification along the dehiscence zone, while IND and ALC are necessary for proper differentiation of the separation layer (Rajani and Sundaresan, 2001; Liljegren *et al.*, 2004). SPATULA (SPT) plays a minor role that is redundant with its paralog ALC in the specification of the dehiscence zone (Girin *et al.*, 2011; Groszmann *et al.*, 2011).

All these genes belong to three large transcription factor families. FUL and SHP1/2 belong to the MADS-box family (Gu *et al.*, 1998; Liljegren *et al.*, 2000); IND, SPT, and ALC belong to the bHLH family; and RPL belongs to the homeodomain (HOX) family (Rajani and Sundaresan, 2001; Roeder *et al.*, 2003; Liljegren *et al.*, 2004). Given that these gene lineages have duplicated at different time points during angiosperm diversification, it is unclear whether the gene regulatory network in *Arabidopsis* can be extrapolated to fruits outside Brassicaceae. Efforts have been made on multiple fronts: (1) by studying the evolution of gene families and identifying duplication time points that have changed the genetic content in specific angiosperm groups; (2) by studying gene function of pre- and post-duplication candidate genes to assess functional evolution; and (3) by taking a comparative developmental approach in families that exhibit different fruit types, and studying how gene expression and function has changed to shape dry and fleshy fruits, and the variation in between. New tools for predicting protein interactions together with more empirical data in non-model plants, hold great promise for better understanding fruit diversification.

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See also: Angiosperm Phylogeny and Diversification. Evo-Devo: Regulatory and Protein-Coding Evolution in Plant Diversification

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Relevant Website

http://phytozome.jgi.doe.gov/pz/portal.html
Phytozome.

O

Operational Sex Ratio

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Glossary

EPP The 'environmental potential for polygamy' is a conceptual scheme for how the spatial and temporal distribution of resources necessary for reproduction allowed certain individuals to monopolize mates; EPP included the degree to which the costs of parental care might limit or enhance mate acquisition.

Estrus A period of heightened sexual receptivity, usually observed in female mammals.

Iteroparity Repeated reproductive events within an individual lifetime.

OSR The operational sex ratio, often defined as "the average ratio of fertilizable females to sexually active males at any time;" designed to measure, in terms of the excess of one sex relative to the other, the level of competition for mates in animal populations at a particular time and in a particular place.

Polyandry An animal mating system in which males mate once but females vary in their mate numbers.

Polygamy An animal mating system in which both sexes vary in their mate numbers to a similar degree.

Polygyny An animal mating system in which females mate once but males vary in their mate numbers.

PRR The potential reproductive rate is the maximum possible rate of offspring production per unit time that an individual in one sex would achieve if unconstrained by the availability of mates.

Sexual dimorphism The condition in which males and females within the same species express distinct phenotypes.

Introduction

The Operational Sex Ratio, or OSR, first defined by Emlen (1976, p. 309) as "the ratio of receptive females to potential mating males at any one time," was designed to measure, in terms of the excess of one sex relative to the other, the level of competition for mates in animal populations. The ease with which OSR could be measured, and its apparent utility as a proxy for the intensity of sexual selection, established it as one of the most widely used metrics in animal mating system research (Andersson, 1994; Klug *et al.*, 2010). Although first defined as a ratio of reproductively active females to males, the OSR is usually expressed as the reciprocal of this value because males rather than females compete for mates in most animal mating systems (Kokko *et al.*, 2012). Moreover, while the simplest quantitative description of OSR in this sense is $N_{♂}/N_{♀} = R_{O}$, where $N_{♂}$ and $N_{♀}$ are the total numbers of males and females within a population (Shuster and Wade, 2003), most measures of OSR specifically include only those individuals who are sexually receptive at a particular time and in a particular place (Ahnesjö *et al.*, 2001; Kokko *et al.*, 2006).

The OSR has proven useful for characterizing mate competition for many species, and it is particularly valuable for characterizing social circumstances within experimental populations (Krakauer, *et al.*, 2011; Weir *et al.*2011; Kokko *et al.*, 2012). However, the relationships between estimates of OSR and actual measures of sexual selection are inconsistent at best (Klug *et al.*, 2010) and at worst are likely to provide inaccurate or misleading information about the actual intensity of sexual selection (Shuster and Wade, 2003; Shuster, 2009). Because the effects of local variation in OSR on sexual selection are accounted for by other more accurate measures, it is now clear that without specific qualification, estimates of OSR cannot be interpreted as equivalent to sexual selection, and are unlikely to account for observed patterns of evolutionary change (Shuster, 2009; Klug *et al.*, 2010; Krakauer *et al.*, 2011; Kokko *et al.*, 2012).

How could such a seemingly useful and important metric as the OSR fall upon such hard times? To answer this question, I summarize below the origins of the OSR as a parameter in mating system research and review the various definitions of the OSR that have appeared in the literature since its inception. I next describe actual measures of sexual selection and compare them with estimates of OSR to illustrate what OSR actually measures and how. Lastly, I explain how data collected as part of mating system research and formerly used to estimate the OSR can be used to generate evolutionarily useful estimates of the effect of sex ratio biases in animal and plant mating systems.

The Origins of OSR

In his study of bullfrogs (*Rana catesbiana*), in which males compete vigorously for access to females, Emlen (1976, p. 309) defined the operational sex ratio (OSR) as: "the ratio of receptive females to potential mating males at any one time." Emlen clearly conceived of the OSR as a measure of the level of competition for mates, and began the convention of expressing the OSR, not as he originally defined it, but rather using the number of individuals in the more abundant sex as the ratio's numerator, and the number of individuals in the less abundant sex as its denominator. Because he calculated the OSR as the ratio of potential mating males to receptive females, Emlen's reported values for OSR for bullfrogs (Table 7, p. 309) were all greater than 1.

A year later, Emlen and Oring (1977, p. 216) modified slightly the original definition to consider multiple measurements, and defined OSR as "the average ratio of fertilizable females to sexually active males at any time." Here too, Emlen and Oring used the OSR as a measure of the level of mating competition, primarily in birds and mammals. However, the OSR was also framed as the primary determinant of mating system organization, with the prediction that (p. 216) "Where the OSR is skewed toward males, polygyny is expected; when the skew is toward females, polyandry should occur."

This statement indicates that the authors, like Darwin (1871, p. 266), considered mate availability to be the prime mover for animal mating system evolution. However, Emlen and Oring's definition of OSR also emphasized how parental care might influence who was available to mate and when. They summarized this concept with 'the environmental potential for polygamy' or EPP, a less easily measured parameter, but one which captured conceptually how the spatial and temporal distribution of resources necessary for reproduction allowed certain individuals to monopolize mates. EPP also addressed concern at that time for the degree to which the costs of parental care might limit or enhance mate acquisition (cf. Williams, 1966; Trivers, 1972). The unique insights of Emlen and Oring (1977) led to an explosion of research on animal mating systems over the next four decades, generating, literally, thousands of articles (over 4500 to date).

In many vertebrate species, the taxon featured in Emlen and Oring (1977), OSR *did* seem to provide a reliable means for visualizing the level of competition among males to monopolize resources necessary for female reproduction. Moreover, given how readily OSR could be measured in the field, and its apparent utility as a proxy for the 'intensity' of sexual selection (see note #23, p. 222), estimates of OSR soon became the standard parameter measured in mating system research, beginning in 1977 and continuing to the present day (reviews in Klug *et al.*, 2010; Kokko *et al.*, 2012; Passos *et al.*, 2014).

Emlen's (1976) field calculation of OSR, rather than Emlen and Oring's (1977) modification of it, is the form for OSR usually encountered in the literature, and it equals the measurable number of mature males divided by the measurable number of sexually receptive females. This version of the ratio serves as a useful instantaneous reproductive competition coefficient (Shuster and Wade, 2003); when OSR > 1, females are rare and competition for mates is presumed to be intense, whereas when OSR < 1, females are abundant and competition for mates is presumed to be relaxed. However, the definition of OSR has not remained consistent among researchers.

Three primary variants of the original concept of OSR now exist: (1) those that change the definition of the ratio itself, (2) those that attempt to incorporate the effects of parental investment within estimates, and (3) those that change how individuals are (or are not) included in estimates of this parameter. Regardless of how OSR is defined, in nearly all cases, OSR is presumed to predict or induce evolutionary responses by the populations in which it is measured.

Changes in Definition

As explained above, the OSR has been expressed as: (1) "the ratio of receptive females to potential mating males at any one time" (Emlen, 1976) and (2) "the average ratio of fertilizable females to sexually active males at any time" (Emlen and Oring, 1977). However, additional definitions include: (3) "the average ratio of males to females who are ready to mate at a given time and place" (Kvarnemo and Ahnesjö, 1996; Kokko *et al.*, 2012), (4) "the reciprocal of the population sex ratio" ($R_O = 1/R = N_{♂}/N_{♀}$; Shuster and Wade, 2003), (5) "the number of males and females ready to mate" (Nyman *et al.*, 2006), (6) "the ratio of matured females to males" (Yamamoto and Edo, 2006), (7) "the ratio of fertilizable females to sexually active males at any given time" (Forbes *et al.*, 2006; Prohl, 2006), and (8) "the relative number of members of each sex willing (or able) to mate at any given time" (Kemp and Macedonia, 2007).

Definition (3) is the most widely used in current literature. It makes Emlen's (1976) characterization more concrete by focusing on the actual numbers of sexually active males and females in the population, instead of the 'potential' numbers that may exist (vagueness from which definitions 5, 6, and 8 also suffer). As mentioned above, it also calls attention to parental care and the spatial and temporal circumstances in which mating competition may occur. Although Emlen and Oring's (1977) definition (2) is often cited as the source of the OSR, it is less used than Emlen's (1976) OSR, because most estimates consider OSR, as Emlen first suggested, at a single point in space and time.

Definition (4) expresses OSR as the reciprocal of the population sex ratio and therefore considers all males and females in the population, regardless of their willingness or ability to mate. Emlen and Oring (1977, p. 216) cautioned against this practice, but as explained below, a focus only on individuals 'ready' to mate is responsible for the failure of OSR to provide consistent estimates of the strength of sexual selection (Shuster and Wade, 2003; Shuster, 2009). The reciprocal of OSR ($= R$) defined as in (4) also has the advantage of expressing the population average number of available mates; per male in conventional mating systems ($N_{♀}/N_{♂}$ = average harem size, H) or per female in role-reversed mating systems ($N_{♂}/N_{♀} = 1/H$), metrics that, unlike OSR, do covary with the opportunity for sexual selection (Wade and Shuster, 2004).

Changes in Emphasis

The focus on parental care in sexual selection theory, beginning with Bateman (1948) and later with Williams (1966) and

Trivers (1972) appears to have influenced the changing view of what measurements of OSR needed to emphasize. Emlen and Oring (1977, pp. 222–223) anticipated these issues but later authors gave them quantitative expression. In particular, because reproductive costs were presumed to be substantial for each sex, Clutton-Brock and Parker (1992) introduced modifications to estimates of the OSR to consider whether individuals were in the 'time in' or 'time out' phase of their reproductive cycle. Similarly, Kokko and Monaghan, 2001; also Kokko *et al.*, 2006) explicitly considered whether costly reproduction caused individuals to be choosy or not when selecting mates.

Mitani *et al.* (1996) accounted for the effect seasonal reproduction had on reproductive availability by multiplying the adult sex ratio (ASR = $N_{adult♂}/N_{adult♀}$; Arnold and Duvall, 1994) by a coefficient that included the product of birth interval, B, and the number of days per year (365), divided by the duration of estrus, c, summed over the number of estrus cycles female experience before conception, n. The OSR estimated in this way was thus specific to iteroparous mammals with conspicuous estrus, such as primates, and was designed to reveal the degree to which iteroparity was influenced by estrus duration within and among species. However, attempts to correlate this value with the degree of sexual dimorphism (a proxy for past sexual selection) in primates required qualification. Although Mitani *et al.* (1996) found a positive relationship between OSR and sexual dimorphism in nonhuman primates, Marlow and Burbesque (2012) found a weaker one in humans, possibly because humans avoid physical combat in mating competition more than other great apes.

Changes in Personnel

Whereas the original definition of OSR focused on all individuals who were potentially receptive (cf. Emlen, 1976), later definitions focused on only individuals who were capable of mating at a particular time and place. In addition to considering the 'time in' and 'time out' phases of reproductive cycles (Clutton-Brock and Parker, 1992), the goal of this adjustment evidently was to partition breeding seasons into episodes that allowed consideration of the effects of competition for resources, competition for mates and the 'potential reproductive rates' (PRR) of breeding adults (Clutton-Brock and Vincent, 1991).

Ahnesjö *et al.* (2001) considered the fact that the ASR would likely be modified by competition for resources (usually among males, but also among females in role-reversed species such as pipefish and shorebirds), resulting in exclusion of certain individuals from breeding, and generating a post-competition ratio of 'qualified' breeding adults the authors identified as Q. Further reproductive competition was presumed to lead to the actual OSR, which described the ratio of actually reproducing females to actually reproducing males, whose contribution to offspring at that time would depend on the PRR of males and of females at that place and at that time. Other similar approaches were proposed by Kokko *et al.* (2006, 2012) and Kokko and Monaghan (2001).

Evolutionary Interpretations

Biases in OSR until recently were widely presumed to have significant evolutionary consequences, and the range of predicted outcomes is considerable (Shuster, 2009; Kokko *et al.*, 2012). The most commonly cited consequence of biased OSR was that proposed by Emlen and Oring (1977, p. 222), i.e., increased variance in mating success and a correlated increase in sexual selection intensity. However, these predicted effects now seem to depend on the species examined. Positive effects on sexual selection appear to exist in bullfrogs (*Rana catesbiana*; Emlen, 1976), St. Peter's Fish (*Sarotherodon galilaeus*, Balshine-Earn, 1996), Gulf pipefish (*Syngnathus scovelli*, Jones *et al.*, 2001), and arthropods, such as dungflies (*Scathophaga stercoraria*, Jann *et al.*, 2000) and garden spiders (*Argiope aurantia*, Foellmer and Fairbairn, 2005). In contrast, a negative effect of increasing OSR on sexual selection was shown in isopod crustaceans (*Paracerceis sculpta*, Shuster *et al.*, 2001), and no effect was shown in humpback whales (*Megaptera novaeangeliae*, Cerchio *et al.*, 2005).

Positive and negative effects of biased OSR have also been reported in the context of sperm competition avoidance. Whereas male-biased OSRs appear to favor such traits in bats (Hosken, 1997), songbirds (Moller, 1988; Møller and Briskie, 1995), and fruitflies (*Drosophila* spp., Pitnick and Karr, 1996), male-biased OSRs appear to have a negative effect on sperm competition avoidance in simultaneous hermaphrodites (Pen and Weissing, 1999) and Orange Sulphur butterflies (*Colias eurytheme*, Kemp and Macedonia, 2007). Mate selection and choosiness appear to be favored by biased OSR in broad and narrow nosed pipefishfish (*Nerophis*, *Syngnathus*, Rosenqvist, 1993; Berglund, 1995), as well as in bushcrickets (*Steropleurus stali*, Bateman, 1997) as are increased mate guarding and mating duration in green stinkbugs (*Nezara viridula*, McLain, 1981), seed bugs (*Lygaeus equestris*, Sillen-Tullberg, 1981), Chinese bushcrickets (*Gampsocleis gratiosa*, Gao and Kang, 2006), and in many Crustacea (Jormalainen, 1998).

Female-biased OSRs were widely presumed to be responsible for sex role reversals and the appearance of polyandry (Emlen and Oring, 1977; Smith, 1984; Berglund *et al.*, 1989; Forsgren *et al.*, 2004; Andersson, 2005; Simmons and Kvarnemo, 2006). Again, however, such patterns are inconsistent across taxa. For example, family sex ratio adjustment has been attributed to local biases in OSR, but the way females respond to biases varies; sex ratio biases coincide with predictions in flowering plants (*Begonia gracilis*, Lopez and Dominguez, 2003), insects (*Nezara viridula*, McLain and Marsh, 1990), silverside fish (*Menidia menidia*, Conover and vanVoorhees, 1990), as well as some oviparous lizards (*Niveoscincus microlepidotus*, Olsson and Shine, 2001; *Eulamprus tympanum*, Robert *et al.*, 2003); but other lizards, even in the same species, respond to biased OSRs in the opposite direction (*Eulamprus tympanum*, Allsop *et al.*, 2006; *Amphibolurus muricatus*, Warner and Shine, 2007).

Similarly, aggressive behavior increases in response to biases in OSR in Japanese medaka (*Oryzias latipes*, Grant *et al.*, 2000; Grant and Foam, 2002), but predicted changes in behavior and hormone levels do not always coincide in St. Peter's fish (*Sarotherodon galilaeus*, Ros *et al*,. 2003). In some salmon, aggression decreased with increasing OSR

(*Oncorhynchus nerka*, Quinn *et al.*, 1996); in zebrafish it remained unchanged (*Danio rerio*, Spence and Smith, 2005). Per capita oviposition rates appear to decrease overall with increases in OSR in *Danio* (Spence and Smith, 2005) and while female body temperature increased with increasing OSR in oviparous lizards, such influences did not affect progeny sex ratio (*Eulamprus tympanum*, Allsop *et al.*, 2006). In ortolan buntings, increases in OSR led to overall population decline (*Emberiza hortulana*, Stifetten and Dale, 2006).

Klug *et al.* (2010, p. 455) called the species-specificity of responses to biases in OSR, "the OSR/mate availability conundrum," and other reviews also document similar inconsistency (Head *et al.*, 2008; DeJong *et al.*, 2009; Weir *et al.*, 2011; Marlow and Burbesque, 2012). However, this observation on mating systems is not new. In describing the source of sexual selection, Darwin (1871, p. 208) stated, "In many cases, special circumstances tend to make the struggle between males particularly severe." This statement suggests that while heuristically plausible, the extent to which OSR biases actually lead to greater competition for mates, and thus stronger sexual selection, is highly variable.

What OSR Does Measure?

The focus on parental care in existing sexual selection theory appears to have been responsible for the changing view of what OSR is and what it should measure. If OSR is designed to measure the level of competition for mates, as stated by Emlen and Oring (1977), and if parental care requires time and energy, forcing individuals to periodically remove themselves from competition, then it does seem reasonable to consider which individuals are available to breed at any particular time. If only certain individuals reproduce at any time, including all males and females in such measurements seems likely to bias estimates of competition intensity. Methods now exist for capturing these relationships in detail (Clutton-Brock and Vincent, 1991; Clutton-Brock and Parker, 1992; Ahnesjö *et al.*, 2001; reviews in Kokko *et al.*, 2006, 2012).

However, measuring competition intensity or reproductive cost are not the same as measuring selection. Ignoring non-breeding individuals, either because they have failed in competition, or because they have temporarily exhausted their resources for providing parental care, omits those individuals whose numbers significantly increase the variance in reproductive success within that sex, which is proportional to the strength of selection. This policy of omission results in two kinds of errors in estimates of actual selection (Shuster and Wade, 2003; Shuster, 2009). When non-breeding individuals are removed from fitness estimates, a significant fraction of the among-group component of the total variance in fitness is omitted, causing the average fitness among mating individuals to be overestimated, and total variance in fitness for the population to be underestimated, respectively. Stronger sexual selection makes this error larger because more of the non-mating population is excluded (Shuster *et al.*, 2013).

A related difficulty involving field measurements of OSR is that instantaneous estimates of OSR often do not distinguish between males who mate and males who do not. As mentioned above, this issue can lead to errors in estimates of the mean and variance in fitness. Moreover, while OSR is presumed to approximate the level of mate competition, such estimates tend to overestimate the average intensity of competition during the breeding season, undermining the utility of such estimates (Shuster and Wade, 2003; Shuster, 2009).

Figure 1 summarizes these issues for two hypothetical breeding populations, each with 5 females and 5 males. For simplicity, each female is presumed to mate only once and have non-overlapping periods of receptivity. Relaxing these assumptions simply makes competition weaker overall. In population 1, each female mates with the same male in a different *j*-th interval (**Figure 1(a)**), whereas in population 2, each female mates in a different *j*-th interval with a different male (**Figure 1(b)**). That these scenarios are distinct in their influence on sexual selection is obvious (one male mates with all of the females vs. each male mates with a different female), yet each case generates indistinguishable instantaneous values for OSR in each interval ($R_{O(j)} = N_{♂}/N_{♀(j)} = 5$), as well as when summed across the breeding season ($R_{O(j)} = 25$).

Also note that the sum of the instantaneous estimates of OSR, $R_{O(j)}$, is 25 times larger than the overall estimates of OSR and of the sex ratio, R, which are identical in value ($N_{♂total}$ / $N_{♀total} = N_{♀total}/N_{♂total} = 1$). The reason for this discrepancy is that instantaneous estimates of OSR ($= N_{♂}/N_{♀(j)}$), tend to overestimate the level of competition, particularly when females are rare. This happens because the average rate of female arrival throughout the breeding season is estimated by the harmonic mean number of females per time interval ($= 1/H_{♀} = (1/T)(1/N_{♀(j)}) = 1$, where $T =$ the number of intervals; see Shuster and Wade, 2003, p. 95), which emphasizes small values. When females are rare, these instantaneous estimates of OSR contribute disproportionally to the aggregate value of OSR, making competition seem stronger than it actually is.

What OSR Doesn't Measure?

Although Emlen and Oring (1977) never claimed to measure sexual selection directly, they did imply the existence of a direct correlation between such selection and OSR. They stated (p. 222), "The greater the potential for individuals to monopolize resources or mates, the greater the intensity of sexual selection and the greater the environmental potential for polygyny." Despite this statement, it is now clear that a primary difficulty with estimates of OSR is that they don't measure selection at all (Arnold and Duvall, 1994; Shuster and Wade, 2003; Jones, 2009; Klug *et al.*, 2010).

Shuster (2009; see also Jones (2009)) summarized methods useful for measuring selection in the context of mating systems. These included (1) the difference between pre- and post-selection phenotypes, divided by the standard deviation of the pre-selection phenotypic distribution, also known as the selection differential (Lande and Arnold, 1983; Shuster and Wade, 2003), (2) the covariance between trait variation and offspring numbers, divided by the standard deviation in trait variation, also known as the selection gradient (Hersh and Phillips, 2004; Klug *et al.*, 2010; this approach can be executed using univariate or multivariate methods), and (3) the standardized covariance between mate numbers and offspring numbers, also known as the Bateman Gradient (Arnold and Duvall, 1994).

(a)

	Intervals w/ females->						
Territories w/ males	1	2	3	4	5		$N_{i.}$
1	0	0	0	0	0		0
2	1	1	1	1	1		5
3	0	0	0	0	0		0
4	0	0	0	0	0		0
5	0	0	0	0	0		0
						Σ	5
$N_{.j}$	1	1	1	1	1	5	
K_j	5	5	5	5	5		
R_j	0.20	0.20	0.20	0.20	0.20	1.00	
Ro_j	5	5	5	5	5	25.00	
$1/N_{.j}$	1	1	1	1	1		

(b)

	Intervals w/ females->						
Territories w/ males	1	2	3	4	5		$N_{i.}$
1	0	0	0	0	1		1
2	0	0	0	1	0		1
3	0	0	1	0	0		1
4	0	1	0	0	0		1
5	1	0	0	0	0		1
						Σ	5
$N_{.j}$	1	1	1	1	1	5	
K_j	5	5	5	5	5		
R_j	0.20	0.20	0.20	0.20	0.20	1.00	
Ro_j	5	5	5	5	5	25.00	
$1/N_{.j}$	1	1	1	1	1		

Figure 1 Estimates of the operational sex ratio (OSR) during the breeding season often fail to distinguish between males who mate and males who do not; they also tend to overestimate the intensity of sexual selection during the breeding season. Two hypothetical breeding seasons illustrate these points; each season (a–b) is represented by a 5 row × 5 column matrix; within each season, the *i*-th rows and the *j*-th columns are each numbered 1–5; the rows represent the territories on which each of the 5 males are established; the columns represent the intervals within which females may mate with males on territories; individual mating females and the pattern of mating in each season are highlighted in red. The total number of females mating with a male per territory is summarized at the right margin by $N_{i.}$; within each *j*-th breeding season interval (each column), the total number of receptive females per interval, $N_{.j}$, divided by the total number of males, K_j, equals R_j, the interval sex ratio, or the average number of females per male. The total number of males, K_j, divided by the total number of receptive females per interval, N_j, equals the interval operational sex ratio, R_{0j}. The effect of each female on overall male competition can be measured by the rate at which females appear in each interval, $1/N_j$. The average rate at which females appear across intervals is estimated by the harmonic mean of the number of females per interval, $1/H_{♀}=(1/T)(\sum 1/N_{♀(j)})=1$, where T equals the number of intervals containing females ($=5$). In season (a), each female mates with the same *i*-th male in a different *j*-th interval. In season (b), each female mates with a different *i*-th male in a different *j*-th interval. Because mating and non-mating males are not distinguished, each season generates identical and thus indistinguishable instantaneous and overall values for OSR ($R_{0j}=N_{♂}/N_j=5$; $\sum R_{0j}=25$; $R_0=N_{♂}/N_{♀}=5/5=1$. Also, because receptive females are rare in each *j*-th interval, in this case appearing one at a time, each instantaneous OSR (R_{0j}) is large and contributes disproportionately to the total OSR, which equals $(N_{♂}/H_{♀})T=\sum R_{0(j)}=25$, and exceeds the overall estimate of OSR by 25-fold. Redrawn from Shuster, S.M., 2009. Sexual selection and mating systems. Proceedings of the National Academy of Sciences of the United States of America 106, 10009–10016.

Each of these methods has strengths and weaknesses. Their primary advantage is that they identify the actual trait or traits under selection, and, based on the magnitude of the differential or the steepness of the gradient, they identify how strongly selection might operate on a particular mating phenotype. In arguing against the use of OSR as a proxy for sexual selection, Klug *et al.* (2010) asserted that such measures were the only satisfactory method for documenting the strength of sexual selection.

However, as noted in Krakauer *et al.* (2011), such methods may also generate erroneous results because traits that appear to be associated with mating success may also covary with other unmeasured traits. Accidental omission of correlated traits does not allow recognition of how the unmeasured trait

may contribute to selection, and may incorrectly assign evolutionary importance only to measured characters (Lande and Arnold, 1983; Wade and Shuster, 2010). Moreover, direct estimates of selection may overestimate the possible response to selection because they may detect significant covariance even when the total opportunity for selection is comparatively small (Hersh and Phillips, 2004).

When less is known about the phenotypes under consideration, and as a first step for detecting whether sufficient opportunity for selection exists (Hersh and Phillips, 2004), (4) the opportunity for selection, I, provides a means for estimating the maximum possible change in phenotype over a single episode of selection, wherein the variance in fitness, expressed in offspring numbers, is divided by the squared average in fitness (Crow, 1958; Wade, 1979; Wade and Arnold, 1980). When even less is known about the experimental system, estimates of the spatial and temporal distributions of mates can be documented by estimating the spatial and temporal crowding of sexually receptive individuals (m^* and t^*; Wade, 1995; Shuster and Wade, 2003) which each are proportional to the opportunity for sexual selection, I_{mates} ($= I_s$; Jones, 2009).

Shuster and Wade (2003; see also Shuster, 2009) showed how this framework, inspired by Emlen and Oring's (1977) graphical representation of the environmental potential for polygyny, can be used to partition the total variance in mating success into spatial, temporal, and sex ratio components that can be used to partition the total opportunity for sexual selection I_{mates}. In this scheme, $I_{mates} = I_{sexratio} + [{}^*I_{mates(t)} - {}^*I_{within(k)}]$, and thus estimates three parameters:

1. $I_{sex\ ratio}$ measures the opportunity for sexual selection caused by temporal variation in the sex ratio. This parameter estimates what the OSR is evidently designed to measure, but cannot because as defined, the OSR overestimates the intensity of sexual selection within intervals in which females are rare (see **Figure 1**).
2. ${}^*I_{mates(t)}$ measures the weighted opportunity for sexual selection caused by temporal variation in the availability of females. This parameter is small or large depending on whether females mate synchronously or asynchronously and is weighted by the fraction of all females that appear in each interval. Here larger fractions per interval contribute the largest effects.
3. ${}^*I_{within(k)}$ measures the weighted opportunity for sexual selection caused by spatial variation in the availability of females. This parameter is small or large depending on whether females are spatially dispersed or spatially clumped, and again, larger fractions of females per male territory contribute the largest effects. Notice too that ${}^*I_{within(k)}$ decreases the value of ${}^*I_{mates(t)}$ because ${}^*I_{within(k)}$ estimates the variance in mate numbers within individual males. Increases in the variance in relative fitness within males lead to concomitant decreases in the variance in relative fitness among males.

OSR in Experimental Situations

Despite the quantitative difficulties described above, estimates of the OSR can provide useful information on mating system competition, provided qualifications are made when these values are interpreted. Krakauer *et al.* (2011) argued that OSR can provide a useful within-species index of instantaneous levels of intra-sexual competition, conditions likely to be perceived and responded to by individuals within field and laboratory populations (Berglund *et al.*, 1986; Mobley and Jones, 2007). Weir *et al.* (2011) found in their meta-analysis of OSR studies that predictable changes occurred in aggression, sperm release, mate guarding and copulation duration, but that the nature of changes depended on the type of mating behavior and the species examined. This result suggests that OSR can be used to make the predictions Emlen and Oring (1977) found useful, provided that the nuances of the experimental system are clearly known in advance.

Summary

The utility of the operational sex ratio as an unqualified proxy for sexual selection intensity appears to be unfounded and the relationships between OSR and actual measures of sexual selection have proven inconsistent within and among species. Thus, estimates of OSR are not equivalent to those for sexual selection and are unlikely account for observed patterns of evolutionary change. Nevertheless, OSR is useful for characterizing experimental populations, and for manipulating competitive circumstances to observe behavior in particular species. Thus, the kinds of information obtained in past and present estimates of OSR and the spatial and temporal distributions of breeding adults can have evolutionary application, provided that the details of the experimental system and how changes in the level of competition among members of each sex are clearly known.

See also: Life History Evolution: The Role of Mating Systems. Polyandry and Female Postcopulatory Choice. Sexual Selection, Theory of

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Origin of Life, RNA World and

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Glossary

Activated nucleotide A nucleotide with chemical leaving group that allows it to polymerize (e.g., a triphosphate or an imidazole).

Aptamer An RNA sequence that has been experimentally selected to bind to a target molecule.

Autocatalysis A chemical reaction in which a molecule (or set of molecules) catalyzes the formation of more of itself (or themselves).

Extremophile An organism that is adapted for life in extreme conditions that would not be habitable by most other organisms.

Group selection Selection that acts on a group of entities as a whole (such as animals living in a social group or molecules inside a protocell) and that favors survival of the whole group, in contrast to selection acting on individual members of a group that leads to competition between the individuals.

Ligase A ribozyme that catalyzes the linking of two RNA strands to make a longer strand.

Polymerase A ribozyme that catalyzes polymerization of ribonucleotides to make RNA sequences.

Recombinase A ribozyme that catalyzes a cross-over (or recombination) reaction between two RNA strands.

Replication A process in which a second copy of a given molecular sequence is created.

Ribozyme A catalytic RNA molecule (as opposed to an enzyme, which is a catalytic protein).

Getting Life Started

One of the most commonly accepted definitions of life comes from a discussion on astrobiology organized by NASA: "Life is a self-sustaining chemical system capable of undergoing Darwinian evolution" (Joyce *et al.*, 1994; Benner, 2010). These scientists were interested in finding a definition that would apply not just to Earth life, but to life on another planet, if we ever find it. It is very significant that evolution was included as a defining feature of life, and it seems appropriate that we should include a discussion of the Origin of Life in this *Encyclopedia of Evolutionary Biology*.

In the last decade, astronomers have detected hundreds of planets in other star systems, and the increasing sensitivity of these observations means that it is getting progressively easier to detect smaller planets that might be similar to Earth. It now seems likely that quite a high fraction of stars have Earth-like planets (Petigura *et al.*, 2013), i.e., rocky planets with a temperature that allows liquid water. One reason to be optimistic that life might be common elsewhere is that life occurs in a very wide range of environments on Earth, with micro-organisms (bacteria and archaea) being particularly versatile in their habitat. Organisms that live in conditions that appear 'extreme' to us are known as extremophiles (Rothschild and Mancinelli, 2001). For example, organisms are found in boiling hot springs at close to 100 °C, crevices in sea ice at close to 0 °C, and lakes of extremely high salt concentration. Although most organisms would die in these extreme conditions, the fact that some organisms can survive there suggests that evolution often finds a way to solve the challenges posed by unusual environments. Typical conditions on other planets might be very different from those on Earth, but the study of extremophiles on Earth tells us that we should not be too narrow in our expectations of what other kinds of planets might be able to support life.

The big unknown is how likely it is that life will evolve, given a suitable planet. It may still be very difficult for a living process to get started, even on a planet where physical and chemical conditions are appropriate for supporting life, if it arises. We still have no way of knowing how many other planets have life, and we cannot rule out the possibility that we are alone in our galaxy (or even the whole observable universe!). However, the timeline for life on Earth gives us another reason to be optimistic. It is known that the Earth and the rest of the solar system formed about 4.6 billion years ago. It is thought that there were many impacts of large meteorites and comets in the early period of the solar system that would have made Earth uninhabitable until about 4.0 or 3.9 billion years ago (Hartmann *et al.*, 2007). There is also fairly strong evidence that life existed 3.5 billion years ago, and some indirect evidence that it was present as early as 3.8 billion years ago (Buick, 2007). Hence, the time window during which life evolved is at most 0.5 billion years, and could be as little as 0.1 billion years (100 million years). Although this may seem like a long time, it is much shorter than the age of the planet. There has been life on Earth for most of the time that Earth has been here. If the time for life to evolve on Earth is in any way typical, then we expect that there are many other planets that are old enough for life to have had a good chance of originating.

Of course, this does not mean that the origin of life is 'easy.' We cannot just throw together a few chemicals in a test tube and expect that a new kind of life will spontaneously appear (otherwise someone would have done it by now!). The origin of life is probably a very unusual event that takes millions of years to occur once on a whole planet. But once is all it takes, given that life can multiply exponentially once it gets started, and can adapt to areas of a planet that are much different to the place where it originated. In summary, the origin of life is not a miracle; it is an unusual event that can be studied from a scientific point of view, with an aim of understanding how it was most likely to have occurred on Earth and how it could occur elsewhere.

Factors Pointing to RNA

Cellular organisms on Earth today use three types of biopolymers (Figure 1). DNA genes are transcribed by protein enzymes to make mRNAs. The mRNAs are translated by ribosomes (whose chief component is RNA) to make proteins. Thus, DNA, RNA, and proteins are mutually dependent on one another for replication. The RNA World hypothesis is that modern organisms evolved from a simpler system that used only RNA. In the RNA world, RNA genes would be copied by RNA catalysts (Joyce, 2002).

In addition to logical simplicity, there are many other factors that suggest this could be true. Although no cellular life forms have genes made of RNA, there are many viruses that use RNA as genetic material. RNA and DNA encode genetic information in the same way. A strand of either nucleic acid can be a template on which a second strand is assembled. Pairing between complementary nucleotides means that sequence information is passed on. When the second strand acts as a template, a new copy of the original sequence is made. RNA viruses replicate by this two-step processes. RNA virus replication is catalyzed by protein enzymes (RNA polymerases) that are encoded by the viral genome. In the RNA World, it is envisaged that there were RNA polymerases that were 'ribozymes' (i.e., catalysts made of RNA). There are no naturally occurring RNA polymerase ribozymes of this type, but there has been considerable progress in synthesizing polymerases in the laboratory, as we will discuss below. Nevertheless, there are many other types of naturally occurring ribozymes, and the ability of RNA to be a catalyst is well established.

The first ribozymes discovered were self-splicing introns (Kruger *et al.*, 1982), which splice themselves out of pre-mRNA sequences without the aid of protein enzymes. For the RNA World hypothesis, the most fundamental naturally occurring ribozyme is ribosomal RNA. From the three dimensional structure of the ribosome, it is clear that the active site for the peptide bond reaction is made of RNA (Nissan *et al.*, 2000). This suggests that the original ribosomes were made only of RNA, and that the ribosomal proteins that are present in modern ribosomes were more recent additions. Protein synthesis also depends on transfer RNAs to decode the gene sequence and messenger RNAs that contain the genetic sequence. The whole process of translation only makes sense if RNAs preceded proteins.

The vast repertoire of catalytic functions that is carried out by modern proteins is impressive. Protein sequences are relatively small and flexible compared to nucleic acids, and the amino acid side chains in proteins contain a much larger range of chemical groups from which catalytic structures can be built than do the bases in RNA. Although proteins may be more efficient and more versatile catalysts than RNA, there appears to be no mechanism in proteins equivalent to complimentary base pairing in RNAs. Thus the information in an amino acid sequence cannot be passed directly from one protein to another. Also, the traditional view of nucleic acids as having a limited range of functions, is becoming somewhat outdated (Breaker and Joyce, 2014), as new discoveries are made regarding nucleic acid functions in cells.

In some versions of the RNA World, it is envisaged that relatively complex organisms with many different kinds of RNA catalysts evolved before the origin of encoded protein sequences (Chen *et al.*, 2007). In this view, the evolution of the ribosome and the genetic code would mark the end of the RNA World. Proteins would then begin to take over most of the roles that were previously catalyzed by ribozymes. A slightly different picture is that amino acids and small peptides were essential players alongside RNA all along (Li *et al.*, 2013), and that there was never a very large repertoire of purely RNA catalysts. Using peptides as cofactors of ribozymes, or even having amino acids covalently linked to ribozymes, is compatible with an RNA World picture of early life, but it is important to remember that long protein sequences could not be encoded before the translation process arose.

The other side of the cofactor argument is that many modern proteins use either single nucleotides or dinucleotides as cofactors that associate with the folded proteins and are essential for their function. This is often seen as further evidence for the RNA World (White, 1976), with the nucleotide cofactors being relics of an earlier phase of evolution.

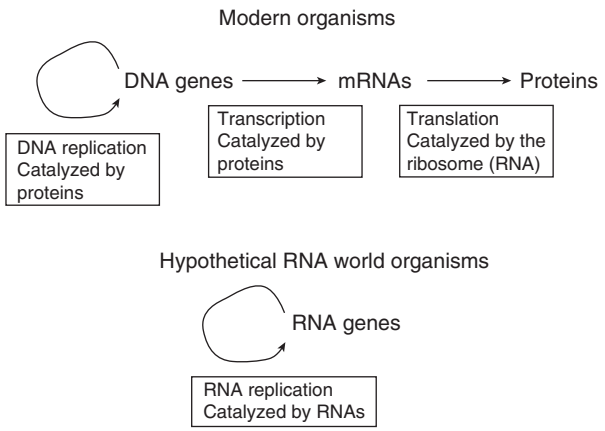

Figure 1 Comparison of the DNA + RNA + Protein system used by modern organisms with the RNA-only system that could have existed in the RNA World.

Alternatives to RNA

The factors discussed in the previous section strongly support the idea of an RNA World phase of life prior to modern DNA/RNA/Protein life, but they do not necessarily indicate that replicating RNAs were the first form of life. Maybe something preceded RNA? A variety of different kinds of biopolymers have been proposed that might do a similar job to RNA (Joyce *et al.*, 1987; Eschenmoser, 1999; Yang *et al.*, 2007; Yu *et al.*, 2012). RNA polymers have a backbone, which is a chain of ribose sugars linked by phosphate groups, and information-carrying side groups, which are the familiar four bases A, C, G, and U. Alternative nucleic acid analogues have been synthesized that have different backbone structure or different bases as side groups. Several of these alternative polymers are able to form double helical structures by pairing of complementary strands; thus, in principle, they might be able to support life. If these alternatives were chemically easier to form

than RNA or more stable in prebiotic conditions, then these might be plausible candidates for pre-RNA life. The alternative polymer would also have to be versatile as a catalyst, and it would have to be able to form a hybrid double strand with RNA. This latter point is important, because if some alternative genetic system got going first, genetic sequences that evolved in the first system would have to be transferred to the RNA system at a later date. If this transfer of information were not possible, then the RNA system would have to reinvent everything from scratch, and the earlier system might just as well not have existed.

The transfer of information from one system to another is known as genetic takeover. This idea makes sense because we know that DNA and RNA can form hybrid double strands. This happens in today's organisms during the transcription of an RNA strand from a DNA template, and during reverse transcription (which is essential for the replication of certain viruses), in which a DNA strand is synthesized using an RNA template. According to the RNA World hypothesis, DNA genes would have been synthesized originally from preexisting RNA genes. The better chemical stability of DNA and possibly the better fidelity of replication of DNA strands are thought to have given an advantage to organisms that evolved to use DNA in this way.

However, there is an important difference between a transfer of information from RNA to DNA, and a hypothetical transfer from a pre-RNA to RNA. In the former case, DNA is only being used as a gene and not a catalyst, so the DNA only needs to acquire the sequence of the RNA and not its function (e.g., the DNA gene for ribosomal RNA does not function as a ribosome). In the latter case, both the RNA and its precursor are genes and catalysts, so the function of the precursor strand as well as its sequence would have to be transferred to RNA, which seems very much more unlikely, as pointed out by Anastasi *et al.* (2007).

The term genetic takeover was originally used in a much wider context by Cairns-Smith (1982), who proposed that information in a mineral crystal might be transferred to a biopolymer system such as a nucleic acid. Another kind of system that might store genetic information is the lipid world (Segré *et al.*, 2001), which consists of globules or molecular assemblies composed of mixtures of different kinds of lipids. These would have information in their composition, but there would be no polymeric gene sequences, i.e., the molecules would not be covalently linked in a chain. An advantage of this idea is that lipid globules could self-assemble, whereas polymer synthesis (including RNA synthesis) is usually difficult because it is 'uphill' thermodynamically. If such molecular assemblies could grow and divide, they would be able to pass on their compositional information to their daughter globules. This idea works quite well in computer simulations, but it is very speculative. Neither the lipid world nor a mineral crystal scenario has been shown to work experimentally, and there is nothing like them in current biology. Alternative biopolymers similar to RNA seem more plausible, although there is no single clear candidate for an alternative. Furthermore, none of the alternatives exists in today's organisms. So if there were a pre-RNA polymer, genetic takeover must have been complete, such that no relics of the earlier system remain. It is tempting to draw an analogy with a possible future in which Earth is covered by self-replicating robots and computer systems after all human beings have died out. More seriously, however, adding extra stages to the proposed history of life makes things more complicated, and is not easy to justify in absence of more clear evidence. Here, we will proceed under the assumption that RNA, or something very similar to it, came first.

Synthesis of RNAs and Ribozymes

Although we cannot go back in time and observe the chemistry and biochemistry that occurred at the time of the origin of life, by doing chemical experiments today, we can show that the required kind of chemistry is possible and reveal what might have occurred at that time. It is helpful to think of four steps of increasing complexity, all of which must be possible if the RNA World hypothesis is true:

1. chemical synthesis of single nucleotides,
2. random polymerization,
3. template-directed polymerization, and
4. ribozyme-catalyzed polymerization.

As we will now discuss, all of these steps have been demonstrated experimentally to some degree.

A single nucleotide is a relatively complex molecule compared to an amino acid, say, or compared to common small molecules like CO_2 or H_2O. Formation of a nucleotide would involve multiple steps, and would be in competition with many other side reactions that produce other slightly different molecules. The difficulty of prebiotic synthesis of RNA has always been the chief reason for doubt of the RNA World idea (Shapiro, 2007). Nevertheless, new mechanisms of synthesis of single nucleotides have been discovered fairly recently (Powner *et al.*, 2009) that make step 1 seem reasonably plausible. Several mechanisms for random RNA polymerization (step 2) have also been studied that can make RNA strands with the aid of clay mineral catalysts (Ferris, 2005) or in association with lipid bilayers (Rajamani *et al.*, 2008). Again, these seem plausible, but we still do not know enough about conditions on early Earth to know at what rates these reactions occurred, what quantities were produced, and what local environment on Earth would have best promoted these reactions.

Random polymerization means that the monomers link in a way that is not guided by a preexisting strand or by a biological catalyst. If different kinds of monomers were involved, they would link in many different ways, so there would not be specific sequences. In contrast, step 3 is template-directed polymerization, in which an existing strand acts as a template on which to assemble the complementary strand. This is often called 'nonenzymatic' polymerization, to emphasize the fact that there is no catalyst (neither a protein enzyme nor a ribozyme) that catalyzes the reaction. Recent work (Deck *et al.*, 2011) has shown that the template-directed extension of a primer is possible with all four RNA nucleotides, significantly improving on earlier work (Hill *et al.*, 1993). In principle, sequence information can be passed from the template to its complementary strand by this process, if the error rate due to

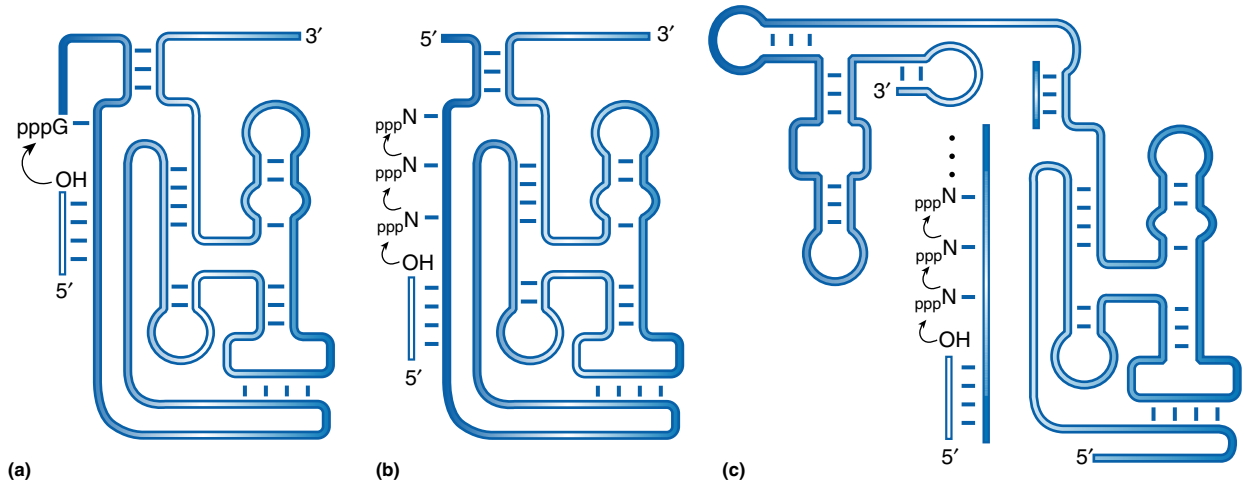

Figure 2 Three steps of *in vitro* evolution of RNA polymerases. (a) A ligase that extends itself by linking an oligomer to its own end (Bartel and Szostak, 1993). (b) A polymerase that extends a primer by addition of single nucleotides, using its own sequence as a template (Ekland and Bartel, 1996). (c) A *trans*-acting polymerase that uses a separate strand as a template to build the complementary strand of the template (Johnston *et al.*, 2001). Reproduced from Joyce, G.F., 2002. The antiquity of RNA-based evolution. Nature 418, 214–221.

incorporation of incorrectly matching bases is not too large (Rajamani *et al.*, 2010).

The origin of life lies somewhere in the gray area between chemistry and biology. Random polymerization seems definitely on the chemical side. Template-directed polymerization is in the gray area that is not quite biological. Ribozyme-catalyzed polymerization (step 4) seems to be on the biological side. In this case, there is an RNA strand that uses another strand as a catalyst to synthesize the complementary sequence of the template. In this case, the rate of the reaction is controlled by the biological catalyst and the sequence information is passed on to the new strand. Such a system would qualify as living, according to the definition of life with which we began this article.

There are no naturally occurring RNA polymerase ribozymes in modern organisms. Even viruses with RNA genomes use protein enzymes as RNA polymerases. The evidence that polymerase ribozymes could have existed is that it has been possible to synthesize them in the laboratory using *in vitro* evolution techniques. In these experiments, functional sequences are isolated from a pool of random sequences using a repeated cycle of copying and artificial selection that mimics evolution. RNA polymerases have been developed through a series of steps of increasing complexity, as shown in **Figure 2**. The simplest of these is a ligase (**Figure 2(a)**) that can link a short oligomer to its own end (Bartel and Szostak, 1993). Building on this, with further mutation and selection, a polymerase was found (**Figure 2(b)**) that extends a primer by addition of single nucleotides, using its own sequence as a template (Ekland and Bartel, 1996). Subsequently, this led to a *trans*-acting polymerase (**Figure 2(c)**) that uses a separate strand as a template to build the complementary strand of the template (Johnston *et al.*, 2001). Ribozymes of this type 2c are a key step forward because the product and catalyst are detachable, unlike 2a, so the ribozyme can catalyze multiple cycles of the reaction, and because, unlike 2b, the template sequence is not specified by the catalyst itself, so the ribozyme can copy whichever template sequences it encounters.

A polymerase of type 2c could have supported replication in the RNA world if it was able to deal with template strands that were as long as itself and if its error rate per base was small enough to avoid the error threshold (as discussed in the following section). The sequence isolated by Johnston *et al.* (2001) was able to extend the primer by up to 14 nucleotides. Subsequent work has increased this to 95 (Wochner *et al.*, 2011) and more recently to 206 (Attwater *et al.*, 2013). This latter case is equal to the length of the ribozyme itself. Although sustained self-replication of its own sequence has not yet been shown, this result is strongly suggestive that RNA-catalyzed RNA replication would have been possible in the RNA World. The step-by-step process of evolution from random chemical polymerization to a living RNA World that we envisaged in this section has been called 'the descent of polymerization' by Levy and Ellington (2001), who draws a parallel with the steps of evolution of the human species.

Evolutionary Theory

Theoretical models can highlight some of the key issues associated with the origin of life and early stages of evolution. As the precise conditions and the precise molecules involved in life's origins are not known, it is not usually possible to have detailed realistic simulations and theories at the molecular level. On the other hand, theoretical models can single out the most important factors that we expect to be relevant in the real world.

An important example of this is the quasispecies theory (Eigen *et al.*, 1988) which emphasizes that populations of molecules are subject to mutations (i.e., copying errors) during replication. In some cases, there is a maximum sustainable error rate, known as the error threshold. If the error rate is below this threshold, high-fitness sequences survive. If it is above this threshold, high-fitness sequences disappear due to accumulation of mutations. The maximum possible error rate per nucleotide is inversely proportional to the length of the

sequence. In modern organisms with large DNA genomes, replication is extremely accurate due to the presence of highly adapted DNA polymerases. Current viruses, with genomes of a few thousand base pairs, are much less accurate than cellular organisms. Early life presumably was still less accurate. This means that sequences would be severely limited in length. There are several ways in which the error threshold problem might be made a little less serious. Firstly, not every mutation in a sequence will destroy its function. There are probably a large number of mutations that are neutral or only very slightly deleterious. Thus, replication needs to maintain a population of related functional sequences rather than just a single exact sequence. Secondly, if sequences with different functions are replicated separately, error accumulation is less severe than if all the sequences are linked and replicated together (as in a modern genome). Nevertheless, accumulation of mutations is a real problem, and we can only conclude that the RNA world would have required an accurate polymerase quite early on, if it were to survive and increase in complexity.

Another important issue is that RNA polymerases, such as those in **Figure 2(c)**, do not copy themselves directly; they use another strand as a template. If the template strand has the same sequence as the polymerase, the polymerase sequence is copied. In the language of evolutionary biology, the polymerase is an altruistic cooperator, i.e., it is providing a benefit to other strands at a cost to itself. While it is acting as a polymerase, it cannot itself be a template for another polymerase. The simplest theoretical models assume a well-mixed solution in which molecules meet one another at random in proportional to their concentration. In this case, the cooperators tend to be overrun by parasitic sequences that can act as templates but not polymerases. Parasites are likely to be produced rapidly because deleterious mutations of the polymerase will produce nonfunctional sequences that can still act as templates. Theoretical models show that cooperative polymerases can manage to survive as long as there is a mechanism that promotes clustering of cooperative sequences together (Szathmáry and Demeter, 1987; McCaskill *et al.*, 2001; Szabo *et al.*, 2002; Takeuchi and Hogeweg, 2012; Shay *et al.*, 2015). One such mechanism is spatial structure that arises if molecules are bound to a surface and interacting with their local neighbors. If diffusion is slow, patches of cooperators form that replicate one another. A second mechanism operates if RNA replication is occurring inside compartments such as lipid vesicles. In this case, compartments that have few parasites can survive and divide, whereas those with many parasites become inviable. Group selection acting at the level of compartments can sometimes overcome individual selection acting at the level of molecules.

We have recently argued that several types of molecular cooperation are important in the RNA World (Higgs and Lehman, 2015). In addition to the altruistic cooperation of polymerases and templates discussed above, molecules can cooperate as part of an autocatalytic set. This is a set of molecules that together can catalyze the formation of each of the molecules in the set. However, none of them is a polymerase that can directly copy its own sequence. Theoretical models show that autocatalytic sets might emerge in a sufficiently diverse mixture of sequences that is able to catalyze reactions such as ligation of shorter strands to make a longer one (Kauffman, 1993; Hordijk and Steel, 2004, 2013). There have also been experimental realizations of this type of autocatalytic set involving ligases (Lincoln and Joyce, 2009) and recombinases (Hayden *et al.*, 2008; Vaidya *et al.*, 2012). In both these cases, non-catalytic precursor strands are assembled into longer catalytic strands. The reaction system requires a continued supply of the precursor strands; hence these systems could not survive in a prebiotic world without an additional mechanism of replicating large numbers of specific precursor sequences. In principle, however, an autocatalytic set might be possible as a scenario for the origin of life if it incorporated reactions that build up longer strands from single nucleotides and short oligomers that could be easily synthesized chemically. An autocatalytic set is complex in the sense that it is composed of many components, but the reactions involved might be relatively simple and specific. On the other hand, a polymerase ribozyme is simple in that it is a single molecule that could replicate many different templates, but the sequence itself might be long and complex. It is not yet clear, from either the experimental or theoretical viewpoint, whether a multicomponent autocatalytic set or a general RNA polymerase is more likely to emerge in prebiotic conditions.

Recombinase ribozymes (Hayden *et al.*, 2008) also illustrate a third form of cooperation. They assemble from shorter strands that are held together via RNA secondary structure formation, and they are not covalently linked initially. These strands are forced to cooperate because none of them is functional on its own. Ribozymes composed of self-assembled shorter strands might be more likely to emerge and more easy to replicate that longer sequences, because the pieces can be synthesized and replicated separately.

The RNA World hypothesis assumes that chemistry is able to create a diverse array of random RNA strands, and that eventually it manages to hit on a small number of specific catalytic sequences that are able to start the replication process. The steps creating RNA polymers, illustrated around the outer ring of **Figure 3**, are presumably slow and inefficient when

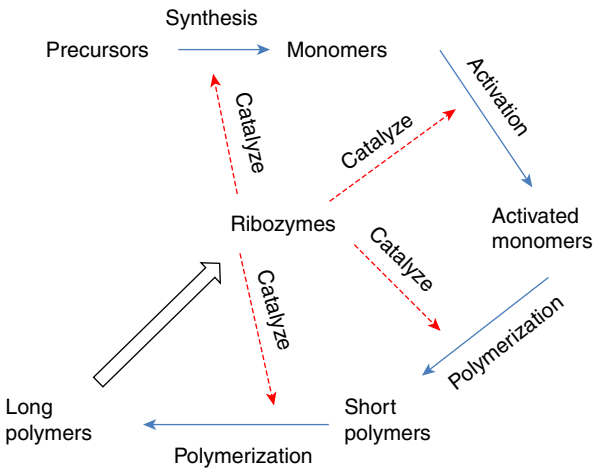

Figure 3 Getting the RNA World started. Before life, the steps leading to RNA polymerization must have occurred slowly and inefficiently. After life arose, all these steps could occur rapidly because they were catalyzed by ribozymes. This creates a living RNA World in which ribozymes catalyze their own formation.

they occur only chemically. However, if ribozymes arise in sufficient quantity, they can catalyze the reactions involved in all these steps. This sets up a feedback loop in which all reactions are rapid because they are catalyzed by ribozymes. We have referred to this as 'jump-starting' the RNA World (Wu and Higgs, 2009). The theoretical modeling shows that this requires concentration fluctuations that arise when only small numbers of molecules are involved initially. The jump from the dead to the living state occurs most easily when there is a spatial structure, such as when sequences are adsorbed to a surface and interact with their neighbors (Wu and Higgs, 2012).

This short article has presented the case that RNA was a key player in the origin of life on Earth and that the first kind of life may have been a system of replicating polymers made of RNA, or some very similar polymer capable of complementary base pairing. Although many alternative scenarios for the origin of life exist, none of them has the degree of support of RNA World hypothesis, which comes both from extensive laboratory based experiments and signals remaining in the way that modern organisms function.

See also: Noncoding RNAs, Origin and Evolution of. Origins of Life, History of

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Origins of Life, History of

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Introduction

The history of the 'origin of life' question is rooted in ancient philosophy and continues in research today. It was a central aspect of a wide range of philosophies, belief systems, and scientific investigations, including in the modern period, as well as the experimental physical and life sciences. This article explores several of the periods and approaches to this question (Strick, 2000). At its core, the search for the elusive answers to this question defines our understanding of our place on the Earth and in the cosmos.

Origin of Life in the Ancient Period

One of the earliest conceptions of the origin of life is found in Aristotle's (384–323 BCE) description of spontaneous generation. He believed that life is created out of a melding of the elements air, earth, water, and fire. In *Generation of Animals* he wrote that, "[a]nimals and plants come into being in earth and in liquid … in all air is vital heat so that in a sense all things are full of soul. Therefore living things form quickly whenever this air and vital heat are enclosed in anything. When they are so enclosed, the corporeal liquids being heated, there arises as it were a frothy bubble" (Aristotle, 1910, Book III, Part 11). Aristotle believed that this 'vital heat' contained the soul, or *eidos*, which was necessary to animate otherwise nonliving matter. It is the soul, which forms the matter into its necessary shape, providing an ancient explanation for the adaptation of species to their environment (Williams, 2010, pp. R84–R85). Recently, some have tried to relate Aristotle's *eidos* to the modern genetics, seeing it as an early forerunner to DNA (Vinci and Roberts, 2005). He realized the role of sperm in fertilization in sexual reproduction, yet he believed that the generative power was in its "vital heat" and that "the semen, if cold, is not generative" (Aristotle, 1910, Book I, Part 7). The details of the reproductive cycle remained outside his observing powers – for example, he concluded that in "testaceous" creatures (mollusks), "one does not generate in another; but they are formed and generated from a liquid and earthy concretion" (Aristotle, 1910, Book I, Part 23).

The Roman philosopher Titus Lucretius Carus (99–55 BCE) proposed a similar doctrine of spontaneous generation. In his epic natural historical poem, 'De Rerum Natura' (*On the Nature of Things*), he did embrace the Epicurean idea that human suffering is caused in part by a dread of the gods, but yet advocated for spontaneous generation, stating that the world takes form without the intervention of the gods (Palmer, 2012, p. 397). He claimed "they feign/That gods have established all things but for man" (Lucretius and Leonard, 1957, Book II). Though he accepted the existence of the gods, Lucretius thought they played little role in the "ordering or continuation" of the world (Palmer, 2012, p. 397). Following Democritus and Leucippus, Lucretius held a materialist atomist view of nature that informed his view on the origin of life. However, as Robert Wardy argued, a belief in an "atomic microstructure" alone does not "compel the theorist to adopt any particular ontology of macroscopic objects" (Wardy, 1988, p. 115). Lucretius, however, explained spontaneous generation due to the constant clashing of atoms that created cascades of combinations and eventually life (Lucretius and Leonard, 1957, Book II).

Seventeenth-Century Thoughts and Discoveries on Spontaneous Generation

During the early modern period, debates on the origin of life reevaluated ancient assumptions about spontaneous generation in terms of origins from nonliving matter and initiated the experimental period of its investigation (Paweletz, 2001, p. 478). Natural philosophers conducted small-scale biological experiments challenging the idea that organisms could arise from sources other than similar organisms – for example, that worms could generate out of decaying meat, or parasites out of dust. Francesco Redi (1626–97), for example, conducted a series of experiments proving that worms and maggots formed not spontaneously, but rather from another biological source. Covering meat with gauze and a perforated dome, he observed that eggs and maggots appeared on the gauze, but not the meat (Mazzarello, 1999, p. 237). He claimed that insects such as mosquitoes and flies deposited eggs that lead to the generation of new organisms (Hawgood, 2003, p. 30). Due to his inconclusive studies of plant galls, however, Redi continued to defend the origin of life by spontaneous generation in other cases. The discovery of microorganisms defined a new era in investigating the origin of life. Redi's contemporary, Marcello Malpighi (1628–94), for example, would explain the cause of galls. Using the newly invented microscope, Malpighi observed the action of microorganisms in them, eventually tracing them to cynipid gall-wasp eggs (Corrington, 1961, pp. 65–66). The still controversial observations of Antonie von Leeuwenhoek (1632–1723), the discovery of bacteria and their biological origin offered scientists a new material category of life to consider in the experimental investigation of origin of life (Corliss, 2002).

Spontaneous Generation and Uniformitarianism in the Eighteenth Century

Eighteenth-century developments in spontaneous generation research stemmed from the work of the early seventeenth century vitalists who, based on observations of toads being born out of 'slime' and maggots out of wheat grains, believed that life could arise out of nonliving matter (Pommerville, 2012, p. 10). Belief in the theory of spontaneous generation began to wane in the seventeenth and eighteenth centuries due

to observations of sperm and embryo development (e.g., Leeuwenhoek) and controlled biological experiments with negative results (e.g., Redi). Following Leeuwenhoek's work in 1677, British clergyman and naturalist John Needham (1713–81) conducted a series of experiments by boiling and stoppering mutton gravy discovering that: "the gravy swarm'd with life, with microscopical animals of most dimensions" (Needham, 1748). The Italian cleric and naturalist Lazzaro Spallanzani (1729–99) criticized this work, proposing that Needham had insufficiently heated the broth, and established a new protocol using open, stoppered, and sealed vials. Spallanzani's open vials teemed with bacteria, the stoppered vials had many fewer, and the sealed vials had none (Pommerville, 2012, p. 11). He argued that this experiment discredited the theory of spontaneous generation, yet Needham and others countered that excessive heating destroyed the vital force, initiating a debate that would persist into the late nineteenth century.

In 1735, Carl Linnaeus (1707–78) published *Systema Naturae*, a classification system for organisms from kingdoms to species. Built on the idea that organisms are more closely related to some than others, his system became a key to determining ancestral relationships in the fossil record. James Hutton (1726–97) in *The Theory of the Earth* developed the geological concepts of gradualism and deep time, which together comprised uniformitarianism, or the idea that "the present is the key to the past" (Hutton, 1795). This notion that the geological record provided "no vestige of a beginning, and no prospect of an end," challenged creationist theories of the origin of life (Hutton, 1795).

Nineteenth-Century Materialism and Cell Division

Naturalists exploring the origin of life question in the late eighteenth to mid-nineteenth century argued from a wide range of materialist frameworks. They considered matter the fundamental substance of nature and all phenomena the result of material interactions. Others favored vitalism, the idea that life is defined by the existence of a "vital spark" (soul) that separates it within the realm of the inorganic or nonliving (Bunge, 1981, p. 17; Normandin, 2006, p. 6). For the materialist Carl Vogt (1817–95), for example, the human soul is only a function of the brain, and thoughts the products of the brain, just as bile is a product of the liver (Rupke, 2000, p. 219). In materialistic views of nature, the existence of soul does not differentiate living from nonliving objects. Among those who believed in materialism were scientists such as Jean-Baptiste Lamarck (1744–1829), who supported the notion that all organisms have an innate ability to change over time (Burkhardt, 2013, p. 796). This hypothesis suggested that supernatural abilities once reserved for God could instead be performed by nature, leading some to believe that matter was the only substance in the world and there was no need for supernatural explanations.

The turn of the nineteenth century witnessed a new wave of evidence that supported materialism. The work of the German physiologist, Theodor Schwann (1810–82) helped define cell theory and garner support for materialism. In 1839, Schwann, informed by Matthias Schleiden's theory that the basic structure of plants consisted of cells, extended it to the animal world. An essential part of their research was the materialist notion that only cells formed cells (Sander, 1996, p. 8). Schleiden discovered that daughter cells are created within the parent cell in 1838. Hugo von Mohl countered with a new theory that cells were not created within, but divided from parent cells to daughter cells. This concept of cell division was first introduced in Mohl's work with green algae in 1837. His investigation showed that cells would split, then grow into full size again as separate cells. The idea that life could crystallize "out of an unspecific nonliving fluid, which can be regarded as a special case of the doctrine of spontaneous generation" was central to these novel theories of cell development (Deichmann, 2012, p. 535).

Alternation of Generations

A community of morphologists in the nineteenth century challenged the established ideas about the relationship between, and thus the origin of animal and plant life. Johannes Steenstrup (1813–97) in his *On the Alternation of Generations* represents a pioneering achievement concerning biological development in the lower classes of animals. His investigations of the 'alternation of generations' in *Medusa* and other invertebrates, he argued, revealed the plasticity and progressive development of species (Steenstrup, 1845, p. 2). His work highlighted novel ideas about cellular origin, but also the development of plant and animal life, from its simpler to more complex forms.

End of Spontaneous Generation

The ancient notion of spontaneous generation persisted into the late nineteenth century, when for much of the scientific community, they were resolved by the work of Louis Pasteur, John Tyndall, and others. Working within a quasi-Darwinian framework, these biomedical experimentalists participated in a series of popular scientific debates with the notable figures serving as the opposition, including Felix Pouchet and Henry Charlton Bastian (Strick, 2000, p. 2). In 1862, Pasteur published the results of his experiments, in which he maintained a sterilized broth in swan-neck retorts that filtered out bacteria and dust particles, but yet allowed in air, proving that decomposition had a biological origin, that is, biogenesis (Strick, 1999, p. 56). At the same time, Pouchet and Bastian provided convincing experimental evidence in support of spontaneous generation. Pouchet's research showed that bacteria growth occurred in "previously boiled and sealed infusions made from … hay," supporting Bastian's theory of heterogenesis – that germs and microbes originated only from inorganic material (Strick, 1999, p. 57–61). While these debates were resolved more politically than experimentally, others contributed new evidence. For example, John Tyndall's light scattering experiments supported the ideas that atmospheric dust consisted of organic materials and germs and likely contaminated Pouchet's experiment (Strick, 2000, p. 22). Pasteur's arguments and long series of experimental evidence led to a consensus among the scientific community that biogenesis was the correct theory for the origin of life.

An alternate hypothesis based on the work of Thomas Huxley, Ernst Haeckel, Carl Nageli, August Weismann, Eduard Pfluger, and others, called abiogenesis proposed that "life emerged during the course of the evolution of matter and life on earth was a natural product of terrestrial processes" (Kamminga, 1982, p. 67–68).

Panspermia

In the nineteenth century, physicists William Thomson (1824–1907) and Hermann von Helmholtz (1821–94) developed panspermia – from the Greek *panspermos* meaning 'containing all kinds of seeds' – a theory of the cosmic origin of life, in which "seeds of life were spread from star to star" (Anon., 2006, p. 54)." In *Worlds in the Making*, Svante Arrhenius (1859–1927) proposed an evolutionary and thermodynamic version of panspermia that discussed the conditions of bacterial spores and plant seeds to determine the likelihood of survival in interplanetary and interstellar space. He was the first to discuss the unearthly qualities of bacteria and the mode of transportation (the force of starlight) of these cosmic voyagers (Arrhenius and Borns, 1908). More recently, Fred Hoyle (1915–2001) and Chandra Wickramasinghe (1939–), arguing from a neo-Darwinian perspective, asserted that bacteria (traveling on comets) could survive in space because they must originate from space (Hoyle and Wickramasinghe, 1996). Thus, panspermia can explain the quick evolution of life on earth.

The Biogeochemical Hypothesis

Up to the 1920s, the question of the origin of life was discussed mostly in biological and chemical terms. The debate hinged on whether life was spontaneously created in one flash of organic synthesis, or whether life formed slowly by a combination of inorganic and organic reactions. The growing field of colloidal chemistry, or the study of homogeneous solutions suspended with microscopic particle, fueled this debate. Biochemists argued whether colloids would be able to effectively trap and concentrate organic precursors in such a way as to create life. The argument then proceeded into a familiar debate over spontaneous generation, which had yet to be resolved. Despite the lack of consensus, one general principle was agreed upon that profoundly changed the discussion going forward: the origin of life could be explained in purely chemical terms (Farley, 1974, pp. 159–163).

This conception of a purely chemical origin of life gave J.B.S. Haldane (1892–1964) and the Soviet scientist Aleksandr Oparin (1894–1980) the opportunity to synthesize the prior debate into one clear hypothesis. In 1929, Haldane, discussing the role geological conditions played in the origin of life, proposed the idea of an anoxic early earth, rich in proto-organic compounds (Kamminga, 1988, p. 7). In chemical terms, organic molecules could arise anew only in the absence of oxygen, which permitted ultraviolet (UV) radiation (energy) to initiate the photochemical organic synthesis (Rutten, 1962, pp. 48–49).

Oparin extended Haldane's concept further, hypothesizing a series of steps in the chemical evolution of life. Drawing on geochemistry, astronomy, evolution, and colloidal chemistry, Oparin developed a grand origin theory. In Earth's early history, he stated, inorganic molecules were broken down by UV rays to form organic molecules and a 'primeval soup' (Miller and Orgel, 1974, pp. 11–12). These molecules would then have coalesced into a coacervate colloid, which would concentrate organics, allow proteins to gradually form, and through competition eventually evolve into the first life forms. This revolutionary synthesis of biology, chemistry, and geology would set the next stage in the experimental era of the study of life's origin.

What Is Life?

During the twentieth century, attempts to define 'life' came to bridge the physical and social sciences and the humanities. Debates became personal, emotional, and often mired in cyclical rhetoric and beyond the reach of practical explanations, no matter the philosophical and scientific contexts. A novel set of questions came from Erwin Schrödinger (1887–1961), who asked in a public lecture *What Is Life*, explored three important issues related to the origin of life: resistance to entropy, permanence of genetic material, and its replication – all of which occurred bounded in the living cell. His work was significant less for its portrayal of contemporary ideas in origin of life scientific research, but more for the influence it had on its investigation in the 1950–70s, and especially molecular genetics (Olby, 1971, pp. 130–135).

Origin of Life: Urey–Miller's Experiments

In 1952, inspired by Oparin's work and advised by Harold Urey, Stanley Miller (1930–2007) conducted a new classic experiment that demonstrated how amino acids and other organic compounds – that is, the basic building blocks of life – could be synthesized under primitive Earth conditions. Miller's idea was a prebiotic synthesis experiment in which an electrical energy source would be discharged on a mixture of methane, ammonia, water vapor, and hydrogen (replicating primitive earthly conditions). During the experiment, a mixture of primitive earthly gases were circulated through a water solution and zapped with electric sparks (substituting for natural lightning). The results of the continuous sparking were 'biochemically significant' compounds such as amino acids, hydroxyl acids, and urea (Miller, 1953). Numerous scientists replicated and expanded these investigations since, finding a large number of amino acids that are integral to life, and many others that are not.

RNA/DNA Biochemistry

In the field of evolutionary biochemistry, two important questions exist: how did life originate, and how did it evolve? In particular, biochemists look at components of a cell to help make that determination. The cell's nucleic acids RNA

(ribonucleic acid) and DNA (deoxyribonucleic acid) are necessary components in the formation of proteins; likewise, proteins are necessary in the formation of nucleic acids. In 1967, Carl Woese (1928–2012) attempted to determine which came first, nucleic acids or proteins, leading to the RNA World Hypothesis. He proposed that RNA was the precursor to life by mixing proteins, RNA, and DNA (Woese, 1967). Following Woese, in 1982, Thomas Cech discovered a type of RNA that can act as a chemical catalyst, scientists did not know whether proteins or nucleic acids came first; however, with this discovery it was determined that some forms of RNA can replicate itself, thereby solving the question that RNA came first in the evolution of Earth's first life (Gesteland *et al.*, 1999).

In 1967, biologist Lynn Margulis (1938–2011), drawing on the Russian tradition, published her theory of symbiogenesis, which added a new dimension to evolution that allowed for the combination of existing genomes in a new symbiotic organism, at both the cellular and organismic levels (Khakhina *et al.*, 1992). Margulis proposed that a major transition in the origin of life occurred when nuclear information transferred between bacteria and eukaryotes (Margulis, 1999).

In the 1980s, Andrew Ellington noted that the chemicals that constitute life formed without living matter first by replicating themselves and later by evolving into reproducing life forms. His investigation of the evolution of dehydrogenase isozymes – enzymes found in single-celled organisms lacking a defined nucleus – led him to develop a method to engineer nucleic acid species known as 'aptamers' through repeated *in vitro* selection. Today, Ellington is extending and accelerating his evolutionary engineering approach to whole organisms and biotechnologies (Ellington, 2008).

'Life' in Recent Philosophical Debates

The question 'What is life?', a long standing topic of philosophical debate, has in recent years become increasingly scientific. Carol Cleland, a leading philosopher at CU Boulder, challenges the traditional approach taken by philosophers and scientists in answering it; instead of looking for 'definitions of life,' she notes, we should develop a general theory of living systems that might exist in the universe – life on Earth is only one possible example of life (Cleland and Chyba, 2007). Informed by recent work in philosophy and evolution, Iris Fry concludes that similarities in Earth's microbiology and biochemical makeup support a theory of common origin (Fry, 2000).

Another issue is the Earth-centric view that is pervasive. In the infinite universe it is a distinct possibility that life could form in multiplicate, with nonliving materials without necessarily being on Earth. Life arising more than once from nonliving materials could occur elsewhere than Earth, but it could also have occurred on Earth. It is within the realm of possibility that even with extraterrestrial life all life could have a similar origin, or not. Microorganisms, for example, might survive interplanetary travel on meteors eventually to land on planetary bodies with environments that can sustain life (Cleland, 2013).

Astrobiology: The Past, the Present, and the Future

The origin of life question, and its corollary the existence of intelligent life, is a primary focus of exo/astrobiology (Brack *et al.*, 2001). By applying the principles of natural sciences (biology, chemistry, and physics) to explain the mechanisms behind the origin and evolution of life, astrobiologists are developing search parameters for life on other planets. Strong programs have existed since National Aeronautics and Space Administration's (NASA) first program in 1959, and continue in numerous institutes, such as Ariel Anbar's team at Arizona State University. The aim is to study the past, current, and future mechanisms that allow Earth to be a habitable planet and how those mechanisms might apply to extraterrestrial life (Des Marais *et al.*, 2008).

Conclusion

The history of the origin of life described above is a necessarily narrow view that excludes equally important perspectives that occurred in religion, literature (especially science fiction), and the humanities as well as many other scientific fields. A comprehensive account of attempts to understand the origins of life would ideally include the ideas of non-Western science and philosophy, especially Islamic, and those of the very earliest civilizations (Babylonia, India, China, and Africa). It would also require a more thorough discussion of the philosophical debates on the origin of life during the early Modern period, including Bernard Fontenelle (1657–1757). It is however an effective summation of the history of scientific attempts to answer this time-honored subject of inquiry.

See also: Biogeography, History of. Biogeography, Microbial. Evolutionary Biology, History of. Origin of Life, RNA World and. Symbiosis, History of

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P

Paleobiogeography and Fossils

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Glossary

Biome Community of animals and plants occupying defined area.
Climograph Graphic summary of key climatic parameters.
Cosmopolitan Citizen of the world, living everywhere or nearly everywhere.
Dispersal The spread of organisms from their sites of origin.
Endemicity Restricted to particular district or area of the world.
Neontologist A specialist researching living organisms.
Obduction The edge of an oceanic crustal plate is thrust over the edge of an adjacent continental plate.
Orogen A zone of mountain building, usually the collision of two continents.
Panbiogeography Connects the disjunct distributions of organisms across the globe.
Phenetic Generally refers to the external features of an organism.
Phytogeography Distribution of plant taxa across the globe.
Province Area of the globe characterized by endemic taxa.
Realm Large areas within ecosystems sharing similar history.
Terrane Fragment of crustal material.
Vicariance The range of an organism is split by barriers.

Introduction

The fossil record stretches back some 3.5 billion years and is a unique archive for the evolution of life on our planet, illustrating how the biosphere has changed through deep time and informing us of possible patterns and trends in the future. The geographic distribution of fossil organisms has played a fundamental role in understanding Earth history, particularly the movements of the continents and the location of diversifications and extinctions together with the track of biotic migrations. All living organisms have a well-defined geographic range, from large to small, usually dependent on factors including climate and latitude. The mapping of current biogeographic patterns and their analysis have informed the parallel investigations of biogeography in deep time. Today, the Earth is divided into six main provinces: Nearctic, Palearctic, Neotropical, Ethiopian, Oriental, and Australasian (Figure 1). These biogeographic provinces owe much of their origin to the ground-breaking and perceptive research of Philip Sclater and Alfred Russel Wallace in the 1880s. Provinces are characterized by 'endemic taxa' that have restricted ranges in contrast to 'cosmopolitan' or worldwide taxa. Provinces today tend to be defined at the species level, while those from the geological past use genera. But continental configurations and positions have changed through time as have faunal and floral provinces. Key to the positioning of the continents and oceans are palaeomagnetic data; the orientation of iron minerals is frozen in a number of types of rocks acting like minute compass needles, indicating the latitude where they formed. In addition, types of rocks such as bauxites, coals, desert sandstones, evaporites, and glacial deposits can also fix the latitudinal position of a continent or microcontinent. The continents have been on the move for at least 2 billion years. The supercontinent of Rodinia formed about one billion years ago in the late Precambrian, breaking up into at least eight continents during the Early Palaeozoic (Figure 2(a)). These recombined to form the supercontinent of Pangea (Figure 2(d)), during the later Palaeozoic, itself breaking up to form Gondwana and Laurasia in the early Mesozoic (Figure 2(e)). Palaeontological data were instrumental in demonstrating the drift of the wandering continents, prior to plate tectonic theory, and such data continue as an integral part of palaeogeographical analysis.

Biogeographic provinces are today delimited by biotic barriers. Firstly, corridors, such as seaways or mountain passes, are always open for migration, secondly, filters restrict the passage of organisms, and thirdly, sweepstake routes are only occasionally open. These three concepts underpin 'dispersal' models. On the other hand continental areas and various types of volcanic arcs, split and move, driven by plate tectonics; this provides a mechanism to amalgamate or fragment provinces forming a basis for 'vicariance' models.

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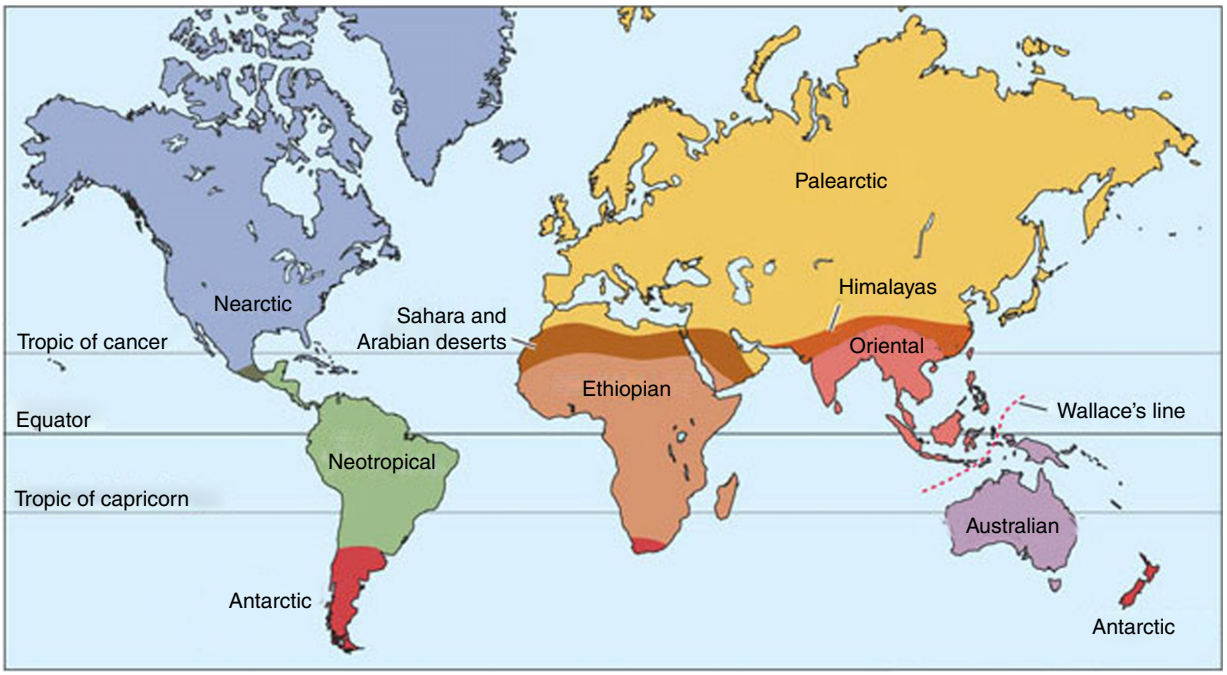

Figure 1 Major biogeographic regions of the world.

Historical Background

Two key approaches have dominated the analyses of palaeobiogeographical patterns of organisms through time. Both are rooted in historical concepts developed by 'neontologists' and both are predicated on the geological process of plate tectonics. The dispersal and vicariance models form two different camps and have two different origins. The Swedish systematist, Carolus Linnæus or Carl von Linné (1707–78) believed that biogeographical distributions were due to the subsequent dispersal of species from an island paradise located in the tropics, where land areas had emerged with the retreat of the seas. In his view all species originated and dispersed from a single 'center of origin,' enabled by ecological conditions. Georges-Louis Leclerc, Comte de Buffon (1707–88), however, considered that the distributions of living organisms were controlled by fluctuating climatic and environmental conditions. He established one of the first principles of biogeography: birds and mammals occupying the same environments but in geographically isolated regions will be different, forming the substance of Buffon's Law. On this basis he classified the globe into the New and Old World. Karl Willdenow (1765–1812) is widely credited with the first major synthesis of plant biogeography, but it was Alexander von Humboldt (1769–1859) who is generally viewed as the pioneer of 'phytogeography'. Following several surveys of the indigenous flora during his expeditions in South America, his key contributions on the biogeography of plants were published and distributed to a more global audience, in the late eighteenth and early nineteenth centuries, gaining wide popularity.

The study of Modern biogeography, however, was initiated by Augustin-Pyramus de Candolle (1779–1841), whose classification of the modern flora defined some 20 botanical regions and introduced the concept of endemism, the basis of biogeography, and the definition of biogeographic units. Deep time, however, was already crucial in the development of the historical dimension to biogeography. Charles Lyell (1797–1875), the 'father of modern geology,' encouraged the development of biogeography in the nineteenth century through his major opus, *Principles of Geology*, first published in 1830–33 (Lyell, 1830–1833). Lyell rejected the ideas of evolution developed by French scientists (such as Jean-Baptiste Lamarck, 1744–1829) and proposed a 'centers of creation' theory to explain the geographical distribution of species. Charles Darwin (1809–82) integrated that approach into his model for evolution, applying the concept of adaptation of organisms across time and space in *The Origin of Species*, first published in 1859. Darwin's (1859, p. 353) tenet "…that the view of each species having been produced in one area alone, and having subsequently migrated from that area as far as its powers of migration and subsistence under past and present conditions permitted is the most probable" challenged both the 'center of origin' and 'vicariance' models, changing the direction of the science. Darwin, however, in his analysis had no mechanism to account for horizontal continental movements (1859, pp. 357–358); convincing evidence for plate tectonics and seafloor spreading was yet to be established. Models involving allopatric speciation and subsequent dispersal thus anchored his theory.

Prior to Darwin's '*Origin*' scientists were beginning to develop a framework for our understanding of modern biogeographical provinces. For example, Philip Lutley Sclater (1829–1913) published on the general geographical distribution of birds in 1858, complementing Alfred Russel Wallace's (1823–1913) fundamental concepts of the geographical

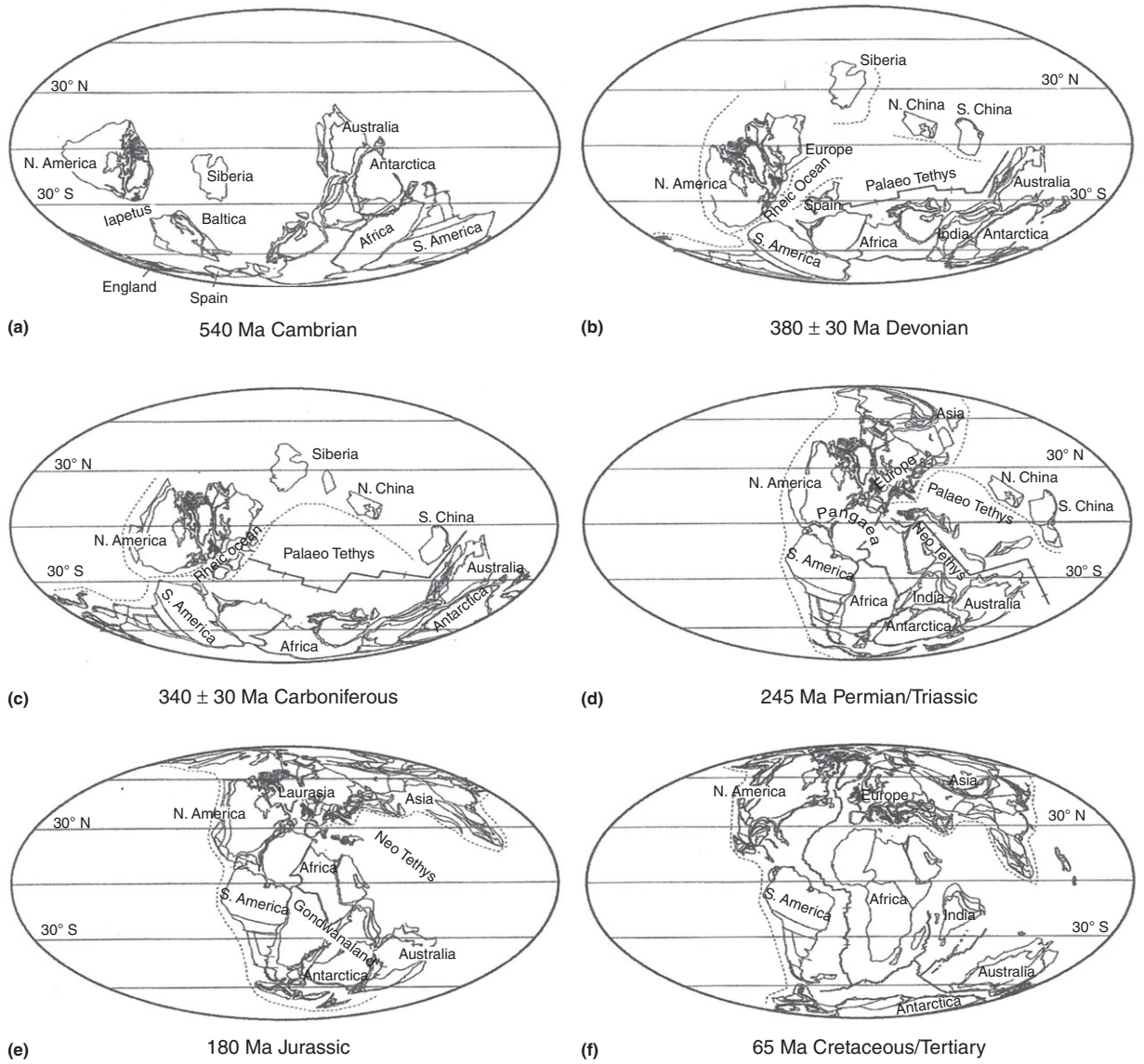

Figure 2 Collage of the main continental plates through the Phanerozoic. The Early Palaeozoic was characterized by low- and high-latitude provinces separated by the Iapetus Ocean. During the Late Palaeozoic, the Rheic Ocean separated Old and New World provinces, whereas the Mesozoic was characterized by Boreal (high-latitude) and Tethyan (low-latitude) provinces. Reproduced with permission from Benton, M.J., Harper, D.A.T., 2009. Introduction to Paleobiology and the Fossil Record. Chichester: John Wiley & Sons Ltd.

distribution of animals in 1876, based on many years of research on the Malay Archipelago. The first nomenclatural terms were introduced in these papers, and they are still widely used today: while Sclater divided the world into six biogeographical 'regions,' Wallace labeled these faunal distributional areas, 'realms.' Both Wallace and Sclater divided the world into six more or less similar units: the 'Nearctic,' 'Neotropical,' 'Ethiopian' units belong to the 'New World,' while the 'Palaearctic,' 'Oriental,' and 'Australian' units were attributed to the 'Old World' (Figure 1). This first major subdivision attempted to divide landmasses into major geographic belts, reflecting affinities and differences among terrestrial biotas, that remains widely accepted today. The major biogeographical units were separated by 'lines.' A prime example of such a sharp boundary between two different biogeographical units is of course 'Wallace's line,' that is located between the Indonesian islands of Borneo and Sulawesi (Celebes), and Bali and Lombok. This line was and is largely accepted, but has subsequently been modified by more recent authors, and represents the limit between the 'Oriental' and the 'Australian' realms.

It was not before the second part of the twentieth century, however, that palaeobiogeographical models integrated the concept of plate tectonics. The German meteorologist Alfred Wegener (1880–1930) proposed the theory of continental drift in the 1910s (Wegener, 1915), based on the similarity of faunas and floras across the southern continents during the Carboniferous and Permian (Figure 3) that had subsequently drifted apart. It was only in the 1960s, that geological evidence for 'plate tectonics' was widely accepted by most geologists.

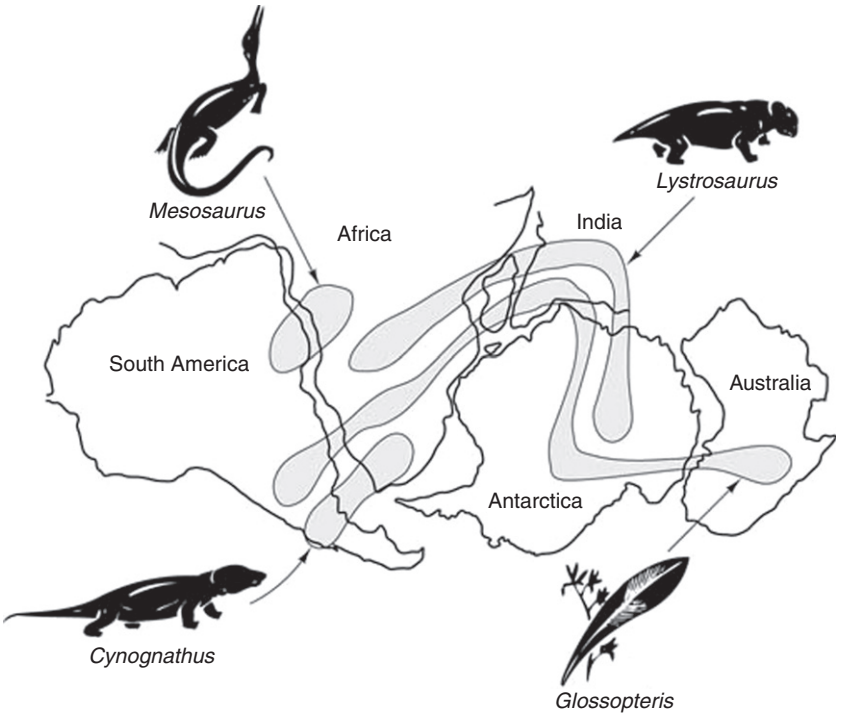

Figure 3 Carboniferous and Permian distributions of the *Glossopteris* flora and the *Mesosaurus* fauna, the Late Permian–Early Triassic *Lystrosaurus* fauna and the fit of Gondwana. The tight fit of Gondwana and the correspondence of fossil faunas and floras across the southern continents suggested to Wegener (1915) and others that South America, Africa, India, Antarctica, and Australia had drifted apart since the Permo–Triassic. Reproduced from Smith, P.L., 1988. Paleobiogeography and plate tectonics. Geoscience Canada 15, 261–279, with acknowledgement.

This paradigm shift integrated the concept of moving continents with the hitherto perplexing distributional patterns of fossil organisms through deep time.

Although in the absence of evidence for continental movements biogeography was dominated by 'dispersalists,' the botanist Léon Croizat (1894–1982) had predicted movements of the continental masses (e.g., Croizat, 1958) prior to the acceptance of plate tectonic theory. He introduced the concept of 'panbiogeography', which consists of an annotated geographical map with lines or tracks that join the known distributions of living, related organisms across the different areas of the globe. For example, tracks of terrestrial organisms traverse the oceans and hence cannot be explained by present-day geography. Croizat introduced the concept of 'vicariance', which is the separation of an ancestral biota into two or more biotas through the formation of a geographical barrier which prevents gene flow between populations. This concept is an alternative to the idea of dispersal from a center of origin, the model promulgated by Linnæus, Buffon, and Darwin. Croizat's ideas were initially rejected by biogeographers, but with the advent of plate tectonics his work was accepted by many, particularly those promoting cladistics methodologies.

Palaeobiogeographers were able to understand and to develop completely new models for the distribution of fossil organisms in the Mesozoic and Cenozoic eras, i.e., for the post-Pangean world. However, pre-Pangean palaeobiogeographic distributions are still more challenging, where biotic data can only frame and test palaeogeogeographic reconstructions.

Some Basic Concepts

Biogeographers and palaeobiogeographers describe and analyze the distributions of a single taxon or groups of taxa. An initial step is to determine the geographical range of a species or assemblage, for example, plotting the data on geographical or palaeogeographical maps. These maps can then provide evidence of physical barriers, such as mountain belts or oceans, delimiting the ranges of different organisms. Patterns of distribution used by biogeographers can be either spatial (e.g., based on altitude, depth, or latitude) or temporal (e.g., based on climatic, or seasonal changes). Since the 1970s biologists have worked in collaboration with mathematicians and statisticians to understand distributional patterns, in particular using the development of sophisticated multivariate statistical techniques that allow quantification of the degree of similarities (or differences) of the distribution of different communities or species. A large number of similarity indices have been proposed in the last few decades. The same methodologies have been applied to the distributions of fossil organisms using a similar range of multivariate analytical techniques (Hammer and Harper, 2006).

There are, however, a number of key terms that have been variably applied to both living and fossil distributional data. For continental environments, the term 'biome' has been introduced and is still widely used by botanists. Biomes include, for example, tropical rain forests, temperate grasslands, or boreal forests or tundras. They capture easily recognizable terrestrial biogeographic regions that can be easily plotted on

'climographs', i.e., simple diagrams depicting the mean annual precipitation versus the mean annual temperature. The term has also been used by palaeobiogeographers for marine fossils (e.g., Cecca, 2002), although the term 'community' is more commonly used by neobiogeographers for marine biotas. Marine communities include, for example, coral reefs, estuaries, upwelling zones, continental shelves, or the open ocean. In addition, 'biogeographic regions' and 'climatic regions' are used to delimit biogeographic units in the oceans today. The distinction of these is more complicated and nuanced, as natural barriers limiting the distribution of species and communities are less evident, the ocean being in places a large and continuous aquatic habitat.

Biogeographic regions of the marine realm are essentially partitioned by water temperature, creating broad latitudinal zones that may be influenced and perturbed by oceanic currents, or interrupted by the presence of continents, islands, or archipelagos. Today, the major 'biogeographic regions' of the world's oceans are, essentially from North to South, the 'Arctic,' 'Subarctic,' 'Northern Temperate,' 'Northern Subtropical,' 'Tropical,' 'Southern Subtropical,' 'Southern Temperate,' 'Subantarctic,' and 'Antarctic' regions. On the other hand, several major 'climatic regions' have been defined. These are the 'Arctic,' 'Northern Boreal,' 'Southern Boreal,' 'Tropical,' 'Equatorial,' 'Northern Total,' 'Southern Total,' and 'Antarctic' climatic regions (Figure 1).

It is particularly challenging for palaeontologists to trace these different units back into deep time, particularly prior to the Jurassic Period, when no *in situ* oceanic crust is preserved: what were the key assemblages or communities, biogeographic units, and climatic zones in the Early Palaeozoic, for example? Since barriers, of various types, play a significant biogeographic role at any given time and may define and preserve biogeographic signatures in more recent distributions. Former areas of 'endemicity' still exist: for example, the disjunct distribution of Modern lungfishes indicates that they were endemic to the ancient supercontinent of Gondwana, which is no longer a geographic area in the modern world.

There is a myriad of terminology within (palaeo-) environmental and (palaeo-) geographical studies, for which no clear rules have been established but for which some common usage of terms exists; to these, the (palaeo-) ecological context adds additional data. The environments that occur on the continental shelf are referred to as neritic, while those in the open ocean are considered pelagic, divided into epipelagic (<200 m water depth), mesopelagic (<1000 m), bathypelagic (<4000 m), and abyssopelagic (<11000 m). In addition, the life mode of marine organisms allows a classification into benthic or planktonic life styles, with some organisms occupying intermediate positions. Benthic organisms are bottom-living animals that may live on the seafloor (epifauna) or below the sediment (infauna). Organisms in the open waters are usually divided into floating (plankton) and freely swimming (nekton) organisms. Understanding the life modes of organisms, in the oceans, is critical in determining their utility in palaeobiogeographic studies. In general terms, planktonic organisms have the most widespread distributions but even within the benthos there are nuances, with those shallow-water assemblages and species have more restricted distributions than those in deeper water. More over even within plankton groups some species may be more widely distributed than others.

With the fragmentary knowledge available on the distribution of fossil species and assemblages, it is generally impossible to fully understand the life mode or the life habitat of marine organisms. It is therefore not always evident to palaeontologists how to apply these terminologies. A common problem for palaeontologists, for example, is to clearly distinguish between the influence of palaeogeographical (e.g., latitude) and of palaeoecological (e.g., water depth) parameters on the distribution of a fossil organism, particularly when dealing with planktonic and neritic species. These variables are often difficult to quantify in deep time and involve some speculation. Nevertheless, the relative extent of animal distributions during the Early Palaeozoic has been sketched out, emphasizing the role of mobility of respective groups of animals and their larvae, and their relationships to biofacies, more specifically water depth (Fortey and Cocks, 2003). This has invited debate as to whether there are 'good' or 'bad' fossils for palaeobiogeographic analyses. Clearly, those with more widespread distributions, commonly occurring in deeper water, are less good geographic proxies than those restricted to shallow water and tied to a particular continent and latitude.

Reconstructing Ancient Geography

Palaeobiogeography had a fundamental influence on our understanding of continental drift and subsequently plate tectonics. The predrift positions of major continental masses can be reconstructed back through time with reference to the contiguity of ancient provinces. But many continents have complex margins of accreted material, such as microcontinents and volcanic arcs, whose origins are unclear. As noted previously, since no *in situ* crust exists prior to the Jurassic Period, palaeogeographers must, using biotic data, climatically sensitive sediments and palaeomagnetic evidence, locate the sites of such arcs and microcontinents within ancient oceans prior to their accretion. Terrane models recognize that mountain belts or orogenic zones consist of a collage of tectonic units with unique geological histories mutually separated by tectonic structures such as faults. Many 'terranes' have their own distinctive biogeographic signatures. Virtually all 'orogens' can now be interpreted in this way.

There is now a large literature on palaeobiogeography. More recent contributions and compilations strongly focus on vicariance (e.g., Lieberman, 2000), the biogeography and palaeobiogeography of the Indo-Pacific and related regions (Renema, 2007) and on data capture, management, and analytical techniques (Upchurch *et al.*, 2011). Deep time is well represented by compilations of the Palaeozoic and its biotas (McKerrow and Scotese, 1990) and of the Early Palaeozoic (Harper and Servais, 2013). These data are supported by a number of readily available computer-based palaeogeographic reconstructions through time, for example, BugPlates, Colorado Plateau Geosystems Inc., and the Paleomap Project. Smart phone apps, for example, Ancient Mobile History, provide an interactive and visual journey through time as the continental configurations and positions changed during the last 550 million years.

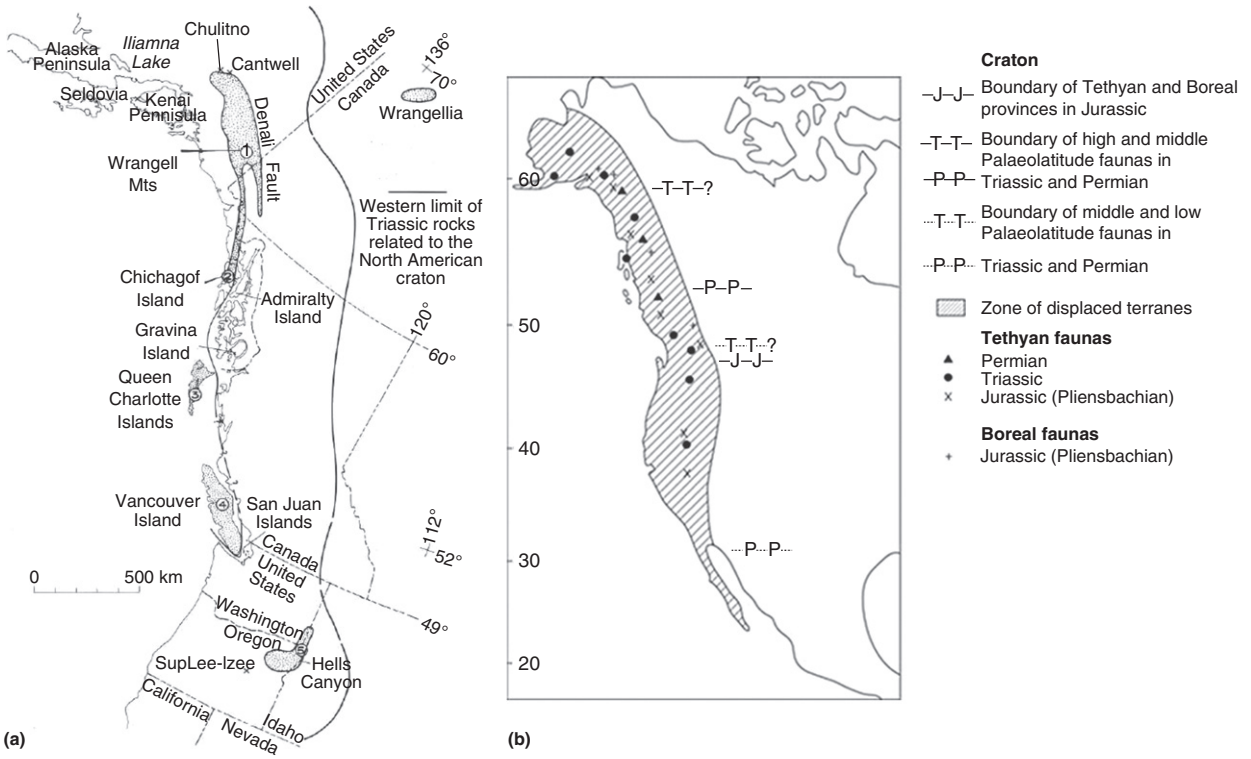

Figure 4 (a) Map showing distribution of Wrangellia and limit of Triassic continental shelf deposits (high-latitude). Reproduced from Jones, D., Silberling, N.J., Hillhouse, J., 1977. Wrangellia—A displaced terrane in northwestern North America. Canadian Journal of Earth Sciences 14, 2565–2577, with acknowledgement. (b) Displaced faunas in terranes within the North American Cordillera together with changing provincial boundaries on the craton. Postulated latitudinal boundaries on the craton during the Permian, Triassic, and Jurassic are indicated and confirm the northern movement of these displaced terranes. Reproduced from Hallam, A., 1986. Evidence of displaced terranes from Permian to Jurassic faunas around the Pacific margins. Journal of the Geological Society 143, 209–216, with acknowledgement.

Critical to any palaeogeographical reconstruction is the understanding of biogeographic patterns in these analyses. (Palaeo)Biogeographic units are generally recognized by key species in common, or genera, families, and higher taxa. More preferable than merely charting the distribution of a single key taxon, is the analysis of large databases of distributional information by multivariate methods (see below). Palaeobiogeographical studies have thus followed three parallel approaches. First, many analyses have focused on the occurrences of key genera or families to help map provincial data. Second, by way of contrast, a number of authors have used the whole-data approach, analyzing statistically all the available data from given time slices using a range of multivariate methods. Third, the biogeographical distributions of taxa have been mapped onto phylogenetic trees for some areas of the phylum. Moreover, the concept and application of provinces have differed between author groups (see below), with some authors basing their provinces entirely on the distribution of taxa, whereas others have tied them to continental palaeoplates.

One classic area for the study of terrane geology is the North American Cordillera. Here the ancient mountain belt on the Pacific-facing margin of the North American continent is composed of a collage of microcontinents or terranes. During the Mesozoic, high-diversity, low-latitude Tethyan province faunas are generally quite distinct from the lower-diversity, high-latitude Boreal faunas and can be used to fingerprint high and low-latitude terranes. The mosaic of terranes, now plastered onto the west coast of the States, probably originating at lower latitudes. For example, in an east – west traverse across these terranes there is a progressive northward displacement of Tethyan-type faunas of early Jurassic age; some of the more exotic, far-traveled terranes may have moved over 1300 km (Newton, 1988); for example, the Triassic rocks of the large, exotic Wrangellia terrane (Jones *et al.*, 1977) originated at low, tropical latitudes and were emplaced northwards along the edge of the North American craton (**Figure 4(a)**) during the Mesozoic. The terranes are distinguished by their biogeographical signatures, narrating a unique pathway through time, simulating Viking funeral ships. Another way of assessing the origins and tracks of these terranes involves plotting the boundaries between the provinces on the craton through time (**Figure 4(b)**).

Analyzing Ancient Biogeography

Two main types of biogeographic analysis are widely used based on either 'phenetic' (dispersal) or 'cladistics' (vicariance) methods. Cladistic methods construct trees by relying on

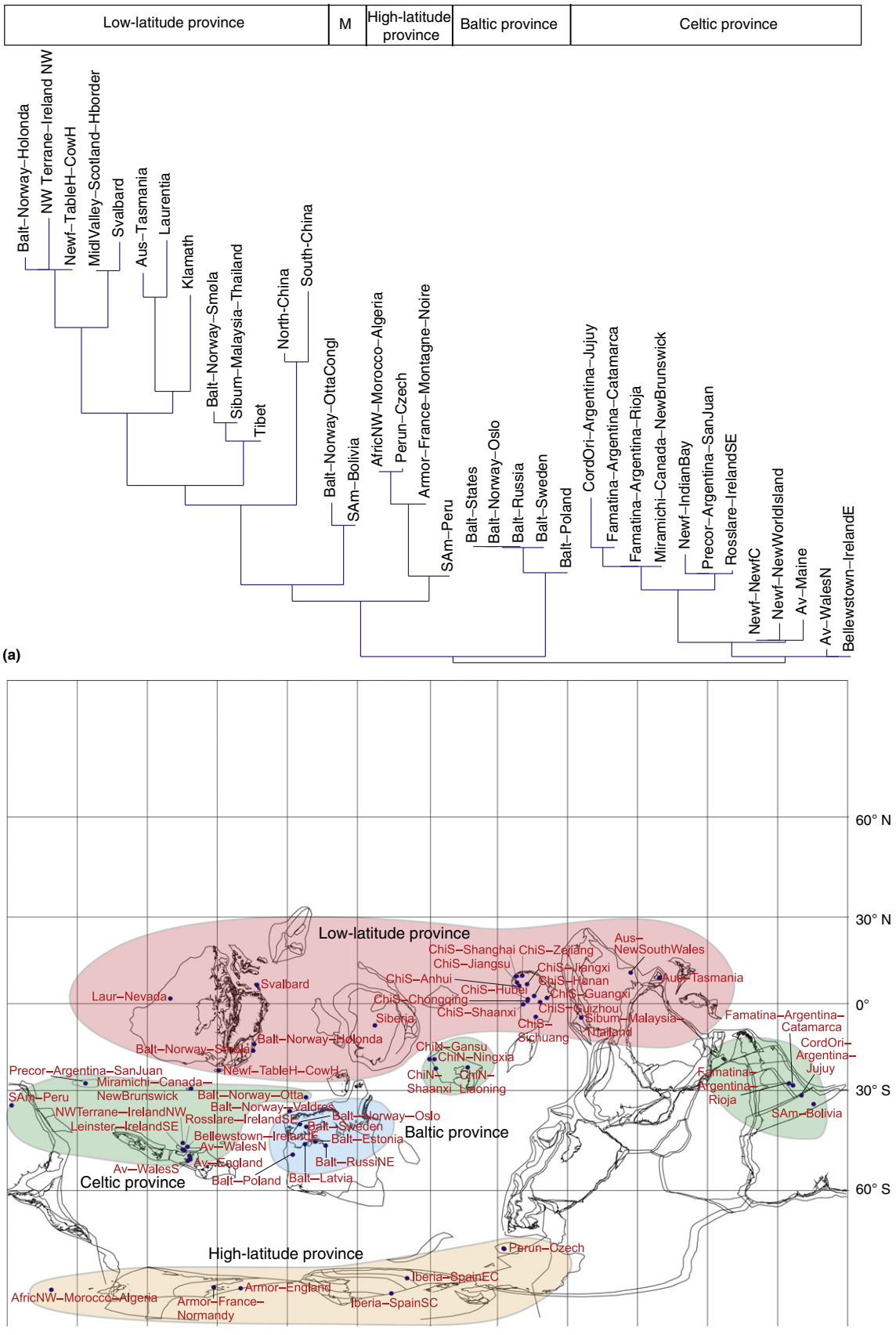

Figure 5 (a) The 'phenetic' cluster analysis of Mid Ordovician brachiopods from the entire World; (b) mapped onto a predrift reconstruction for this interval. Reproduced from Harper, D.A.T., Servais, T., 2013. Early Palaeozoic Biogeography and Palaeogeography. London: Memoir of the Geological Society, with acknowledgement.

assumptions about ancestral relationships as well as on current data, i.e., a province has since fragmented with the creation of subprovinces characterized by new endemics, essentially analogous to apomorphies in taxonomic cladistics. Phenetic methods construct trees by considering the current states of characters without regard to the evolutionary history that brought the species to their current phenotypes; phenograms are based on overall similarity. There are a large number of distance and similarity measures to choose from (Shi, 1993; Hammer and Harper, 2006). A few of the commoner coefficients are listed below:

$$\text{Dice coefficient} = 2A/2A + B + C$$

$$\text{Jaccard coefficient} = A/A + B + C$$

$$\text{Simple matching coefficient} = A + D/A + B + C + D$$

$$\text{Simpson coefficient} = A/A + E$$

A is the number of taxa common to any two samples, B is the number in sample 1, C is the number in sample 2, D is the number of taxa absent from both samples, and E is the smaller value of B or C.

On the basis of an intersite similarity or distance matrix a dendrogram can be constructed linking first the sites with the highest similarities or the closest distances. When the distance or similarity matrix is recalculated to take into account the first clusters, additional sites or genera are clustered until all the data points are included in the dendrogram. Clearly the first clusters have the greatest significance and less importance is usually attached to later linkages. Alternatively some form of ordination, for example, principal components or coordinates analyses can be used to display the similarities of sites based on the taxon compositions of localities (Harper, 1992; Hammer and Harper, 2006). This approach, using total data, has proved useful for defining ancient provinces, based on clusters of taxa (usually genera) characteristic for given ancient provinces. Mapping of the provinces onto base maps forms the basis for understanding the changing distribution of provinces through time. A cluster analysis is presented for a large matrix of fossil brachiopods from all various localities throughout the world some 450 million years, when the world was very different; the key clusters are mapped onto the Mid Ordovician globe (**Figure 5**).

Biogeographical Patterns through Deep Time

Prior to the Jurassic Period there is no oceanic crust preserved in place, most now has been obducted onto continental margins or squeezed into mountain belts during plate collisions. Nevertheless a large spectrum of biogeographic units has been established for much of the Phanerozoic based on a variety of fossil groups, ranging from a complexity of provinces during the Great Ordovician Biodiversification Event (GOBE: Harper and Servais, 2013) to more clearly latitudinally controlled during the Mesozoic, with generally Boreal and Tethyan

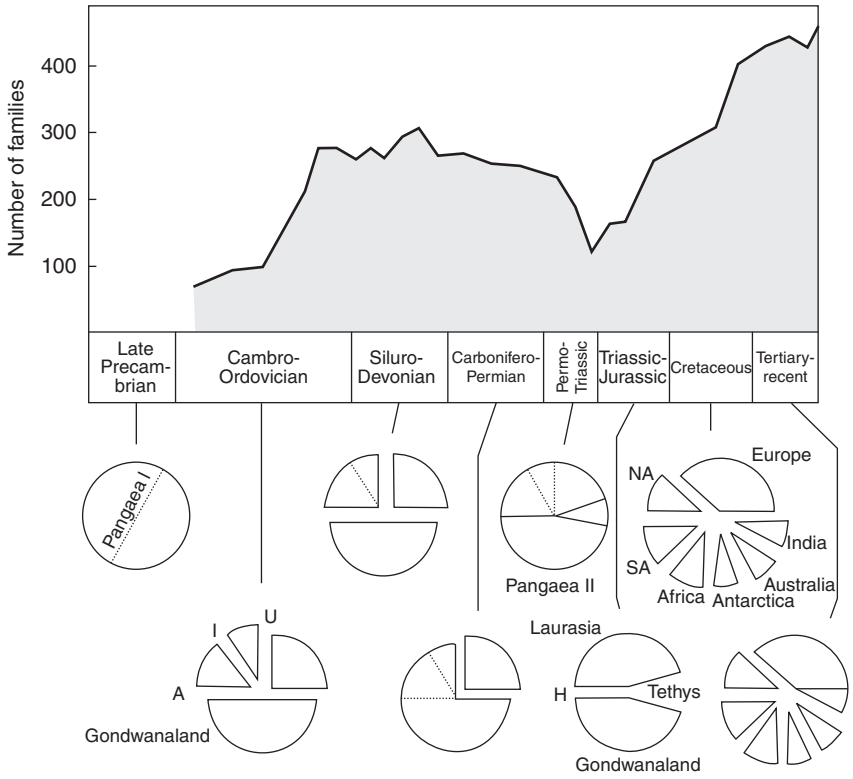

Figure 6 Changing familial diversity of skeletal benthos through time in relation to plate configurations: high diversities are apparently coincident with times of greatest continental fragmentation, for example, during the Ordovician, Devonian, and Cretaceous–Cenozoic. A, pre-Appalachian-Variscan Ocean; H, Hispanic Corridor; I, Iapetus Ocean; U, pre-Uralian Ocean. Reproduced from Smith, P.L., 1988. Paleobiogeography and plate tectonics. Geoscience Canada 15, 261–279, with acknowledgement.

provinces. During the GOBE, the wide dispersal of continental, microcontinents, and volcanic arcs provided mechanisms for allopatric species, increasing dramatically the total marine (γ) diversity of the planet. Similar radiations on land were coincident with the construction of Pangea as forests spread, together with terrestrial, animal-based communities in the later Palaeozoic. Biogeography and climatic gradients are also related to patterns of changing biodiversity. In broad terms low latitudes support high-diversity faunas; biodiversity decreases away from the tropics toward the poles. Studies on modern bivalve, bryozoan, coral, and foraminiferan faunas show marked increases in diversity toward the equator, and since many cool-water species breed later in life, individuals may be larger than their tropical counterparts. The latitudinal diversity gradient has also been found in paleobiogeography. For example, diversity gradients across early Jurassic ammonoid faunas have proved informative in palaeogeographic reconstructions; Tethyan faunas occupying tropical belts were generally more diverse than counterparts at higher latitudes.

Many authors have suggested that changing plate configurations, oscillating between fragmentation and integration, have affected biodiversity through time (Figure 6). For example, the huge early Ordovician radiation (GOBE) of marine skeletal faunas may be related to the breakup of Gondwana (Harper, 2006), the end Ordovician may be associated with an amalgamation of microcontinents and terranes (Rasmussen and Harper, 2011), while the end-Permian extinction event coincides with the development of the supercontinent of Pangaea,

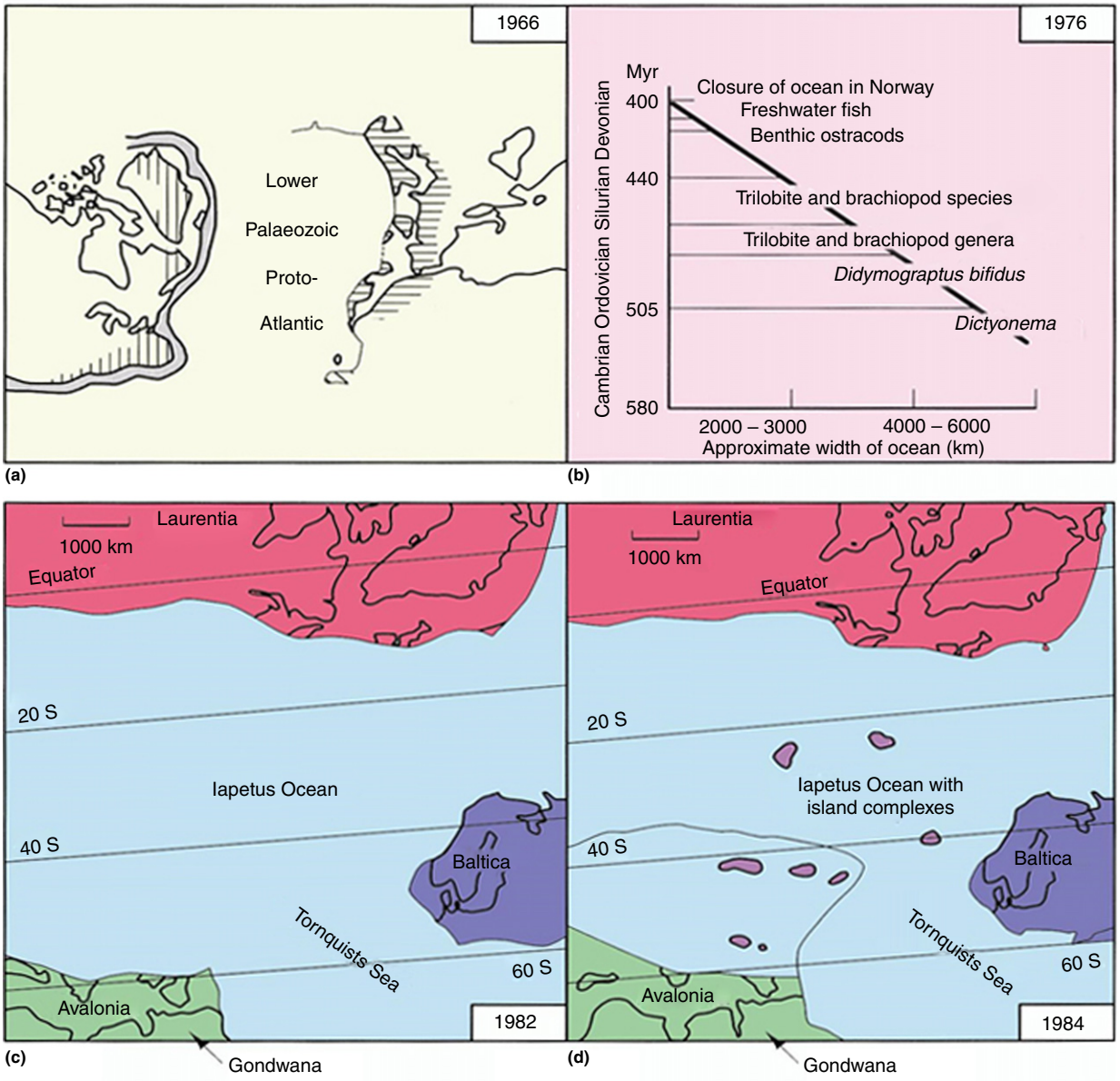

Figure 7 Changing ideas on the development of the Early Palaeozoic Iapetus Ocean and its faunas: (a, c, d) palaeogeographic reconstructions; (b) the mobility of organisms across a closing ocean. Figures 7(a)–7(d) reproduced from Harper, D.A.T., 1992. Ordovician provincial signals from Appalachian-Caledonian terranes. Terra Nova 4, 204–209, with acknowledgement.

and changes in the seaways within and around it (Shen and Shi, 2002); more recent diversifications have occurred during the late Mesozoic fragmentation of this supercontinent.

Islands are key players in biogeography. The majority of fossil island complexes are now entrained in the world's mountain belts where the remnants of oceanic crust occur together with associated microcontinents and volcanic arcs. Islands have fulfilled roles as both cradles and museums of taxa, volcanic arcs, and microcontinents providing the locus for species pumps (e.g., Darwin's finches and tortoises) and conversely peripheral isolates (e.g., the Dodo of Mauritius), exposed to extinction. Moreover the role of islands as stepping stones for both marine and terrestrial biotas is well substantiated. Patterns of migration, by larval island hopping during two of the most active magmatic and tectonic periods, have been established in the Ordovician (e.g., Jin and Harper, 2015; Rasmussen, 2011) and the Cretaceous (e.g., Harper *et al.*, 2005; Skelton *et al.*, 2013).

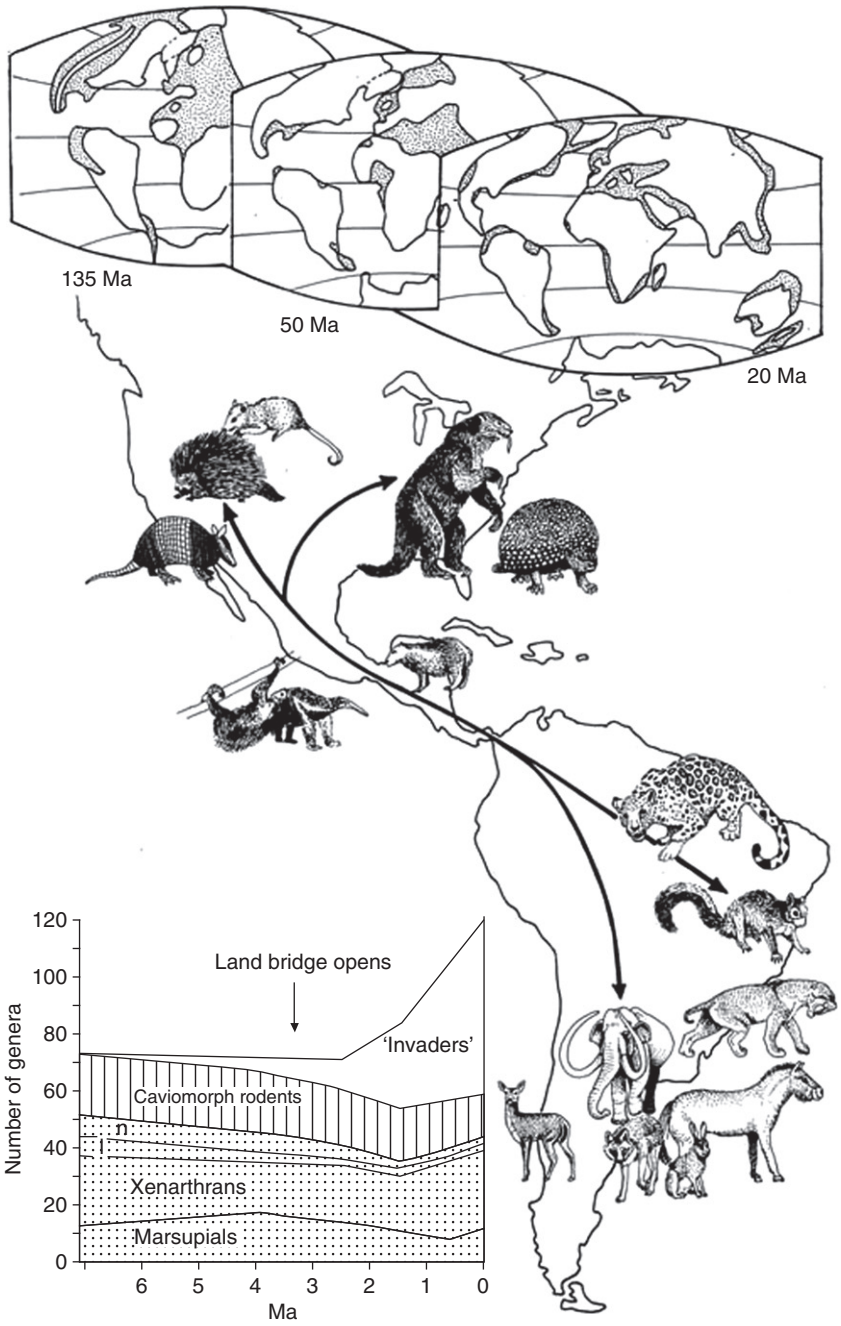

Figure 8 The emergence of the Isthmus of Panama promoted the great American biotic interchange (GABI) between North and South American terrestrial vertebrates together with the radiation of the shallow-water marine benthos of the Caribbean Sea. l, litopterns; n, notoungulates. Reproduced with permission from Benton, M.J., 2005. Vertebrate Palaeontology. Chichester: John Wiley and Sons Ltd.

The Ordovician history of the Lower Palaeozoic Iapetus Ocean provides an ancient laboratory to study the origin and evolution of a marine ecosystem punctuated by islands (**Figure 7**). Tuzo Wilson's classic study in 1966 (Wilson, 1966), in the vanguard of plate tectonic theory, depicted a two-dimensional ocean with orthogonal opening and closing between Europe and North America. The ocean first opened during the late Precambrian with the breakup of a supercontinent, and developed during the Cambrian. 10 years later McKerrow and Cocks (1976) presented a score chart of faunal mobilities against time during ocean closure. Thus, at its widest in the late Cambrian, possibly extending as much as 4000 km across, only planktonic graptolites were similar on both sides of the Iapetus. But as the ocean closed, first pelagic graptolites and later the mobile and eventually fixed benthos, the trilobites and brachiopods, could cross the seaway. By the late Silurian, benthic ostracodes scuffled their way across and by the Devonian, with more or less complete closure, freshwater fishes were similar in Europe and North America. Almost 10 years on Fortey and Cocks (1982) described the ocean in terms of a low-latitude Laurentian continent, a high-latitude Gondwana, and Baltica. The smaller Avalonia rifted from Gondwana during the late Cambrian – earliest Ordovician and together with Baltica moved toward Laurentia during the Ordovician. Neuman (1984) filled the Iapetus Ocean with islands, small suspect terranes with peculiar faunas of endemics together with taxa having otherwise mixed provincial affinities. More recent contributions by Trond Torsvik and his colleagues (Torsvik, 1991) have emphasized that Baltica rotated anticlockwise as it moved toward the equator picking up various terranes as a nappe pile on the edge of the craton. These island complexes functioned as both species pumps and refugia (Harper and Mac Niocaill, 2002; Harper *et al.*, 2008). Both cladistic and phenetic techniques have been used to analyze the large amount of distributional data from within and around the Iapetus Ocean, all confirming in broad terms current palaeogeographic reconstructions of the ocean system (McKerrow and Scotese, 1990; Harper and Servais, 2013).

The removal of oceanic barriers by, for example, land bridges during continental convergence, can also have dramatic consequences for both sides of a closing seaway. For example, the emergence of the Isthmus of Panama has connected North and South America; however it has isolated the Atlantic and Pacific oceans. South America was essentially isolated from North America for most of the past 70 million years, and was dominated by diverse specialized mammalian faunas consisting of marsupials, edentates, unique ungulates, and rodents. However, 3 million years ago the emergence of the Isthmus of Panama provided a land bridge or corridor between the two continents and many terrestrial and freshwater taxa were free to move across the isthmus; the Great American Interchange (GABI) allowed the North American fauna to invade the south and essentially wipe out many of the continent's distinctive mammalian populations (**Figure 8**). South American mammals were equally successful in the north and some such as the armadillo, opossum, and porcupine still survive in North America. Webb (2006) has discussed the dynamics of the interchange suggesting immigrants from the larger area of North America outnumbered those from the south.

See also: Biogeography, History of. Paleobiological Revolution, History of

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Paleobiological Revolution, History of

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Glossary

Konstruktions-morphologie Also known as constructional morphology, later further elaborated as morphodynamics is a paleobiological theory pioneered by Adolf Seilacher that radically situates fossil and modern species in their ecological and adaptive milieux with particular emphasis on understanding not only the role of natural selection but especially how species' adaptive and evolutionary possibilities are constrained by factors such as the materials available to build their bodies, and the developmental pathways they inherit from their ancestors.

Morphospace A construct developed to include the sum total of all physical and environmental possibilities that could potentially exist in the entire biological realm. Among salient issues of interest is the great quantity of morphospace not filled by any extant or extinct organism. For example, why are there no clams with three shells?

Neontologist A scientist who studies extant species without regard for the fossil record.

Orthogenesis An almost universally discredited theoretical construct descending from an original basis in Lamarckian explanations in which trends in evolutionary history result from an internal driving force that causes evolution to unfold along constrained trajectories, often moving toward greater and greater complexity. Orthogenetic views generally leave very little room for natural selection to shape the course of evolution. The idea flourished in the late nineteenth and early twentieth centuries, primarily among paleontologists.

Phylogenetic constraints Fundamental systems of development, architecture, and biochemistry that limit the directions future evolutionary change can take due to the past evolutionary history of the lineage. For example, bilateral symmetry or deuterostomy or the organizational effects of major gene families.

Phylogenetic patterns Large-scale topology of evolutionary histories arising within and among clades.

Saltation A largely discredited theory that speciation happens very rapidly, due to either macromutations or large-scale chromosomal shuffling, sometimes via a one-generation leap.

Speciosity A speciose clade is an evolving lineage consisting of a large number of species.

Vicariance biogeography Vicariance is a process whereby the geographic range of a species or higher taxon, or ecological community, is split into independent subpopulations by the formation of a physical or biological barrier. These daughter populations maintain parallel ecological roles and accumulate changes due primarily to stochastic factors such as genetic drift. For example, Asian and African rhinoceroses or elephants.

Variously lauded and maligned, the rise of paleobiology as a discipline marked a major shift within the broad field of evolutionary biology in the 1960s and 1970s. Paleobiology – as much a movement as a discipline – called for a revolutionary integration among subfields marginalized by the Anglo-American modern synthesis of the 1930s and 1940s, and cleared the way for ascendant disciplines such as evo-devo.

Although the term was first coined by Yale paleontologist Charles Schuchert (1858–1942) in 1904, and saw occasional use from time to time, it was not widely recognized until a group of paleontologists led by Tom Schopf convinced the Paleontological Society to found the quarterly journal *Paleobiology* in 1975.

The Prehistory of Paleobiology

In addition to Schuchert, historians, and scientists alike have advanced a variety of candidates as founder of paleobiology.

Transylvanian aristocrat, adventurer and Albanian sovereigntist Baron Franz Nopsca von Felső-Szilvás (1877–1933), attempted to develop a new discipline he called Paleophysiology. This terminology captures many elements of the later paleobiology movement, especially the push to envision extinct species not as stages, trends, or tendencies on an evolutionary trajectory but as fully formed entities enmeshed in complex ecological communities to which they were adapted just as well as modern-day organisms are to their current environments. Inspired to become a paleontologist by dinosaur bones found by his sister on the family estate, Nopsca may have seen a parallel between these organisms as paleohistorical actors and the claimed transhistorical nationhood of Albania as an intrinsic entity. As historian of science David Sepkoski has documented (Sepkoski, 2012), promoters of paleobiology have frequently been heavily and often very consciously influenced by the philosophic influences of their views on human history (**Figures 1** and **2**).

Viennese paleontologist and eventual Göttingen professor Othenio Abel (1875–1946) mounted large expeditions in Europe, Africa, and the Americas primarily concerned with vertebrate paleontology. His efforts to promote a theoretical grounding in post-Lamarckian orthogenesis led him to entirely internal mechanisms for evolution and beyond that to a mystical romanticism that at least teetered on the verge of the supernatural (Abel, 1929, 1931; Beurlen, 1932, 1937, 1949). It is not clear to what extent his scientific work aligned with his Naziism but both interests revolved around expectations of inexorable broad trends in history, frustrated by local

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Figure 1 Baron Franz Nopsca von Felső-Szilvás (1877–1933).

Figure 2 Baron Franz Nopsca von Felső-Szilvás (1877–1933) in Shqiptar warrior costume circa 1913.

conditions – such as adaptation to local environment in paleobiology and what was called the 'judaification' of the universities in human history. Working with Louis Dollo (1857–1931), the two developed their view of paleobiology and in 1928, Abel began publishing the German language journal *Paläobiologica*. It appears they developed the term without having heard Shuchert's prior use (Figure 3).

Synthetic Paleobiology

Due to Abel's association with Naziism, as well as with Lamarckian notions, the term paleobiology did not become a

Figure 3 Othenio Abel (1875–1946).

part of the established lexicon associated with the synthetic theory of evolution. Well before the modern synthesis and unlike most North American paleontologists, German paleontologist Rudolf Wedekind and his student Otto Schindewolf (1896–1971) opposed Lamarckian inheritance of acquired characters and stayed abreast of developments in genetics, particularly Richard Goldschmidt's work. Goldschmidt (1878–1958), perhaps uniquely among geneticists in the early third of the twentieth century maintained an equal regard for the importance of microevolution and macroevolution. Although Schindewolf and Goldschmidt were saltationists, they did accept a role for natural selection and they embraced both the continuous variation of the Morgan/Chetverikov school of genetics, and the macromutations that Hugo DeVries (1848–1935) had credited as the source of new species. Without naming Abel's views specifically as paleobiology and writing entirely within the German view of synthetic evolution, Schindewolf forcefully rejected the views of paleontologists such as Abel and Karl Beurlen (1901–85) and skewered any suggestion of nonmaterial influences in his synthetic work *Paläontologie, Entwicklungslehre und Genetik: Kritik und Synthese* (Reif, 1994, pp. 439–440).

> Wherever the bases of firmly established knowledge are missing or seem to shake, mystical lines of thought expand. In the case of evolution a wrongly conceptualized holism has seduced authors to abandon causal research and strict scientific analysis. Irrational circumlocutions have replaced clear terms and approaches, without providing any heuristic value....For the time being, scientific analysis of organic evolution is limited by the assumption that mutations (of the genes) are ultimate causes. The irrational remainder cannot be resolved any further with the epistemological means available to natural science.

Schindewolf instead stated his goal to unite all evolutionary disciplines within a mechanistic framework: "On this general basis will be given a robust synthesis and a mutual completion of the discoveries from the experimental, exact-inductive methods of research on heredity and from the historic-deductive character of paleontological research" (Schindewolf, 1936, p. 90). Although the spirit of paleobiology was abundantly in evidence, the term was not.

The Paleobiological Impulse

Unlike the view of evolution in Germany, which placed paleontology in a more central theoretical role, proponents of the new synthetic theory in English-speaking countries tended to focus instead on genetics following the work of evolutionary genetics such as Theodosius Dobzhansky (1900–75). The emphasis on genetics and the turn toward an evolutionary biology as a whole, did not escape the notice of paleontologists and evolutionary theorists such as George Gaylord Simpson, who argued vigorously for the importance of paleontology and did the most to bring his area in line with the synthetic theory as it was emerging in the early 1940s: "Not long ago, paleontologists felt that a geneticist was a person who shut himself in a room, pulled down the shades, watched small flies disporting themselves in milk bottles, and thought that he was studying nature." Simpson said geneticists viewed the paleontologist "like a man who undertakes to study the principles of the internal combustion engine by standing on a street corner and watching the motor cars whiz by" (Simpson, 1944, pp. xxvii–xxviii). However, he said, those of differing evolutionary disciplines were finally able "to realize that we do have problems in common and to hope that difficulties encountered in each separate type of research may be resolved or alleviated by the discoveries of the other."

Following along these lines, paleontologists such as Bernhard Kummel (1919–80) encouraged their paleo students to maintain contact with 'neontologists.' An important example is Kummel's student David Raup (1933–2015), who worked nearly as much with zoologist Ernst Mayr (1904–2005) as with Kummel. Raup's 1956 dissertation drew evolutionary conclusions about living echinoids and found close parallels in fossil lineages.

By 1960, the word paleobiology had shed any mystical and political associations and was again available for usage as an indication of an outlook in which extinct organisms could be seen as arbiters of biological theory – interesting and valuable in their own right – rather than as mere documentation that evolution had happened. Setting off a flurry of intellectual activity along these lines, Gus Cooper in his 1958 presidential address to the membership of the Paleontological Society announced that "paleontology is in reality paleobiology" (Cooper, 1958).

This proclamation served as a wake-up call to the field of paleontology that times were changing. In the larger scheme of US paleontology in the 1950s and early 1960s, evolutionary theory was not a major bone of contention. Implementing the goals of the modern synthesis might have been interesting, but most PhD students in geology prepared their dissertations envisioning careers as biostratigraphers for oil companies. Many of these students' dissertations were not significantly different in focus or methodology from those done in the twenties. As long as the evolutionary patterns preserved in the fossil record were consistent and reliable predictors of the location of oil-bearing deposits, practitioners did not need to concern themselves directly with the biological processes acting to produce these patterns of evolution.

But, increasingly, young paleontologists were fascinated by theory and looked to their field to offer exciting new perspectives. David Raup focused his attention on one of the most challenging puzzles in the field – the stunning diversity in shape and size among fossil and extant gastropods. The result was a tour de force in which Raup reduced this great multiplicity to just four parameters. Raup invented a computer model to generate gastropod shell patterns on an oscilloscope. *W*, represented the size ratio of successive generating curves; *D*, the distance of the generating curve from the axis, and *T*, the proportion of the height of one generating curve that is covered by each ensuing turn. Adjusting these parameters created forms resembling oysters, snails, whelks, and other mollusks. What had been a bewildering array of variation – assumed to be due to the action of natural selection building new adaptations to specific local contexts – was tamed by a mathematical computer model. Morphology could be subject to simple constraints on architecture, rather than the all-encompassing action of selection. Raup's coiling parameters were a revelation about the power of paleobiology (Raup, 1962, 1966).

Entering graduate school with Norman Newell (1919–2004) at Columbia University just as Raup's model was making a splash, young Stephen Jay Gould (1941–2002) threw himself into the study of invertebrate morphology. A dinosaur aficionado since the age of 4, Gould became increasingly enthusiastic about the evolutionary synthesis in high school, especially through Simpson's *Tempo and Mode in Evolution* (1944) and turned his attention toward invertebrates as good indicators of patterns and processes at work in evolutionary transformations. At Columbia and the associated American Museum of Natural History, Gould had access to spectacular fossil collections and coursework with hero of the modern synthesis, Theodosius Dobzhansky. Precocious and prolific, Gould believed in pushing the envelop on everything he put his hand to. Newell encouraged him to search broadly and introduced him to the work of Schindewolf and his student Adolf Seilacher (1925–2014), who had done extensive field work in contact with Preston Cloud's (1912–91) US Geological Survey group. Seilacher was developing his theory of *konstructions-morphologie* (later expanded to morphodynamics), including a model that came to be called Seilacher's triangle, in which organisms were perceived through a three-way causal nexus of multiple constraints on variation: functional (including ecology, selection, and adaptation), phylogenetic (including developmental), and fabricational (material and architectural). Seilacher had a gift for imagining extinct organisms fully situated in their home environments and this led to a radically detailed paleobiological vision – and many more questions. Why was morphospace so sparsely populated, and largely in clumps. Many morphologies and ecological roles seemed to offer highly beneficial evolutionary opportunities. Would these neglected spaces become populated in the fullness of time? Or were there evolutionary constraints that natural selection could never overcome? That no matter how advantageous such lifeways might be, the adaptive landscape offered no route to them due to the limits placed on lineages by virtue of their prior evolutionary history? To better understand the lifeways of extinct organisms, Seilacher soon collaborated with Raup on computer models to generate trace fossils such as tracks, trails, and burrows.

Gould's primary interest throughout his career was the evolution of form and he became fascinated by Wentworth

Thompson's (1860–1948) and Julian Huxley's (1887–1975) work on allometry and maintained a correspondence with Huxley throughout graduate school. Huxley provided a fateful boost to Gould and to the paleobiological revolution when he recommended that the prestigious Cambridge Philosophical Society journal *Biological Reviews* request a review article on allometry from Gould, who was still only a graduate student at the time. A domino effect from this review ensued establishing Gould's reputation as a promising paleontologist, and contributed to his professorship at Harvard and tenure before age 30.

Niles Eldredge (1942) had been an undergraduate at Columbia University when Gould entered graduate school and the two became friends, sharing a passion for theory – including one of Eldredge's special enthusiasms, speciation. By the time Gould moved on to Harvard, Eldredge had become Newell's heir apparent at the American Museum of Natural History. Gould and Eldredge together had a platform from which to help launch paleobiology's new fortunes a few years later via a new theory on speciation – punctuated equilibria.

Paleobiology's New Start

Concomitant with theoretical movements in the 1960s, the practice of paleontology shifted away from an emphasis on detailed monographs specializing on individual taxonomic groups (e.g., horses, trilobites, scleractinian corals, and trigonian clams) – documents mirroring the interests of petroleum geologists as they tracked morphological changes through the stratigraphic column. The post-Sputnik generation of paleontologists focused on picturing fossils more holistically as living organisms – as producers and products of energy flow within ecological communities, on patterns in the history of life. At the same time they sought a theoretical grounding in the same vein as the physical sciences. They mounted a reductionist search for evolutionary mechanisms as inferred by comparisons across taxa. Thomas Schopf (1939–84), for example, wanted to find the 'gas laws of paleontology.' Stephen Jay Gould later described Schopf's ambition: "Tom had a sublime vision of regularity. He yearned to convert an empirical field, manifestly short of ideas to unite its fascinating particulars into a science based on experiment, construction of null hypotheses, rigorous testing and the possibility of rejection to move forward toward an agreement that all could share" (Gould, 1984). As the United States increased the funding and profile of science in the 1960s, the paleobiological community expanded, with a concomitant move from contemplative isolation to vigorous collaboration.

Yale University played a pivotal role in the transformation from taxonomically oriented, monographic paleontology to an interactive, dynamic paleobiology. A critical mass of enthusiastic students including Richard Bambach, Peter Bretsky, Jeremy Jackson, Jeff Levinton, Jerry Ricekin, Steve Stanley, Sara Stuart, Ken Walker, and undergraduate Robert Bakker among many others began working very closely with evolutionary ecologist G. Evelyn Hutchinson (1903–91).

Although it was the students' coordination with Hutchinson that really caught fire, the changes at Yale were in large part instigated by paleontologist Lee McAllister. Inspired by the growing funding for science in the wake of Sputnik, and with degrees in business and philosophy and an amateur interest in geology, McAllister saw the subfield of paleontology as an underachiever that lacked leadership, just the sort of field for a young man looking to make a splash. Securing a PhD in short order with a dissertation on Devonian bivalves, he landed a position at Yale, vowing that if not admitted to the National Academy of Sciences by age 40, he would quit science and go into administration. McAllister's students came to number among the most prominent paleontologists in the US, but he was not to achieve his goal of the National Academy. McAllister went into administration after the Paleontological Society awarded the first Schuchert Prize in 1973 to David Raup, who was by then recognized as a brilliant theorist who combined mathematical wizardry with sensitivity to organic form. Raup soon thereafter was inducted into the National Academy of Sciences, and McAllister became a highly successful dean at his alma mater, Southern Methodist University. McAllister taught at Yale for scarcely a decade and had no more than a dozen students, yet these students were highly productive and went on to play prominent roles in the budding new paleobiology movement (Princehouse, 2003).

McAllister added the bracing air of ambition to the mix, urging students to investigate new sources of evidence, so much so that the students objected to what they saw as less than pure-hearted motives, but even radical dinosaur paleobiologist Bakker admits McAllister must get credit for it. Whomever and whatever was responsible for the dynamic spirit of Yale paleobiology, the chemistry was certainly there for this young group of students in the sixties (Princehouse, 2003).

McAllister's first graduate student was Richard Bambach, who studied, he said, "seafood through time" (Bambach, 1993). Love led Bambach to his initial interest in paleontology. In eighth grade, he developed a crush on classmate Ann Cooper, daughter of Smithsonian brachiopod paleontologist G. Arthur 'Gus' Cooper – who later announced the modern project of paleobiology. Spending time at the Cooper residence, young Bambach was captivated by all the books on fossils, and fell even harder for paleontology than he had for Ann Cooper. Subsequently, Bambach enrolled as an undergraduate geology major at the Johns Hopkins University in 1952, working with Harry Volks and Tom Amsden. There followed a stint at the Smithsonian, then the Navy, and then graduate school at Yale where Bambach worked with McAllister, Hutchison, Don Rhodes, and John Ostrom, receiving his PhD in 1967. At Yale he had the opportunity to participate in geological field work, especially with Chuck Masters, a graduate student employed by Amoco, mapping Cretaceous facies changes in Northwest Colorado. In the paleobiological and paleoecological atmosphere what otherwise in traditional biostratigraphic hands might have been more useful to the petroleum industry than to evolutionary theory, Bambach's dissertation on Silurian *Arasaque* clams from Nova Scotia sent him down to New Haven harbor to make ecological comparisons with modern clams, much as Raup had done with echinoids. McAllister had been searching the literature for ecological data on clams and, somewhat in the spirit of numerical taxonomists Robert Sokal and Peter Sneath, had developed an index of morphology, ecology, and behavior.

Bambach and fellow graduate student Steve Stanley xeroxed his notebooks, noted conflicts, and refined the analysis. In 1972 Stanley drew on this to develop the hypothesis that predation stimulated rapid evolution of novelties in the Cambrian explosion, forcing prey animals to evolve defenses such as shells, rapid swimming, and burrowing. These had secondary effects in opening up evolutionary possibilities: hard shells led to filter feeding, and burrowing introduced animals to new food resources. In 1975 Stanley introduced the highly influential concept of species selection.

The paleobiological approach in South Africa goes back at least to the days of Raymond Dart (1893–1988), who emphasized the ecological adaptations of *Australopithecus*. Getting her PhD at the University of the Witswatersrand shortly before Stanley's species selection paper appeared, Elisabeth Vrba (1942) was well equipped to join the fray. Like Stanley's macroevolution work, her turnover-pulse hypothesis explored higher level macroevolutionary phenomena, such as vicariance biogeography, extinction, and speciation arising especially due to major changes in climate. Her work on exaptation with Gould was highly influential in helping paleobiologists conceptualize the meaning(s) of adaptation (Gould and Vrba, 1982).

In California interest in paleoecology also caught on, coupled with a focus on biodiversity. There, the movement was spearheaded by James Valentine, who credited G.G. Simpson as his primary influence: "He was an evolutionary theoretician; ...he showed that the fossil record...could be used as a source of evolutionary information from which theories could be built" (Princehouse, 2003, p. 217). Valentine's landmark 1967 paper on mathematical models and 1973 book on evolutionary paleoecology were seminal. From there he followed his morphological interests with a focus on systematics, addressing questions such as how to define complexity. This led him to a theory based the evolution of fundamental body plans. On his view the only objective measure of complexity is the number of distinct tissue types generated in the developing embryo. These serve as the basis for the origin of phyla and the enduring shared fundamental organization of their constituent members.

A Chancy Young Man's Game

Throughout the fifties, dissertations were written and paleontology inspired by the modern synthesis appeared to be flourishing. Simpson's early work bringing paleontology to the synthesis was influential as well as inspirational. But young paleontologists increasingly felt the synthesis was moving toward a more restrictive, gradualistic, selectionism that tended to relegate paleontology to a bookkeeping role. They felt other subfields expected them to document the fact of evolution, but not to be a potent source of evolutionary theory. Following the 'central dogma' of genetics that information flows from the genes to the rest of the organism, another central dogma seemed to be developing in the discourse of evolution – that theory flows from genetics to other evolutionary subdisciplines. Bambach credits Simpson's early *Tempo and Mode in Evolution* (1944) as fundamentally important to his vision of the emerging field of paleobiology, whereas he found Simpson's later *Major Features of Evolution* (Simpson, 1953) less important and inspiring. Young paleontologists respected Simpson immensely, but they also worried that perhaps Simpson had sold the field short.

Into that rather orderly social scene burst some self-described 'young turks' looking to turn the study of evolution on its head – as well as some who were simply following up what seemed most interesting to them. The beginning of the modern era of paleobiology was highlighted by the near-simultaneous appearance of four major works between 1967 and 1969: Norman Newell's paper on the episodic nature of extinction, Peter Bretsky's summaries of marine invertebrate assemblages through time, *The Fossil Record*, a proceedings volume from a conference cosponsored by the Geological Society of London and the Paleontological Society that provided the first comprehensive database designed specifically for studies of the nature of the history of life, and James Valentine's work charting the diversification through time of microscopic forams, and various metazoa. As with noting exactly when speciation takes place in a gradually evolving lineage, it is difficult to identify just when the change in question took place in the field of paleontology. It had not arrived yet in 1960, but the new research program was in full swing before 1975 when the movement gave rise to a new flagship journal *Paleobiology* edited by Tom Schopf.

Thomas J.M. Schopf had a thoroughgoing commitment to science and its methods and processes. He combined this broad orientation with a deeply felt desire to turn paleontology into a source of theory that would be recognized as such by scientists in any field. In his understanding this meant working up theories – especially mathematical theories – and testing them empirically, taking the risk of being wrong in a straightforward Popperian manner. Gould admired the way Schopf consciously directed all the energy he could muster to developing and promulgating his rather radical view of life's order. Gould later summed him up: "Tom Schopf was a brave man." Schopf said his main motivation was "to bring the approaches and information of biology to bear upon paleontology." He was especially concerned with bringing theory into paleontology "to make that field less anecdotal, more rigorous and less tied to descriptive work" (Gould, 1984). Although Schopf's gas laws of paleontology did not catch on, Bambach and others credit Schopf's contribution as a major driver of the effort to pioneer the new field of paleobiology (Princehouse, 2003).

At Ohio State, Schopf wrote his thesis on conodonts, and had a life-long interest in ectoproct bryozoa. Although Schopf did not have the social and curricular foundations of the students from the East Coast schools, he absorbed the spirit of the modern synthesis and learned a great deal of population biology on his own. Schopf's autodidacticism continued throughout his life. When the new genetic sequencing techniques were first being developed, Schopf took a sabbatical to apprentice himself in a molecular biology lab at the California Institute of Technology. His father, William J. 'Jim' Schopf (1941–), was a paleobotanist for the United States Geological Survey in Columbus, Ohio who instilled in Tom a nearly obsessive work ethic in a milieu of very conservative paleontology. This combination eventually landed him a position at the University of Chicago. Tom Schopf and his

brother, J.W. 'Bill' Schopf, were inspired by their father's example to want to make major contributions (Princehouse, 2003). For Bill Schopf, this meant exploring the very earliest organisms. For Tom Schopf, always competitive with his brother, it meant a conscious effort to reinvent the field. To turn it into a 'chancy young-man's game' (Gould, 1984).

Establishing Paleobiology

Schopf was midwife to the new movement. He used, in Gould's words, "socially impolitic ways to seek laudable goals" (Princehouse, 2003, p. 207). He organized people, brought them together, and convinced them to work together – even though he, himself, did not have the most charismatic personality. He organized key conferences, big and small, edited the influential volume *Models in Paleontology*, that contained Eldredge and Gould's first punctuated equilibria paper, and he created the journal *Paleobiology*. More than any other single person, Schopf shaped the movement. He was a ruthless editor, rejecting half the manuscripts submitted to *Paleobiology*, a practice unheard of in paleontology in those days. As Neil Shubin said, he "simply didn't have a off switch" (Shubin, 2013). He was single-minded in his efforts to move the profession into a sound theoretical channel.

Schopf sought to develop the practical aspects of the new theoretical field. A major focus was to systematically bring together people whom he thought could pioneer new methodologies and theories for paleobiology. Schopf targeted Gould, in part due to his revolutionary interests but aided by his proximity when both were in Woods Hole. This gave Gould a deeper understanding of Schopf than those who met him only at conferences. Schopf's students at Chicago found him a hard taskmaster, but intellectually very exciting. Instead of sticking just to the fundamentals of the field, Schopf's introduction to paleobiology class explored cutting-edge theory. Students asked themselves who were the then relatively unknown Raup and Gould that Schopf referred to so much. His discussions of theory, grand pattern, and Seilacher's *konstruktions-morphologie* were a little daunting to students, but they did not realize just how unusual their education was. Rebecca German later commented: "For Tom to embrace that was far more revolutionary than we realized" (Princehouse, 2003, p. 209).

A pivotal moment in the history of paleobiology took place over winter break in 1972 when Schopf invited Raup, Gould, and Dan Simberloff, a math major turned ecologist and former grad student of E.O. Wilson (1929–) with an interest in paleontology, to the Marine Biological Laboratories (MBL) at Woods Hole, Massachusetts to take a sharp look at clade diversity with an eye to applying population genetics to the fossil record. Raup said Schopf had the idea that if he could put "a small group, maybe representing several subdisciplines, together in a quiet environment conducive to brainstorming that we might be able to come up with some new and better ways of looking at the history of life" (Raup in Princehouse, 2003). Simberloff had been present at a similar retreat, which had resulted eventually in MacArthur and Wilson's island biogeography theory. This was Schopf's model for such a meeting, and beyond that he was hopeful that an expanded model could be created by substituting speciation rates and geologic time for the immigration rates and short-range time of the island biogeography model. Gould described Schopf's role in the collaboration: "Tom was just a real go-getter. You can't say he was a politician, because he was too undiplomatic a personality. He wanted to get things happening and liked to do it institutionally if he could."

A relatively rare combination at the time, young Simberloff was quantitatively inclined as well as a naturalist and made use of a prized possession, an early Texas Instruments programmable calculator. The four discussed the state of the field and proposed potential models and methods for four days, with Gould's student Jack Sepkoski (1948–99) joining on the last day. One feature of the disciplinary landscape that impressed them was the colossal database that paleontology and associated stratigraphy represented and with which very little had been done except for traditional taxonomic studies for monographs and informing biostratigraphy for petroleum companies. Much of the comprehensive *Treatise on Invertebrate Paleontology* had been published by that time and using it as a data source Sepkoski was already putting together databases working with Gould. Sepkoski later joined forces with Raup at Rochester and the University of Chicago to produce complexly integrated paleobiological work on biodiversity and extinction throughout the history of life on earth, including the controversial result that mass extinctions occur at regular 26 million-year intervals. They interpreted this regularity to suggest an extraterrestrial phenomenon such as a twin star to our sun. If found, Raup wanted it to be named Nemesis. Alternatively, Gould suggested Siva, the Hindu god who destroyed with one hand and created with the other – since mass extinctions usually cleared the way for the evolution of novel forms.

The MBL group wanted a fresh look that was quantitatively based. The idea developed to explore a null hypothesis in which everything in the history of life was simply random. As Gould (Princehouse, 2003, p. 213) put it: "Any non-random model requires that you make a causal theory about the world, and we wanted to see what you could do minimally."

Schopf's intuition that putting the right people together with an explicit mission succeeded. Though they did not come away from the meeting with a manifesto per se, their perception of the field's importance and its potential reoriented their thinking and that orientation soon bore fruit. Raup, who had experience with mainframe computing took the equations Simberloff produced from the meeting and developed full-fledged computer programs to produce simulations of evolutionary history. This became known as the MBL model.

The MBL model relied on Monte Carlo random number techniques. They wanted to break evolutionary biodiversity down to its minimal sufficient parameters. Natural selection was to be avoided at all costs. The lineages would have three possible fates each generation: a bloodline could go extinct, or it could stick together as one lineage, or it could split into two lines. At the beginning of each simulation, random numbers were generated to use as 'seeds' for each clade. At first the chance a lineage would persist into the next generation and branch was greater than the risk of going extinct. When the 'island' reached an equilibrium point beyond which the carrying capacity was exceeded, the program switched over to giving each lineage an equal chance of branching or extinction

to keep the population right around this stable size. Within the set of species that survived, each line had an equal chance of splitting or continuing solo into the next generation. The run ended when any of three things happened: a set length of time ran out; all lineages went extinct; or when the population reached 500 lineages – which was all the computer's memory could handle!

Higher taxa were formed when a lineage reached a certain speciosity. These clades where then represented in the visualization diagrams long in use in paleontology. These 'spindle diagrams' charted the birth and death of a higher taxon. In real-world paleontology, the spindles were defined by points marking the first and last known occurrence of the group in question and the breadth of body of the spindle indicated its relative florescence. The simulations produced interesting results – familiar patterns including evolutionary radiations and mass extinctions. Traditionally, species with similar lifeways were not expected to share a niche. So when a pattern emerged from the fossil record in which one clade waxed simultaneously with another's waning, the assumption was that this was driven by natural selection's competitive exclusion principle. The MBL simulations allowed another possible interpretation: that these were just ships passing in the night.

A series of papers resulted, demonstrating that even in a random system, patterns would emerge that paleontologists were accustomed to interpreting as due to natural selection building Darwinian adaptations. Most stunning to Raup and Gould was a project the two worked on to simulate the evolution of morphology per se, not just biodiversity. To their astonishment they found emergent properties arising without any selection toward that goal but as an intrinsic element of complex systems (Raup and Gould, 1974). This was very unexpected and ran sharply counter to the the primary perception of causality in biology and paleontology at the time. The work was highly controversial but provided an alternative model against which to evaluate claims of long-range adaptation and inexorable adaptive trends in the history of life. The ultimate goal was not just building abstract models, but in comparing them to actual data. Gould set Sepkoski – also skilled with computers – to work tabulating every known occurrence of every fossil species known into a form suitable for computer analysis. A decade later he was still at it. Eventually Sepkoski produced what Gould felt was sorely needed in the field: hard data against which to compare the null hypothesis MBL model.

In California, James Valentine and Jere Lipps among others were posing similar theoretical questions about what could be inferred from the enormous mass of paleontological data that had been published by Preston Cloud's USGS group in successive volumes of the *Treatise on Invertebrate Paleontology* and in the many new journals appearing, such as *Lethaia* (which published Seilacher's triangle paper) and *Micropaleontology*. Valentine and his students rigorously explored the heuristic value of random models, tying them in with increasingly sophisticated understandings of the fossil record, population biology, and genomics. Similar interests and approaches led to good-natured and productive competition between the Raup–Sepkoski group in Rochester and Chicago and the Valentine–Lipps group in California. Both groups generated a host of innovative hypotheses about causes behind the large-scale patterning of biodiversity, mass extinctions, and other prominent features of the history of life. These new traditions were soon seen in such developments as the integrative biology department at Berkeley and Neil Shubin's laboratory at Chicago, a hybrid where fossils are prepared alongside wet lab experiments on embryology and development.

Paleobiology Comes of Age

Central to the paleobiological revolution were not so much the establishment of new institutions and technologies (though computers, and the founding of the journals *Lethaia*, *Palaeos*, and *Paleobiology* played significant roles) but a new preoccupation, largely on the part of paleontologists, with questions of a particular sort. Where the modern synthesis centered on the explanatory power of natural selection acting on copious random mutation, the new approach concerned parameters. What opportunities and constraints existed in genetic, developmental, and ecological systems? How did these evolutionary parameters bear on the long-term patterns of the evolutionary history of life on earth? Can process and pattern be uncoupled? If not, then how isomorphic are they and why?

The 'young turks' pursued the direction mapped out by G.G. Simpson's major synthesis work *Tempo and Mode in Evolution*. They read widely across evolutionary disciplines, but did not acquiesce to the role they perceived as having been assigned to paleontology. Michael J. Benton commented that some "will recall palaeontological meetings at which fun was made of the work of statistical palaeontologists. Some … were based on valid concerns, but others were founded on a pessimistic view of the potential of palaeontology" (Benton, 1999). Echoing Cooper's (1958) address, Bambach indicated that paleobiologists "practice an exceptionally broad interdisciplinary subject. We are no longer the 'handmaiden to stratigraphy" (Princehouse, 2003, p. 224).

The power and prestige of paleobiology was greatly enhanced beginning in the 1990s when the fields of genomics and evolutionary developmental biology ('evo-devo') began providing additional understanding of the mechanisms of morphological variation. Although paleobiologists, especially Gould (1977), played some role in inspiring the evo-devo movement, its force came largely from within the fields of molecular biology. That the new framework had been developed quasi-independently from multiple different evolutionary subfields bolstered the claims – and reputations – of those who had come to similar conclusions based on fossil evidence. Genomics and evo-devo supplied a physiological basis for phenomena such as quick morphological change, mosaic evolution, widespread parallelism among related lineages, etc. Issues that until recently had been the almost exclusive province of paleontology. The paleobiological revolution had come full circle and unified with these new fields to produce a richer, more adequate, more fully synthesized evolutionary theory.

See also: Biogeography, Human. Cambrian Explosion: A Molecular Paleobiological Overview. Developmental Paleontology and Paleo-Evo-Devo. Paleobiogeography and Fossils

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Parallel and Convergent Molecular Evolution

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Glossary

Adaptation It refers to long-term evolutionary modification and maintenance of functional traits or molecules in an organism in response to natural selection. Adaptation does not necessarily imply optimization because a trait may have constraints in how much it can be modified, and also because it is sufficient to improve a trait until it is 'good enough.'

Ancestral reconstruction It is the process by which ancestral traits or sequences that no longer exist in living organisms are inferred. In recent decades, ancestral protein molecules have been reconstructed and expressed in the laboratory to test their function and obtain more direct inference of how functions have evolved over time. Because of the multiplicative accumulation of uncertainty in ancestral reconstruction among all sites in an alignment, because of coevolution or epistatic interactions among sites, and because of laboratory experimental uncertainty, such inferences cannot be assumed to be exact reconstructions of sequence and function, but are often presumed to be strong indicators of trends in functional evolution.

Branch pairs They are two branches (sometimes called edges) on a phylogenetic tree that are compared as paired lineage segments for which the amount of convergent evolution can be measured.

Convergent evolution It occurs when two biological traits in two separate lineages independently evolve to similar end points. At the molecular level, a key goal is to distinguish between convergent events that have arisen because of adaptation, and possibly neutral convergent events that occurred by random drift and were thus not driven by positive natural selection.

Divergence It refers to the process of traits or sequences becoming more different between lineages over evolutionary time by the accumulation of differences in each lineage that are not convergently replicated in the other.

Evolutionary distance It refers to the expected number of substitutions that occurred between species or along branches of a phylogenetic tree. Evolutionary distance in neutral evolution is the product of the mutation rate and time, and thus its expectation is proportional to time if the mutation rate is constant, although the outcome of actual number of substitutions along a lineage is subject to stochastic variation.

Homology It refers to traits in an organism that are descended from a common ancestor. In molecular evolution, in gene families that have incurred duplications, homologous genes are divided into orthologs, which are only related by speciation events, and paralogs, which are related by gene duplication events.

Homoplasy It is a cladistic term that refers to traits that cannot be resolved as having occurred just once on a phylogenetic tree, and thus must have arisen by convergence. Such traits cannot be resolved parsimoniously with other traits on a phylogenetic tree, and thus confound phylogenetic inference using cladistics methods.

Long branch attraction It is the phenomenon by which the accumulation of nonadaptive convergent events, or homoplasies, which is predictably greater between longer branch pairs than between shorter branch pairs, will lead to false phylogenetic signal that tends to 'attract' or falsely join long branches if the true phylogenetic signal is not sufficiently strong.

Multiple sequence alignment It is the process by which positions in different sequences are ordered with respect to each other such that all corresponding sites in the alignment represent inferred homologous positions.

Neutral evolution It is evolution that occurs by random drift, meaning that the probability of fixation of new alleles is proportional to their starting frequency. At sites that evolve neutrally without selective constraint (and without certain interfering mutation repair processes), the substitution rate is expected to be equal to the mutation rate.

Parallel evolution It is sometimes used as a synonym for convergent evolution and homoplasy, or may refer to convergent evolution that occurs in experimental replicates, or in closely related species under similar ecological pressures to adapt. At the molecular level, it may sometimes be used to indicate convergent evolution with the same or similar molecular mechanism, or involving the same genes, and at the amino acid level it may refer to convergent evolution from the same ancestral amino acid. In this article we advocate that the term should be deprecated.

Phylogenetic inference It is the process by which evolutionary relationships, or branching order and timing, among species or sequences, is inferred.

Position In a functional molecule such as a protein it may refer to the amino acid location along the sequence and in three-dimensional space. Positions in difference sequences may be related to each other as inferred homologous sites in a sequence alignment.

Sequences They refer to the ordered components of biological molecules such as DNA (made up of four different deoxyribonucleic acids), RNA (made up of four different ribonucleic acids), or proteins (made up of twenty different amino acids).

Sites In an alignment of amino acids or nucleotides it refers to aligned positions that are inferred to be homologous, and thus related by substitutions alone rather than insertion or deletion events.

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Introduction

The idea of convergence is central to the study of evolution because it addresses the key concepts of adaptation and replicability. Roughly, convergence happens when two biological traits in two separate lineages independently evolve to similar end points. The concept can cover a broad range of evolutionary events, from convergence of function and morphology to gene duplication, expression levels, and sequence. At the organismal scale, convergence is usually assumed to involve adaptation in response to similar selective pressures from the environment, but at the molecular scale this is not always so. There is a great deal of excitement to the current study of molecular evolutionary convergence because in the age of genomics, large amounts of information are becoming available that can be used to elucidate the molecular mechanisms of phenotypic convergence. Researchers can now ask detailed questions about whether the convergence of aquatic mammals to life in the sea (Foote *et al.*, 2015), echolocation in bats and dolphins (Parker *et al.*, 2013; Liu *et al.*, 2010), or song in different groups of birds (Zhang *et al.*, 2014) involve similar genes expressed in similar locations with similar amino acid changes.

Integrating Molecular Convergence from Molecules to Phenotypes

To understand and dissect the mechanisms of convergence, it is necessary to consider how phenotypic convergence of organisms is achieved across multiple organizational levels, from molecules to phenotypes. Adaptive pressures act on organisms as a whole, and the response will be integrated from amino acid changes that alter functional aspects of proteins, regulatory and transcriptional changes that alter when and where molecules are expressed, and interactions among system components. We discuss here just a few of the many examples available in the literature, particularly pointing out a couple of noteworthy recent genome-wide convergence analyses.

Molecular convergence of amino acids in proteins was first observed in lysozyme as an adaptation to expression in the acidic environment of the stomach as a digestive enzyme in cows (ruminants) and colobine monkeys (Stewart *et al.*, 1987). This was later augmented by observation of amino acid convergence in RNAses in the same environments (Schienman *et al.*, 2006; Zhang, 2003). A recent example is that of convergence in the molecule prestin, involved in adaptation to echolocation in dolphins and bats (Liu *et al.*, 2014; Davies *et al.*, 2012). The example of adaptive convergence at positions involved in modulating proton transport in cytochorome C oxidase between snakes and agamid lizards (Castoe *et al.*, 2009) is the largest and densest (per amino acid position) example of convergence that we are currently aware of, and is large enough to allow characterization of the types of amino acids and positions involved in convergence (Figure 1). The adaptive burst in the ancestor of all snakes is also notable for having generated follow-on convergent events in the subsequent phylogeny of snakes, some of which are also convergent with amino acid changes in the ancestor of another tubular legless squamate, *Rhineura* (Castoe *et al.*, 2008). These events provide examples of convergence to the same amino acid at the same position, physicochemical convergence to amino acids with the same functional group (hydroxyl, or -OH) at the same site, and convergence to add positively charged amino acids in a tightly clustered region at the base of a proton channel (some at the same amino acid position and some at different positions).

Convergence on the genome level has been observed in laboratory experiments involving phi bacteriophage, *E. coli* in high temperatures, and yeast under nitrogen starvation (Wichman *et al.*, 2000; Tenaillon *et al.*, 2012; Hong and Gresham, 2014). A general observation under the conditions of these studies (involving selection on a large number of possible genes) is that although adaptive changes were observed repeatedly in the same genes (i.e., convergent usage of genes), it is rare to observe convergence of the same amino acid at the same position in the same gene because there are so many alternative adaptive responses. Foote and colleagues (Foote *et al.*, 2015) considered convergent evolution in the much larger mammalian genomes that adapted to a marine environment. Echoing the viral and microbial results, they showed that while there was widespread convergence between three different marine mammals at the amino acid substitution level, they were unable to separate what might have been adaptive from the noise of what is expected with such a large number of statistical comparisons. Further analysis of genes showing signs of positive selection in multiple marine mammal lineages detected convergent changes plausibly related to adaptation to a marine environment, but the point remains that the proportion of convergent changes is small, and many adaptive responses are not convergent in replicates under both experimental and natural conditions.

Molecular adaptation is also thought to occur rapidly through regulatory change. Zhang and colleagues assessed expression levels of genes associated with song learning in birds and found that increased expression levels of certain genes were often convergent in the song-learning nuclei (Zhang *et al.*, 2014). In a somewhat more complex example, convergent evolution of the transcription factor SP1 in mammals and birds (to different amino acids, but at the same position) appears to have convergently altered its structure and binding specificity. This then caused convergent evolution at hundreds of SP1-regulated binding sites in both mammals and birds (Yokoyama and Pollock, 2012). This system further demonstrated follow-on convergence in regulatory networks, as there were later convergent amino acid substitutions in homologous SP transcription factors in various mammal and bird lineages that co-regulate some of the same regulatory modules by binding to SP1 binding sites. Notably, these convergent changes in the co-regulatory paralogs were at the homologous position as the older convergent changes in SP1, and to the same amino acid as in chicken SP1.

A Conceptual Understanding of Convergence and Parallelism

Despite its importance, and despite its deceptively simple definition, the exact meaning and characterization of convergence is not always clear. This echoes the lack of clarity

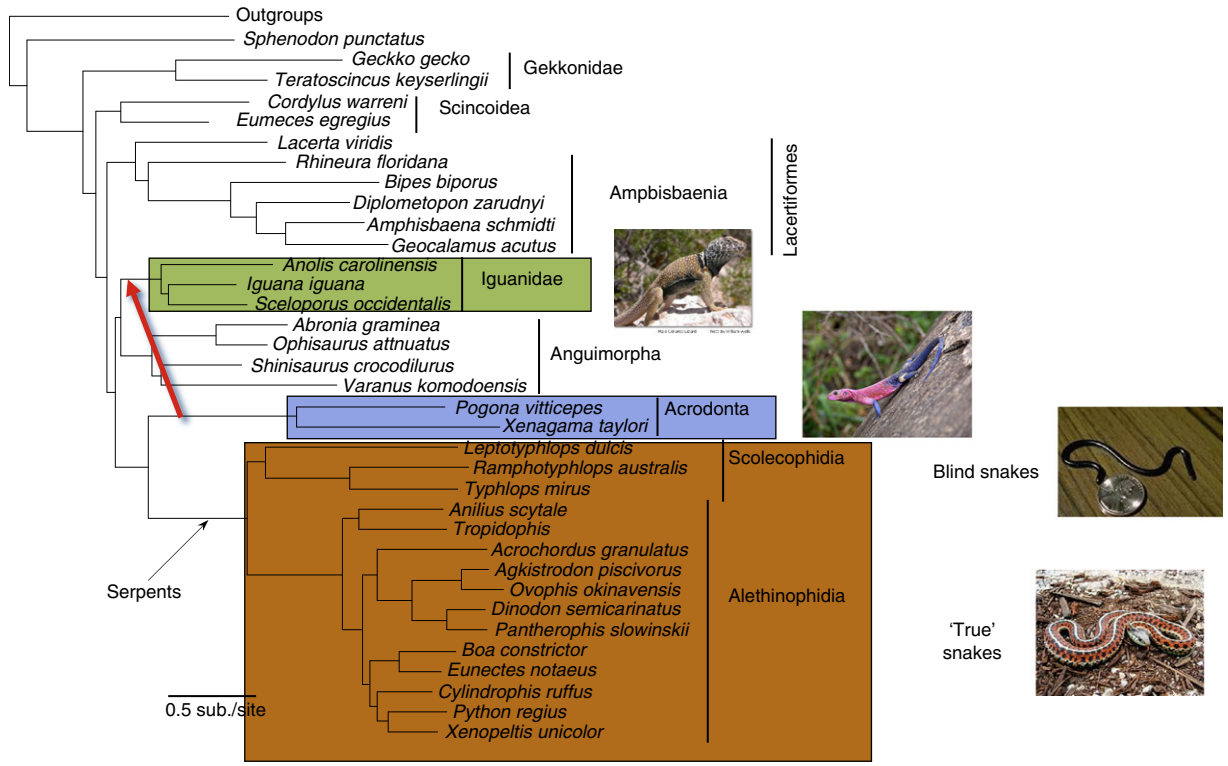

Figure 1 The effect of convergence on phylogenetic tree reconstruction. This figure shows that the mitochondrial data links the acrodont lizards (blue box) in a sister relationship with the snakes (orange box), although most nuclear and morphological evidence suggests that they should be grouped with iguanid lizards (green box), as shown by the red arrow. Detailed analysis showed that the mitochondrial phylogeny was driven by convergent amino acid substitutions at otherwise usually conserved sites. When the 500 codons that most support the mitochondrial tree are removed, the remaining mitochondrial data suggests the same tree as the nuclear data. A similar result was seen if the top 5% most convergent amino acid sites are removed. Modified from Castoe *et al.*, 2009. Evidence for an ancient adaptive episode of convergent molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America* 106(22), 8986–8991.

surrounding the term 'adaptation' itself, which can be difficult to distinguish from selection and can be notoriously difficult to prove. The uncertainty over what is and is not convergence is further confounded by the common use of the term 'parallel evolution,' which is sometimes used as a synonym of convergence or in different ways that can appear mutually exclusive to one another. The intended meaning of the authors is not always clear. At the organismal level, parallel evolution embodies the idea that closely related organisms might respond to similar selective pressures in similar ways. As discussed in a comprehensive review by Arendt and Reznick (Arendt and Reznick, 2008), the idea of parallel evolution thus sometimes applies exclusively to phylogenetic considerations (how closely related the two species are), exclusively to mechanistic considerations (whether the pathways used to effect the phenotypic change are the same), or some mixture of the two. These authors argue strongly and convincingly for the general use of 'convergence' and deprecation of the use of 'parallel evolution,' a usage also followed in the comprehensive review by Christin, Weinreich, and Besnard (Christin *et al.*, 2010) and at the MapOfLife website (mapoflife.org). At the molecular level, the use of parallel evolution has sometimes been focused down even to the trivial level of the individual amino acid, adding another usage to distinguish the cases where the common ancestor is the same (parallel) or different (convergent) (Zhang and Kumar, 1997). Using the same data to argue nearly the opposite of Arendt and Reznick, Rosenblum and colleagues (Rosenblum *et al.*, 2014) advocate for using the term 'convergence' only for the phenotypic level, and 'parallelism' only for the molecular level.

The clearest message from these discussions in the literature is that the difference between parallel and convergent evolution is unfortunately muddled, highly controversial, and unlikely to be settled soon. From a practical standpoint, this means that a reader interested in the subject will need to search for both terms, and will need to read carefully to try to understand the usage intended by each individual author, to the extent that it is clear.

In this article, we will follow the usage of Arendt and Reznick, using 'convergence' as the general term and advocating that the use of the controversial term 'parallelism' should be deprecated. This is strongly motivated in part by our own studies of convergent evolution in mitochondrial genomes (Castoe *et al.*, 2009) and on coevolution and epistasis (Pollock *et al.*, 2012), and in part because it makes writing on the topic simpler and more clear. In considering convergent amino acid changes in proteins, for example, we are most concerned with which convergent amino acids caused a convergent structural or functional change, and not whether the convergent events on each lineage arose from a common

ancestor. Because of coevolution and epistasis, the effect of an amino acid replacement may be dependent on the entire protein, and the effect cannot be assumed to be constant over time. Furthermore, given the variation in the evolutionary process across positions in a protein, it is clear that sorting sites by whether or not they have a common ancestor has the perhaps unintended consequence of biasing the groups depending upon rates of evolution at each position. The rate at which sites evolve affects their tendency to contribute to adaptive convergence because of the well-known relationship between function and conservation: the most functionally important positions are usually most conserved and also most likely to be utilized to effect adaptive change (Castoe *et al.*, 2008).

The position we take on usage should not be construed to imply that the distinctions that authors are trying to make when they use the term 'parallel evolution' are unimportant. On the contrary, they involve extremely important questions about how adaptation works, what matters in terms of the changes that occur during an adaptive event, and the degree to which adaptive pathways are replicable. It is clear that the answers to these questions are tied up in the degree of constraint in the adaptive potential of the biological system, and how those constraints change over time. What functional modifications are theoretically accessible to a system, or to an individual protein, at any point in evolutionary time? How does the realm of accessible modifications change over time? Do slightly different adaptive pressures applied to an organism result in similar or very different molecular evolutionary responses? What amount of functional change in a protein or a regulatory system as it evolves over time will lead to different evolutionary responses to similar adaptive pressures? It is our belief that rather than to say an adaptive event 'is parallel' as opposed to convergent, it is better to be clear on precisely what questions are being asked and answered about the degree of taxonomic similarity between the lineages in question and the degree of functional similarity between any of the molecular components.

Discriminating Adaptive and Nonadaptive Molecular Convergence

Perhaps the greatest problem in the study of molecular convergence is how to discriminate adaptive molecular convergence from nonadaptive molecular convergence. There have been a number of claims of large-scale adaptive molecular convergence over the last decade (Parker *et al.*, 2013; Rokas and Carroll, 2008) that have later been thoroughly refuted (Castoe *et al.*, 2009; Thomas and Hahn, 2015; Zou and Zhang, 2015). The primary reason for these mistaken claims is that commonly used evolutionary models do not adequately predict expected levels of convergence under neutrality (Castoe *et al.*, 2009), although in some cases use of indirect tests of convergence and failure to apply proper comparative controls may also have played a role (Thomas and Hahn, 2015; Zou and Zhang, 2015). It has been thought that more realistic models that vary across sites may be adequate to predict convergence levels (Lartillot *et al.*, 2007), but it has been recently shown that the situation is complicated and may require models that vary over time as well as across sites (Goldstein *et al.*, 2015). Unfortunately, such complex models are not well specified with existing datasets, and we can expect intense future analysis and development of approaches to determine the best way to predict molecular convergence using models of amino acid substitution.

In the absence of good predictive models, an alternative approach is to use empirical observation. Such an approach is based on the idea that we expect adaptive molecular convergence between lineages (or branches on a phylogenetic tree) to be rare or moderately rare, whereas we expect that nonadaptive convergence is everywhere. Thus, in principle we can infer the amounts of convergence levels among branch pairs and then determine which branch pairs have excessive convergence relative to the main distribution. However, one additional factor to be considered is that we expect the amount of convergence to somehow be scaled by the total amount of evolution on each of the two branches being compared. To see this, consider that to be convergent, a pair of substitutions must occur at the same site on both branches, and the probability of substitution at each site and branch will be proportional to the total amount of substitution along that branch.

An example of such an empirical approach was developed and implemented by Castoe and colleagues in their analysis of adaptive convergence between ancestral snakes and agamid lizards (Castoe *et al.*, 2009). This approach avoids the direct dependence of convergence on branch lengths by making use of a discovered strong general correlation between convergence (abbreviated C) and paired divergence (abbreviated D), defined as substitutions in two separate lineages that independently evolve to different end points (as opposed to the same end point in convergent events). In this analysis, we found that predicting convergence from branch lengths was much worse than prediction from paired divergence events, which may be related to inaccuracies in the evolutionary model used to predict the mitochondrial phylogenetic tree as well as variation in rates among sites and over time, which may have made the average branch lengths inapplicable to the convergence predictions for the sites involved.

The idea that the ratio C/D of paired convergence and divergence events should be constant and would thus arise naturally from the data makes some degree of intuitive sense. This is because given that paired substitutions at the same site on two different branches have occurred, all else being equal the probability that such events are convergent or divergent should be equal among branch pairs. However, it should be considered that all else is not necessarily equal, in that the ancestral amino acids may have changed over evolutionary time, the composition of the sites that make up the paired substitutions may have changed, and the evolutionary process at some or all sites may have changed. We recently found that nonadaptive amino acid convergence rates do decrease over evolutionary time of separation between branch pairs, with dependence on the specific ancestors involved, the rate of substitution at the sites involved, and a fluctuating evolutionary process due to epistasis or coevolution (Goldstein *et al.*, 2015). Thus, these are important interacting effects, and it is a major challenge to incorporate them adequately in future predictions of adaptive convergence.

Molecular Convergence, Ancestral Reconstruction, and Phylogenetic Inference

Phenotypic and molecular convergence can both in principle be inferred through the observation that distantly related groups of species contain similar traits, while many other species, more closely related to each of the convergent species than they are to each other, do not. However, as we begin to understand how molecular convergence events have led to phenotypic convergence, we generally need to pinpoint when the convergent events occurred with as much accuracy as possible. In other words, we need phylogenetic trees with diverse representation of relevant species to break up critical branches on the trees as much as possible, and then we need to infer on which branches the putative convergent events have occurred. It is useful to do this to understand the timing and possible environmental correlates, but it is essential to do this to limit the large numbers of neutral or unrelated substitutions that are bound to occur on long unbroken branches. To make these inferences, one must also perform ancestral reconstruction to estimate the state of each position at nodes (branching points) of the phylogenetic tree, a process that has error and which may be biased at the sequence as well as the functional level (Krishnan *et al.*, 2004; Williams *et al.*, 2006).

Although phylogenies are necessary to dissect the mechanisms of molecular convergence, both adaptive and nonadaptive convergence may interfere with phylogenetic inference. The example of Castoe and colleagues (Castoe *et al.*, 2009) makes it clear how adaptive convergence can positively mislead phylogenetic reconstruction by systematically and falsely linking distant branches. This can be understood by seeing that if there are a lot of convergent events, the false signal at the sites involved may overwhelm the true phylogenetic signal at the more reliable neutral and well-behaved (or at least not systematically biased) sites. It may then be more parsimonious to resolve the changes on a tree that would make the convergent sites appear to have substituted only once. This problem is made worse by the tendency of adaptive convergence to occur at functionally important sites that are otherwise conserved, and thus often thought to constitute a more reliable signal than variable sites. Although we expect adaptive and convergent events of the magnitude and density of the snake example to be rare, smaller events are more difficult to detect, may be more common, and if they occur between closely related and difficult-to-resolve branch pairs they may easily overwhelm the true phylogenetic signal.

Nonadaptive and possibly neutral convergence should have less obviously deleterious effect on phylogenetic inference because it is widespread and not focused on particular lineages. However, if the substitution models used in phylogenetic inference do not adequately predict levels of convergence (often called homoplasy in this context because the effect is worst for cladistic methods such as parsimony that do not have an explicit model), then there will be bias toward falsely joining long branches. Thus, the observation that most current models inadequately predict nonadaptive convergence levels is of considerable concern. Furthermore, the recent discovery of exceedingly high convergence levels in closely related lineages, with a decrease in these levels over time, is also of obvious concern (Goldstein *et al.*, 2015). Because this is also expected to be unbiased toward any particular branch pair, it appears that the greatest concern is that it adds considerable noise to inference of the most difficult phylogenetic branching problems, and that certainty and confidence in some phylogenetic results may be poorly understood.

Conclusion

Although there are open questions in how to best detect molecular convergence and how to improve our models to understand it better, genome-wide studies as well as focused biochemical studies have begun to reveal general mechanisms of how molecules effect phenotypic convergence. These are exciting times for the study of molecular convergence. Broad generalizations are beginning to emerge, but big questions remain about when and why adaptation to environmental conditions repeats itself in different organisms with different levels of relatedness. Elucidating the details promises to keep evolutionary biologists occupied for many decades to come.

See also: Adaptive Molecular Evolution: Detection Methods. Ancestral Reconstruction: Theory and Practice

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Parallel Speciation

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Glossary

Biological species concept Species concept according to which a species is defined as a group of individuals that are reproductively compatible with one another, while being reproductively isolated from other such groups.
Candidate genes Genes that have been shown to be associated with a trait of interest in previous work. Here, traits of interest will be those that show adaptive divergence or may reflect barriers to gene flow. Candidate genes may be inferred from observations in the same or other taxa.
Epistasis The effect of an allele is affected by the genotype at another locus.
Gene expression The process by which genetic information is translated into gene products. The amount of mRNA transcribed from a gene gives an indication of the extent to which a gene is 'used' in the cell.
Genome scan Method that is used to identify genomic regions potentially affected by divergent selection ('outlier loci'). It utilizes the fact that effective gene flow in such genomic regions is reduced, increasing measures of differentiation relative to the rest of the genome. However, results need to be treated with caution as other processes (e.g., background selection, local adaptation without gene flow) might produce outlier loci as well.
Homologous traits Traits (in different taxa) that originate from the same ancestral trait.
Phylogenetic species concept Species concept which uses a phylogenetic tree to define a species.
Pleiotropy When a single allele affects multiple phenotypic traits. This can lead to constraints on the evolution of these traits.
Quantitative trait locus (QTL) mapping Method by which genomic regions affecting a trait of interest are identified. Crosses of individuals with different trait values are used to generate individuals in which the genomic background is randomized, so that a statistical association between trait and genes underlying it can be detected.
Reinforcement Process in which the evolution of prezygotic barriers (e.g., mate choice) between two interbreeding populations is favored when hybrids are less fit due to the presence of postzygotic barriers.
Reproductive barrier Trait or genomic region that contributes to reproductive isolation, reducing effective gene flow between populations. Reproductive barriers may act at the pre- or postzygotic stage.
Standing genetic variation Genetic variation is present within a population. Adaptive standing genetic variation can facilitate rapid and recurrent adaptation as novel mutations are not needed.

What is Parallel Evolution?

Selection can drive the evolution of phenotypic traits. However, phenotypic change does not necessarily mean that a trait is under selection. Changes can evolve by chance, especially in small populations. A pattern that represents strong evidence for the role of selection is when the same trait evolves repeatedly in the same direction in independent populations, always associated with the same environmental transition (in space or in time) (Figure 1). For example, sticklebacks have repeatedly lost their plate armor when colonizing freshwater habitats from an ancestral marine population. If this loss had been observed only once, chance could be a reasonable explanation – for example, the founder population colonizing freshwater might by chance have been different from the average of the ancestral marine population. However, because the pattern has been observed repeatedly in multiple, geographically distant locations, it is most likely generated by a nonrandom process – selection.

The repeated evolution of similar changes in homologous phenotypic traits is referred to as 'parallel evolution.' However, the exact definition of the term varies across the literature, sometimes narrowing down its scope, for example, to cases where closely related taxa are concerned, or where the same gene is responsible (see Box 1). We stick to the classical definition in this text, arguing that phenotypes are what is under selection. Taxa may be closely related (populations of the same species) or very distantly related.

At least two pieces of evidence are required to demonstrate parallel evolution driven by selection. (1) Repeated associations of a certain phenotype with a certain environment. This will require sampling across multiple locations, characterization of environmental factors and phenotyping of individuals. (2) Evidence that the phenotype has evolved repeatedly (as opposed to a single origin, followed by the colonization of multiple locations; Figure 2(a), Box 2). For this, phylogenetic information is often necessary. However, even if the same phenotype appears repeatedly, in distinct clusters in a phylogenetic tree, evidence for parallel evolution is not conclusive. This is because gene flow between geographically close populations in different environments may obscure the true phylogenetic relationships (Figure 2(b)). Combining molecular data and modeling is a possible approach toward resolving this issue (e.g., Butlin *et al.*, 2014; see Box 2). Tracing the correct phylogeny is particularly important in systems where instances of parallel evolution are closely related and gene flow is possible, which is expected for many cases of parallel evolution.

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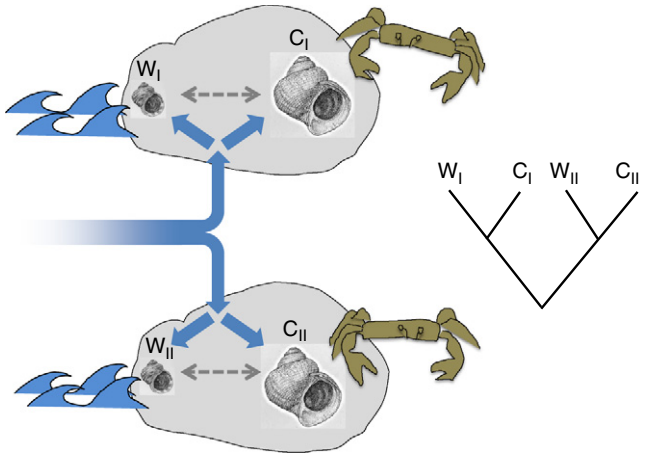

Figure 1 Parallel evolution is the repeated evolution of similar phenotypes under similar regimes of selection, in two or more geographic locations.

Box 1 Definitions of parallel evolution

Simpson (1961) – 'The independent occurrence of similar changes in groups with a common ancestry and *because* they had a common ancestry.' In contrast, convergence involves the evolution of similar characteristics without common ancestry.

Wake (1991) – While convergence is driven by external factors (selection), parallelism is the combined effect of external and internal (genetic) factors.

Zhang and Kumar (1997) – Parallel evolution happens when the same substitution at a nucleotide site occurs in two independent lineages, derived from a common ancestral state. In contrast, convergent evolution occurs when the same derived state is reached from different ancestral states.

Wood *et al.* (2005) – Parallel genotypic adaptation: The same gene evolves to affect a trait in the same way, in independent populations; different substitutions may be involved as long as the same gene is affected. Convergent evolution happens when nonhomologous loci affect the same trait.

This article – Parallel evolution is when homologous traits evolve in a similar fashion in independent populations or species. This contrasts with convergence, which is when nonhomologous traits evolve to become increasingly similar.

Parallel evolution is interesting because it can demonstrate the role of selection in divergence (and speciation, see below). Especially in systems with a large number of 'replicate' populations (e.g., Bernatchez *et al.*, 2010; Johannesson *et al.*, 2010; Roda *et al.*, 2013a), parallel evolution offers great potential to understand how selection shapes adaptive phenotypes and how repeatable/deterministic evolution is (e.g., Soria-Carrasco *et al.*, 2014). In the following, we discuss evidence for parallel evolution and parallel speciation. Now that various genetic and genomic tools are available, researchers also begin to understand the genetic mechanisms underlying parallel evolution. Shared standing genetic variation, novel mutations and gene flow may all play a role in generating shared phenotypic patterns (Johannesson *et al.*, 2010), as we discuss in the final section of this article.

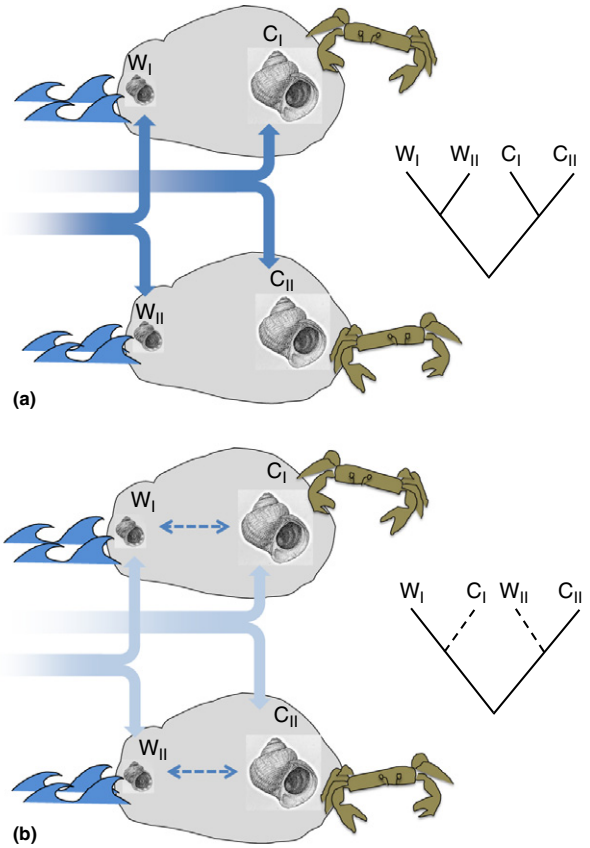

Figure 2 Separate evolution of distinct phenotypes (ecotypes) followed by secondary overlap in distribution in two or more geographic locations results in a phylogenetic relationship between phenotypes different from a parallel relationship (a). If hybridization is possible (ecotypes not completely reproductively isolated) ecotypes will become locally similar and the apparent phylogeny might look similar to a parallel phylogeny (b).

Evidence for Parallel Evolution

Under controlled conditions in the lab, the repeated application of the same selection pressure has shown parallel

Box 2 Single vs. multiple origins

An alternative hypothesis to parallel evolution of pairs of phenotypes is a single origin followed by secondary overlap in distribution of the two phenotypes, along with local hybridization in multiple locations (Grahame *et al.*, 2006; Johannesson *et al.*, 2010; Bierne *et al.*, 2013). To separate single origin and multiple (parallel) origins of phenotypes requires more than a simple glance at a phylogenetic tree as secondary gene flow may have removed the traces of the true phylogenetic origin of each local population (see **Figure 2**). Approximate Bayesian Computation (ABC) provides a toolbox (e.g., Wegmann *et al.*, 2010) for a more formal test of the two hypotheses. Different historical demographic models can be constructed and compared using a number of statistic measures obtained from input of comprehensive molecular data (e.g., heterozygosity, F_{ST}, Tajima's D). This approach was successfully used by Butlin *et al.* (2014) to separate two demographic models; 'old divergence' and 'parallel divergence' of ecotypes of *Littorina saxatilis*. However, the evolutionary history of divergence may be complex, necessitating a lot of simplification when formulating demographic models and making final resolution challenging.

phenotypic evolution in multiple studies (see e.g., Rice and Hostert, 1993). Even though a multiple-colonization scenario can often not be completely rejected (Bierne *et al.*, 2013), many cases from nature also suggest parallel evolution.

These empirical studies have provided evidence for parallel evolution in response to various parallel selection pressures, including biotic as well as abiotic factors; for example, predation regimes, water chemistry, or food availability (Elmer and Meyer, 2011). Parallel evolution can affect a variety of morphological, physiological, or behavioral traits – for example, color patterns (Manceau *et al.*, 2010), toxin resistance (Soong and Venkatesh, 2006), or courtship behavior (Boughman *et al.*, 2005) in animals, and tolerance to soil conditions in plants (reviewed in Ostevik *et al.*, 2012). Sometimes complex phenotypes involving multiple traits have evolved in parallel. For example, in the marine snail *Littorina saxatilis*, divergent ecotypes have evolved on rocky shores in multiple locations across Europe, adapted to crab predation versus wave action (Johannesson *et al.*, 2010). Adaptations include changes in shell morphology, size, and behavioral traits, among others.

Empirical studies have also shown that parallelism is a continuum. In a given system, some traits may evolve in parallel, while others do not. For example, lake-stream stickleback pairs show parallel divergence across locations in many traits, but in this case armor traits do not evolve in parallel, probably because of predation differences among lakes (Kaeuffer *et al.*, 2012). Such patterns may be explained by the fact that there are multiple axes of selection in each location (here: each lake), some of which may be similar across locations (generating parallel evolution), while others may be different (generating nonparallel patterns). Even if traits evolve in the same direction in different locations, the extent of change may differ. This may be because the strength of selection is not the same, or because different populations face different constraints and trade-offs, or are differently affected by genetic drift and/or gene flow. Alternatively, different populations might just have had a different amount of time available to adapt. For example, *Astyanax* fish have repeatedly colonized cave environments, and phenotypic changes in response to the cave environment have occurred repeatedly, including reduction of eyes and pigmentation, both of which are not necessary in the dark caves (Jeffery, 2009). However, while old cave populations show pronounced eye reduction, populations of more recent colonists retain a higher variability in eye traits (Strecker *et al.*, 2012).

Parallel Speciation

Divergent selection might not only cause distinct phenotypic adaptations, it might also generate reproductive barriers between populations, thereby contributing to speciation (Nosil, 2012). The role of divergent selection in speciation has long been under debate, and its relative importance compared to other processes is still not clear (Butlin *et al.*, 2012). Parallel speciation – the parallel evolution of reproductive barriers – can provide strong evidence (Johannesson, 2001).

Divergent selection between populations may automatically introduce some postzygotic reproductive barrier, through selection against immigrants or hybrids (Nosil, 2012). However, this barrier will mainly be restricted to the genomic regions under selection as well as tightly linked sites (Charlesworth *et al.*, 1997), while having little effect on gene flow across the rest of the genome (unless selection is extremely strong) (Barton, 1983). Still, divergent selection may drive reproductive isolation beyond this effect by introducing additional barriers, thereby initiating or facilitating the speciation process (Schluter and Nagel, 1995; Nosil, 2012). For example, in benthic versus limnetic sticklebacks, body size is under divergent natural selection but also important in mate choice (Boughman *et al.*, 2005). In this example, both a premating and a postmating barrier are generated by divergent selection. Similarly, divergent ecological selection might cause individuals to prefer the habitat they are best adapted to, also generating nonrandom mating (Funk, 1998). There are a variety of other mechanisms by which divergent selection may generate reproductive barriers, supported by variable amounts of evidence (for an extensive review see Nosil, 2012).

One of the strongest pieces of evidence for the role of divergent selection as a driving force in speciation occurs where reproductive barriers emerge in parallel in independently evolving populations across similar environmental transitions. Any other mechanism of speciation (e.g., divergence via environment-independent sexual selection; Lande, 1981) would not predict repeatable patterns.

Finding conclusive evidence for parallel speciation in natural systems can be challenging. Schluter and Nagel (1995) defined three criteria: (1) Phylogenetic independence. As for parallel evolution, phylogenetic data (mostly mtDNA sequences in the past, but now large numbers of markers across the genome), and potentially modeling, are necessary to establish that reproductive barriers evolved repeatedly. (2) Reproductive isolation between populations inhabiting different environments. Reproductive isolation involves pre- and/or postzygotic barriers in addition to the extrinsic barrier that is automatically generated by divergent selection alone. (3) No (or less) reproductive isolation between

populations from similar environments. Furthermore, it is important to identify the adaptive mechanism in order to demonstrate the role of natural selection.

According to these criteria, if divergent ecological selection leads to reproductive isolation, individuals from similar environments should be less reproductively isolated, even if they originate from distant geographical locations. In contrast, individuals from different environments should show reproductive isolation even if they are geographically and phylogenetically close. Consequently, at this stage there is a conflict between the biological species concept (which would argue that populations with little reproductive isolation, i.e., those from the same environment, represent a species) and phylogenetic species concepts (which would argue that phylogenetically close populations, i.e., those from same location but different environments, represent a species). Points (2) and (3) require tests of reproductive isolation, ideally pre- and postzygotic. Many studies, however, mainly test prezygotic isolation, using mating trials in the lab (e.g., Rundle *et al.*, 2000).

If same-environment populations are geographically close, gene flow between them might occur and eventually lead to their fusion (e.g., Richmond and Jockusch, 2007). Alternatively, location-specific divergence processes (e.g., reinforcement; Butlin, 1987; Servedio and Noor, 2003) may introduce reproductive barriers that also generate isolation between populations from the same environment (Ortiz-Barrientos *et al.*, 2009), violating criterion (3). Similarly, 'same-environment' populations might differ with regard to environmental characteristics other than the ones under study, leading to divergence between these populations. For these reasons, demonstrating examples where parallel speciation has reached completion may be difficult. However, evidence for the early stages of parallel speciation has been found (but does not indicate whether speciation will reach completion or not) and will be discussed below.

Evidence for Parallel Speciation

Demonstrating phylogenetic independence is not a problem for laboratory experiments under controlled conditions, in some of which evidence for parallel evolution of reproductive barriers could be found (see Rice and Hostert, 1993; Nosil and Harmon, 2009). For example, Kilias *et al.* (1980) maintained *Drosophila melanogaster* lines under different environmental conditions and observed that premating reproductive barriers evolved between lines from different, but not from the same environment. However, not in all studies could parallel evolution of reproductive barriers be observed, and sometimes barriers evolved in some, but not other lines (Nosil and Harmon, 2009). Evolution may find different ways to respond to the same selection pressure, and in that case barriers may not evolve in parallel.

With respect to field populations, there are many putative cases of parallel evolution of reproductive barriers in animal taxa. The marine snail *L. saxatilis* is an example in which all three criteria explained above have been tested. *L. saxatilis* has repeatedly evolved distinct ecotypes, one of which is adapted to the wave action on the steep cliffs it inhabits, while another one lives in boulder fields or other crab-inhabited areas and is adapted to crab predation. Both ecotypes occur in close proximity (tens of meters) and are connected by gene flow. A recent study combining multiple molecular markers and ABC modeling supports the hypothesis that the ecotypes have evolved repeatedly and potentially in the face of local gene flow in distinct geographical locations (Sweden, Spain, and the UK), and in independent regions within each country (Butlin *et al.*, 2014) (criterion (1)). Both genetic and behavioral data show that reproductive barriers have evolved between individuals from different environments, even if they are geographically in contact (Hollander *et al.*, 2005; Panova *et al.*, 2006; Johannesson *et al.*, 2008) (criterion (2)). Finally, mate choice experiments have shown less isolation between populations of the same ecotype from geographically distant populations than between those of different ecotypes from the same geographic location (criterion (3)) (Hollander *et al.*, 2005).

Similar to *L. saxatilis*, other examples of parallel speciation are characterized by multiple occurrences of similar environmental transitions. These often involve selection imposed by interaction with other species, for example, different predation regimes (Nosil *et al.*, 2002; Langerhans *et al.*, 2007). Systems showing parallel speciation may be characterized by geographical features restricting migration between same-environment populations, allowing for a certain degree of independent evolution. For example, multiple fish taxa have repeatedly evolved benthic and limnetic forms in different lakes, adapted, for example, to different food sources and predators (Schluter and McPhail, 1992; Bernatchez *et al.*, 2010). Another example is the evolution of morphological divergence and reproductive barriers between *Gambusia* fish inhabiting predator-free versus predator-containing blue holes (Langerhans *et al.*, 2007).

A trait that has been associated with parallel evolution in multiple animal systems is body size (e.g., in sticklebacks and *Littorina* snails; McKinnon *et al.*, 2004; Hollander *et al.*, 2005). Because this trait is often differently selected across environments (e.g., due to predators with different feeding strategies), but may also be involved in mate choice, it might be predestined to generate reproductive barriers repeatedly.

There are fewer known examples of parallel speciation in plants, and for these, evidence is often less strong (Ostevik *et al.*, 2012). Putative plant examples are often associated with adaptation to different soil conditions, such as metal tolerance in locations close to mining sites (Ostevik *et al.*, 2012). Notably, it has been proposed that recurrent formation of hybrid species in sunflowers may in part be supported by adaptation to a specific habitat type (Schwarzbach and Rieseberg, 2002), that is, may have a component of parallelism. Although examples of parallel speciation are less common in plant species, there are nice examples of parallel evolution of traits involved in local adaptation (e.g., Roda *et al.*, 2013a), which impede gene flow at and nearby selected loci.

Molecular Basis of Parallelism

It is important to note that parallel changes in phenotype, or the parallel evolution of reproductive barriers, may be generated by different underlying mechanisms in different locations. The genetic basis of parallel evolution has

received increasing attention in recent research, as various molecular techniques now allow for the study of large numbers of genetic markers. One question is to what extent parallel evolution is caused by the same genes, and maybe even substitutions in the same nucleotide position (Elmer and Meyer, 2011).

At least three factors determine the extent to which the genetic basis of parallel phenotypic patterns is shared (e.g., Johannesson *et al.*, 2010). (1) Shared standing genetic variation. When parallel evolution occurs in closely related taxa, genetic variation dating back to the time before the populations split may still be present (Barrett and Schluter, 2008). Shared adaptive genetic variation may be utilized by selection in multiple locations. In this case, the same nucleotide positions in the same genes will be under selection across locations. (2) Gene flow. A small amount of gene flow might be sufficient to transport strongly adaptive alleles between locations (Morjan and Rieseberg, 2004; Hedrick, 2013). Similar to standing genetic variation, the same genes and nucleotide positions will then be under selection in different locations. (3) Novel mutations. Adaptation might be based on mutations that occurred after the split from the common ancestor. If different genes are involved in the adaptive process in different locations, distinct novel mutations are a likely cause. However, novel mutations might also occur in the same genes, or even the same nucleotide position, in particular if there are genetic constraints that favor mutations in certain genomic regions (Renaut *et al.*, 2014).

Theory predicts that the relative importance of these processes depends on multiple factors. In closely related taxa, adaptation from shared standing genetic variation is more likely than from novel mutations (Barrett and Schluter, 2008) for two reasons – closely related taxa share most variation and new adaptive mutations take time to emerge. Furthermore, the genetic architecture of the trait evolving in parallel might play a role. If a trait is controlled by a large number of genes (polygenic), many possible genetic changes may lead to similar phenotypic outcomes, thereby increasing the potential for independent genetic solutions. Changes in a trait controlled by a single gene would likely involve the same genomic region repeatedly. However, even if multiple genes control a trait, these might vary with regard to their potential for adaptive evolution. For example, some genes might be constrained by a low mutation rate, pleiotropic effects on other traits, or epistatic interactions with other genes. These effects might concentrate adaptive evolution on a small number of genes (Stern and Orgogozo, 2009), and, if they are shared among populations, make a shared genetic basis of parallelism more likely. This again should increase the probability of a shared genetic basis in closely related taxa, as their genetic setup is more similar.

Studying the genetic basis underlying parallel phenotypic traits is now possible with increasingly comprehensive methods, focussing on candidate genes, large numbers of markers, or whole genomes (Elmer and Meyer, 2011). There are several ways to understand in how far parallel evolution is due to evolution of the same genomic regions.

Candidate gene approaches focus on genes that are known to be associated with a trait of interest at least in some taxa. Sequencing such genes in multiple taxa may reveal whether similar genetic changes have occurred in populations with similar phenotypic changes. Some studies have also gone a step further and genetically modified such loci to find more direct evidence for their functional role (Colosimo *et al.*, 2005). The disadvantage of these approaches is that other, unstudied loci may contribute to the evolution of a given trait as well.

Mapping approaches (e.g., quantitative trait locus (QTL) mapping) allow for the identification of the genetic basis of phenotypic traits even if no candidate loci are available. The extent to which the identified genomic regions are shared will inform about the similarity of the genetic basis across populations (e.g., Rogers and Bernatchez, 2007).

An alternative approach is a genome scan, which uses genetic markers (or whole genomes) to identify genomic regions potentially affected by divergent selection between populations connected by gene flow (Luikart *et al.*, 2003). If such divergent genomic regions are shared among multiple instances of parallel evolution, a similar genetic basis may be involved (e.g., Hohenlohe *et al.*, 2010; Jones *et al.*, 2012; Soria-Carrasco *et al.*, 2014). Genome scans also have the potential to detect divergent loci underlying unstudied/unknown parallel traits. Analogous approaches can be used to detect whether parallelism is caused by similar changes in gene expression (St-Cyr *et al.*, 2008).

Studies using these and related methods have detected evidence for all of the three mechanisms explained above. Studies focussing on phenotypic traits known to evolve in parallel have often found that standing genetic variation plays a great role in parallel adaptation, potentially related to its capacity for rapid evolution (as opposed to novel mutation). For example, alleles at the *Opsin* gene are involved in multiple cases of divergence between cichlid fish in different light environments (Seehausen *et al.*, 2008), and the same alleles at the *Eda* locus were repeatedly associated with stickleback plate armor reduction (Colosimo *et al.*, 2005). There is also some evidence for the role of gene flow; for example, an adaptive allele of the arginine kinase gene, associated with adaptation to sheltered (as opposed to wave-exposed) habitat, has spread among multiple locations in the snail *Littorina fabalis* (Kemppainen *et al.*, 2011). Some evidence for the role of the same *de novo* mutation occurring repeatedly has also been found, for example, in the repeated adaptation of sand goby rhodopsin to similar light environments (Larmuseau *et al.*, 2010).

However, there are also examples where parallel adaptations are based on different mutations within the same gene, or even in different genes. The pelvic reduction repeatedly associated with stickleback freshwater colonization is based on different mutations in the *cis*-regulatory region of the same gene (*Pitx*) (Chan *et al.*, 2010), and parallel shifts in *Arabidopsis* flowering times may be caused by different nonfunctional alleles at the same locus (Shindo *et al.*, 2005). Different genes are involved in the repeated evolution of light coloration in beach mice in adaptation to dune habitats (Hoekstra *et al.*, 2006; Steiner *et al.*, 2009).

A recent meta-analysis of mapping and candidate gene studies suggests that parallel evolution is frequently caused by the same genomic regions (Conte *et al.*, 2012), which could be explained either by shared standing genetic variation, gene

flow, or independent mutations in the same genomic regions. The results may indicate that evolution is constrained and only a limited number of genes are available to change a given trait.

However, genome scan studies, which do not focus on specific traits but on the sharing of loci under divergent selection across the genome, often find relatively small proportions of shared loci, even among closely related or geographically close instances of parallel divergence (Kautt *et al.*, 2012; Deagle *et al.*, 2012; Perrier *et al.*, 2013; Gagnaire *et al.*, 2013; Roda *et al.*, 2013b; Soria-Carrasco *et al.*, 2014; Westram *et al.*, 2014; Ravinet *et al.*, 2016). This might suggest that location-specific divergence processes play a greater role than what would be expected from observed patterns of phenotypic parallelism. However, even if sharing is limited at the level of SNPs or small genomic regions, it has sometimes been found to be higher at the level of genes or genomic regions. Some recent studies have found that sharing may be increased at the level of genetic or functional pathways, which again highlights that evolution may find different molecular solutions that generate similar phenotypic functions (e.g., Roda *et al.*, 2013b; Renaut *et al.*, 2014).

Hence, it is too early to draw conclusions about the relative importance of different genetic mechanisms underlying parallel evolution and speciation. However, in agreement with theoretical predictions, there is some correlation between the age of divergence and the probability of gene reuse (Conte *et al.*, 2012). Nevertheless, there are many known exceptions to this pattern – even very distantly related taxa may use the same genes or even nucleotide positions for adaptation, as observed in divergence between light and dark coloration, where the same gene (*Mc1r*) is used across a wide variety of taxa (reviewed in Arendt and Reznick, 2008; Manceau *et al.*, 2010). On the other hand, closely related taxa (populations within species) may use different genetic mechanisms to generate similar phenotypes (e.g., Renaut *et al.*, 2011). The extent of a shared genetic basis may depend on a complex interplay between the age of divergence, the complexity of the genetic setup, the extent of present connections via gene flow, mutation rates and effective population sizes, genetic constraints, and chance.

A large part of the work on the genetic basis has focused on parallel evolution in general, rather than on the traits most important for speciation. Much more comprehensive research is therefore also needed to identify the loci and the mechanisms underlying the parallel evolution of reproductive isolation.

Conclusion

Parallel evolution and parallel speciation provide strong evidence for selection as the driving force in divergence and speciation. Many putative examples have been found in nature. However, a lot remains to be done – for example, distinguishing parallelism clearly from a single origin can be difficult (**Box 2**). Advances in modeling as well as experimental approaches will bring more clarity. There is also little information about how frequently similar selection pressures actually do lead to parallel evolution, as opposed to cases where evolution finds completely different solutions to the same problem. We begin to understand the genetic basis of parallel evolution, but the relative contributions to genetic change of standing genetic variation, novel mutation, and gene flow, as well as the role of constraints, and stochasticity, need much further study.

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See also: Ecological Speciation and Its Consequences. Speciation Genomics

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Parsimony Methods in Phylogenetics

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Glossary

Alignment The process of hypothesizing homology for DNA or RNA sequences by arranging them so that columns represent corresponding (homologous) positions.
Apomorphy The advanced or derived state of a character.
Character A unit of information that is used in phylogenetic reconstruction. A structure or feature that is hypothesized to be homologous among taxa.
Clade A monophyletic group of taxa on a tree.
Cladistics The approach to classification that emphasizes clade membership over degree of difference.
Cladogram A character diagram in tree form for a group of taxa.
Consensus tree A tree that summarizes the information among a group of other trees.
Heterochrony Relative shift in development among different parts of an organism.
Homology Sameness; correspondence of a structure or feature among organisms because of its presence in a common ancestor.
Homoplasy Lack of perfect fit of a character to a tree; incorrect homology assessment due to convergence or reversal.
Long branch attraction Inaccurate grouping of long branches as a group because changes on the branches are interpreted wrongly as synapomorphies.
Maximum likelihood A method in which a model is used to evaluate phylogenetic trees such that the likelihood (probability of the data given the tree) is maximized.
Monophyly A property of a group of taxa when the group contains a common ancestor and all of its descendants.
Occam's Razor The rule, attributed to William of Occam, that observations should be explained with as few assumptions as possible.
Ontogeny The developmental pattern of an organism.
Optimization A method for determining the character state assignments at nodes of a tree.
Outgroup A taxon used to determine the character polarity for a group on taxa under analysis.
Paraphyly The property of an assemblage of taxa when the assemblage contains a common ancestor but not all of its descendants.
Phylogenetic reconstruction The act of producing a hypothetical tree of evolutionary relationships for a group of species.
Plesiomorphy The primitive state for a character.
Polarity The direction of a character, whether a state is primitive or advanced.
Synapomorphy A shared, derived (advanced) character state that unites a group of taxa.
Taxon (taxa) Any taxonomic unit from species up through kingdom.
Taxonomy The discipline of building classifications of biological taxa.
Terminal A taxon; the biological units in a phylogenetic tree.
Tree A depiction of relationships among taxa.
Weighting The assignment of differential importance/influence of evidence in an analysis.

Parsimony

Parsimony is a method for the construction of hierarchic character patterns for biological taxa. The resulting patterns, called cladograms, can be interpreted as approximations of the phylogenetic history for a group of taxa. A distinctive aspect of this approach is the preservation of the unit nature of character evidence from the original data to the final cladogram. This provides for straightforward assessment of character transformations on the tree. The approach called 'cladistics' is today often taken to be synonymous with the parsimony method, although the term was first applied to the taxonomic approach of basing groups strictly on clade membership and is therefore more properly a taxonomic approach rather than one of tree construction. For additional treatments of and perspectives on parsimony see Felsenstein (2004), Schuh and Brower (2009), Wiley and Lieberman (2011), and Wheeler (2012).

History and Philosophy

The parsimony approach to tree construction was developed during the time that explicit character-based methods of phylogenetic reconstruction were being codified. The German entomologist Willi Hennig was a key figure in the development of these methods; many of the individual elements that he employed existed before Hennig's work, but he synthesized them into a cogent methodology. Hennig's (1950, 1966) approach to phylogenetic reconstruction was not an explicitly parsimonious one in that he employed a parsimony algorithm, but it was implicitly so. He used careful analysis of individual characters and plotted putative homologies on trees in a way that best reconciled the characters to each other. Moreover, Hennig had a rule for hypothesizing homology that has come to be known as the Auxiliary Rule. It states that if instances of a feature among species pass the worker's criteria of similarity, then they are to be hypothesized as homologous. In other words, one does not hypothesize nonhomology without a justification for doing so; this is an application of parsimony. Subsequent workers built on Hennig's foundation and argued for parsimony as an explicit criterion for preferring particular topologies. James S. Farris is a key figure in the development of theoretical and practical aspects of parsimony.

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The central idea behind parsimony follows from the general principle in science that the simplest explanation for a phenomenon – the one that requires the fewest ad hoc assumptions – is to be preferred. The origin of this notion is often attributed to William of Ockham (ca. 1285–1349), although Boehner (1990) cites an earlier statement of this notion in a manuscript by Odo Rigaldus (d. 1275). The use of parsimony as a framework for phylogenetic analysis is also rooted in the idea of falsification – that individual characters are hypotheses that test each other. Putative homologies that pass this test are corroborated, while those that do not are falsified. However, the character pattern remains a hypothesis and is always subject to further test by the addition of new information.

Parsimony can be viewed as one of a larger family of maximum likelihood (ML) approaches, one that occupies an extreme on that spectrum. Rather than specifying individual parameters such as character state frequency and rate of change that comprise a model under typical ML approaches (including Bayesian analysis), parsimony employs a minimal model, equating all character changes in terms of cost, under the rationale that we do not know how costs should differ. Further, by not incorporating a priori assumptions via models, we obtain a pattern that is most appropriate for interpretation of process, since assumptions about it were not already included. This minimal model has been termed the 'no common mechanism' model and it has been shown to be equivalent to an ML analysis where each character has its own instantaneous rate matrix (Tuffley and Steel, 1997). Because it does not use a more complex model, parsimony does not attempt to account for unobserved character state changes to the extent that ML does. This means that at times it may suffer from the misinterpretation of autapomorphies on long branches as synapomorphies that unite them, the phenomenon known as 'long branch attraction,' although ML is also susceptible to this. A key assumption of the ML approach is that the model employed is correct. In the end, the choice between parsimony and model-based methods such as ML is a decision between the potential benefits and costs of models – whether a minimal approach, with its potentially unbiased solution with respect to process, is judged better or worse than the more complex model approach, which, to the extent that the model is correct, may give a more accurate solution. The choice may depend on the data under study. Whereas at least some molecular data might fit a model well because of the physicochemical mechanism of change that underlies those data, morphological data would be expected to be difficult to model, since we may have no particular expectation for a regular pattern of character change.

A pertinent question is whether the use of the parsimony approach implies the belief that evolution is itself parsimonious. Those who advocate model use will often say that it does because on the spectrum of models, it is the one that fits a scenario where evolution would take the shortest path, and one uses a model that one believes will fit the system. However, from another point of view, one that views parsimony as the minimal model possible, it does not – it is rather the most model-free way of analyzing the data and is not intended to imply anything about the process. From this perspective, it is an implementation of Occam's Razor in attempting to account for the character pattern using the fewest ad hoc hypotheses of character change. In this way, the most parsimonious tree is also the one with the most explanatory power (Farris, 1983).

The Basic Procedure

Evidence

A parsimony analysis begins with the scoring of character information from an assemblage of species (or higher-level taxa). The methodology was developed with morphological characters in mind, but it may be used for any data that can be coded as characters with discrete states. Individual characters are hypotheses of homology; they are proposed based on similarity of form and relative position for a feature observed in the collection of study species. The parsimony approach works with raw character data rather than with distances derived from them, meaning that inherently distance-based datasets are not amenable to analysis with parsimony.

In order to be informative, a character must have variation among the species of interest. The alternative (and mutually exclusive) instantiations of a character among the taxa in the study are called 'character states.' The characters are scored for each species in the study and the information is organized into a taxon x character matrix.

With morphology, the judgment of which variable unit in the organisms constitutes a character is a somewhat subjective one because of the complexity of structural features. The key is to recognize corresponding minimal units of structural variation among the study species. DNA sequences are more straightforward in that there are only four possible states – A, C, G, and T. However, the more complicated task lies in deciding how these states correspond among the multiple sequences when insertions and deletions have occurred. In other words, sorting the states into characters is the challenging part. The process of doing this is known as 'alignment' and there are various approaches to it. The resulting DNA alignment, as with a morphological character matrix, remains a hypothesis of homology, since it is a proposal of historical relationships among features.

Trees

Next, the character matrix is evaluated for fit to the different possible trees that can be constructed from the group of study taxa or 'terminals.' In all but the simplest of cases this must be performed by computer because the number of possible trees increases exponentially as the number of terminals increases. The number of unrooted trees for $n \geq 3$ terminals is

$$\frac{(2n-4)!}{(n-2)!\,2^{n-2}}$$

For just a few terminals, it is possible to evaluate all possible trees by constructing them and counting the number of steps required to accommodate all character changes on each tree. Even with continually increasing computational power, for all datasets except those with a small number of terminals the number of trees is too large to proceed with this type of exhaustive evaluation. Instead, branch-and-bound or heuristic

methods of searching tree space must be used. These normally involve creating a starting tree and then manipulating it by repositioning branches, altering character weights iteratively, or other methods of seeking to escape local optima in the landscape of possible trees.

Once a reasonably thorough search of the tree space is completed, the optimal trees – those that require the fewest character changes – are saved and interpreted. If a single tree is optimal, this is straightforward. However, it is often the case that numerous optimal trees will be recovered, especially for data matrices with many terminals. In this case, it is useful to use consensus trees to aid in interpreting the trees. The most useful of these is the strict consensus, in which only clades that are present in all of the most parsimonious trees are present. This provides the harshest assessment of the information in common among all of the trees. Adams consensus trees are useful for detecting taxa that are unstable in placement among sets of optimal trees.

Character Polarity

Analysis of a data matrix results in an optimal tree or collection of trees. These are fundamentally unrooted, in that they have no particular direction with respect to character change. In order to interpret groups of taxa on the trees in a historically meaningful way, it is necessary to provide directionality to the tree. Early on in systematic studies, decisions about character directionality or 'polarity' were often made on a character-by-character basis, based on assumptions by the investigator from presumed knowledge about how a particular character behaved in other groups, by the distribution of states in species at the presumed center of origin of a group as opposed to other areas, by which state appeared first in the geological record, or by the number of species that had one state as opposed to another ('common-is-primitive'). By the time of the codification of the parsimony method in the 1970s, the generally usable approaches had been reduced to ontogeny and the outgroup method. The ontogenetic criterion relies on the sequence of states being observable in development, and states that plesiomorphic states will appear earlier in development than apomorphic states. This approach is often described as the direct criterion in that it depends on processes that have stored information on the historical sequence in the developmental sequence. It is not infallible, however. Ontogeny is susceptible to heterochronic shifts, such that an apomorphic feature may be eliminated from the end of a sequence, making it appear to be absent, although it is not plesiomorphically so. The approach that is perhaps most widely used is one that is sometimes referred to as an indirect approach because it does not utilize a stored historical record in the way that ontogeny does. Rather, it uses other extant (or fossil) taxa to polarize the characters. The idea is that using a species or group of species that is outside of the ingroup can serve to determine the plesiomorphic state for characters in the ingroup. If there is more than one state for a character among the ingroup species, the one that is also present in the outgroup is defined as plesiomorphic. Because it uses species outside of the ingroup, it is referred to as the outgroup method. It is indirect ultimately because it is a relational method that essentially pushes the question of polarity to a higher, or more general, level. The outgroup method suffers from problems as well. The most crucial one would be if an ingroup taxon were to be chosen as the outgroup. This would direct at least part of the tree in the wrong way. Because it is relational, the method assumes that there is always another taxon that may be chosen as outgroup, but since the number of species in the tree of life is finite, at some point the question cannot be pushed to a higher level – in other words, how do we root the whole tree of life with this method? It may also be the case that for any particular character, the apomorphic state was gained independently in the outgroup and in the ingroup, which would make it appear as though the truly apomorphic state in the ingroup is plesiomorphic. For this reason, more than one outgroup is usually included in an analysis.

Tree Properties and Metrics

The tree that results from a parsimony analysis is a network of species or higher taxa that are connected in a mathematically optimal arrangement. Nodes on the tree represent hypothetical ancestors (Figure 1), which may or may not correspond to a taxon that actually existed. The assignment of character changes to nodes on the tree can be determined via a process called optimization. This process utilizes parsimony (or another approach) to propose an optimal arrangement (or arrangements if more than one) of characters at the nodes. From this pattern, the optimal placement of character transformations on the tree is determined. This reveals those characters whose homology statements have been confirmed and those that are shown to be homoplastic – indicating lack of perfect fit to the tree because of convergence or reversal. Advanced character states (apomorphies) that unite branches (clades) on the cladogram are called synapomorphies and are evidence for relationship in a cladistic framework. Apomorphies that appear only once on a tree indicate a perfect fit and require no ad hoc hypotheses to explain their distribution. Apomorphies that appear two or more times on the tree indicate that the initial hypothesis of homology – that all of the instances of that state identity are due to a single origination in a common ancestor – has been falsified because occurrences beyond one require ad hoc hypotheses (of convergence or reversal) to explain the character pattern. That is not to say that

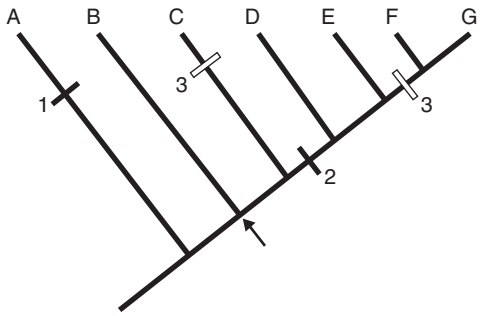

Figure 1 A cladogram (tree) showing a hypothetical ancestor (node, at arrow), an autapomorphy (character 1), a synapomorphy (character 2), and a homoplastic character (character 3).

multiple instances of a specific state change on a tree cannot all function as synapomorphies if they all serve to unite groups of species. An apomorphy that appears on a single terminal is an autapomorphy and provides no evidence for relationship, but may be of interest for study of character patterns. Under what is known as the process of reciprocal illumination, falsifications of homology can prompt the researcher to look again at the character states as they are manifest in the study collection to reassess whether they appear to be truly the same, perhaps in the light of a more detailed analysis. If they continue to be indistinguishable, then the coding should remain as it was, but if differences in the states as they appear in the different groups are judged to be significant enough, one may be justified in coding them as different states.

It is useful to describe a tree in terms of the fit of its characters. The degree of homoplasy can be assessed for individual characters by calculating the consistency index (CI; Kluge and Farris, 1969), which is

$$\mathrm{CI} = m/s$$

where s is the number of steps for that character on the tree and m is the minimum number of steps (= the number of states in that character – 1). It ranges between $0 < \mathrm{CI} \leq 1$. The ensemble consistency index for the whole dataset is just

$$\Sigma(m)/\Sigma(s)$$

This gives one measure of the fit of the data to the tree. Another such measure is the retention index (Farris, 1989), which takes into account how well characters function as synapomorphies on the tree even though they may be homoplastic. The formula for the RI is given as follows:

$$\mathrm{RI} = (g - s)/(g - m)$$

where g is the maximum number of steps possible for a character; it is calculated by assuming that each taxon that has an apomorphic state acquired it independently, such that the character is mapped as an autapomorphy for each. Like CI, RI has a range of $0 < \mathrm{RI} \leq 1$. In this case, characters that function strongly as synapomorphies (i.e., with fewer autapomorphies) will have high RI and those that do so weakly will have low values.

Character Weighting

Although parsimony analysis is most often performed with equal weighting for all characters, various ways have been developed to incorporate weighting into the analysis in order to utilize assumptions about character change. The simplest approach is to select individual characters to be upweighted or downweighted a priori if there is reason to believe that they should be emphasized or de-emphasized relative to others. Some broader weighting approaches are occasionally described as different 'types' of parsimony, but are in fact just weighting schemes. They can be depicted as transformation matrices (Figure 2) and typically are used to differentially weight particular classes of transformations. The method known as 'Dollo Parsimony' is based on the assumption that parallel acquisitions of a feature are less probable that a gain and a loss of the same feature. Hence, in Figure 2, transformations from 0 to 1 are upweighted to high cost, such that the algorithm will attempt to place the character changes in a way that the parallel changes are minimized. Another approach is known as 'Camin-Sokal Parsimony,' in which reversals are prohibited; here, infinite weights are given to reversals. The most general approach to coding via a transformation matrix is to specify transformation costs between any two states, which is sometimes known as 'generalized parsimony.'

When a character is coded as having multiple mutually exclusive states, a choice can be made between organizing the states into a particular transformation series (additive/ordered coding) or allowing the transformation between any two states to be of equal cost (unordered/ nonadditive coding), in which case it is the remainder of the characters that will determine the series for that character. The nonadditive option should be used unless there is a clear justification for ordering of the states. Note that there is a difference between having an ordered character and specifying its polarity. In most cases, what we are really specifying with an ordered character is character state 'adjacency,' which accounts for the cost of transformation between particular states rather than direction, since polarity of the character will usually be determined via the outgroup or ontogenetic method.

Other methods of weighting that act on the fit of characters to the tree have been developed. The first of these successive weighting (Farris, 1969) is an iterative procedure that downweights characters based on the level of their homoplasy, the idea being that good characters are those that are most consistent with the tree. The analysis is run multiple times, each time downweighting the characters proportionally to their homoplasy, until no further changes in the tree occur. An alternative approach is known as implied weighting (Goloboff, 1993). In this method, characters are weighted according to their fit to the tree and no iterations are performed. The best tree under this procedure is the one with best fit to the weighted characters. Fit is determined by a weighting function that may be adjusted by specifying the magnitude of a weighting constant, k. Increasing k penalizes characters less as their homoplasy increases (i.e., most of the penalty is in the

	0	1	2
0	–	1	1
1	1	–	1
2	1	1	–

Non-additive

	0	1	2
0	–	1	2
1	1	–	1
2	2	1	–

Additive

	0	1	2
0	–	N	N
1	1	–	N
2	2	1	–

Dollo

	0	1	2
0	–	1	2
1	∞	–	1
2	∞	∞	–

Camin-Sokal

	0	1	2
0	–	1	2
1	2	–	1
2	5	1	–

Generalized

Figure 2 Character transformation matrices. In each case the character transforms from the state on the left to the state on the top. The number of steps for each transformation is indicated. 'N' under Dollo refers to an arbitrary large number.

first few additional steps in a character). There is no non-arbitrary way of choosing k and exploration of a range of k values will indicate how sensitive the analysis is to its value.

Uses of Parsimony Trees

A cladogram can be used as the basis for a classification, as well as for studying character change and diversification, biogeography, and coevolution. Although different approaches to classification may use the pattern differently, a key concept is monophyly. A monophyletic group comprises a common ancestor (a hypothetical ancestor on a cladogram) and all of its descendants. Most taxonomists now recognize only monophyletic groups of species as real groups that are eligible for naming. Paraphyletic assemblages are defined based on shared plesiomorphies. Although they may be useful in everyday language (e.g., reptiles, invertebrates), they do not designate groups that have the same historical status as monophyletic groups.

See also: Consensus Methods, Phylogenetic. Distance-Based Phylogenetic Inference. Phylogenetic Invariants. Rooting Trees, Methods for. Schools of Classification. Searching Tree Space, Methods for

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Pathogen Epidemiology

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Glossary

Basic reproductive number Often written R_0, this is the average number of onward transmission events resulting from the introduction of a single index case into a completely susceptible population.

Coalescent The Coalescent is a retrospective population genetic model that attempts to trace all alleles of a gene in a sample population to their most recent common ancestor (MRCA). This produces a gene genealogy. Coalescent theory seeks to understand the statistical properties of this genealogy under different selective or demographic scenarios.

Incidence New cases of disease occurring within a specified time period.

Malthusian fitness Where the population is growing in size, individuals that reproduce more quickly gain an advantage and will come to predominate even if their total net number of onward infections is less than more slowly growing competitors.

Phylodynamics The use of phylogenetic data to infer elements of epidemiological processes that have given rise to it, by examining the shape and topology of the genealogy as estimated from sequence data (see 'The Coalescent').

Prevalence The frequency of a disease in a population at a particular time point. Often expressed as a proportion or percentage.

Reproductive number Also known as the reproductive ratio or rate, this is the average number of onward transmission events produced from each infection.

SIR model In an SIR model hosts move from the susceptible compartment (S), to the Infected (I) and then (if they survive infection) into a separate resistant (R) compartment. This allows us to model immunity, by allowing hosts in the R compartment to be less likely to be successfully infected. An extra level of complexity can allow immunity to wane over time such that eventually hosts in the R compartment return to the S one – resulting in an SIRS model.

SIS model In an SIS model, hosts initially susceptible (S) become infected (I) and upon clearing the infection immediately becomes susceptible once more (S). Such simple models may be used where little or no immunity results from infection, or may be a useful approximation in the case that antigenic variation on the part of the pathogen is so rapid that immunity is negligible.

Superspreader An individual who infects a disproportionately large number of secondary hosts relative to the majority of cases of infection.

Zoonoses Any case where transmission of infectious agents occurs between different species of animals, most often used when disease is transmitted from nonhumans to humans.

Modern infectious disease epidemiology contains two distinct intellectual lineages: mathematical models are used to explore disease dynamics and the consequences of interventions (Anderson and May, 1991; Grassly and Fraser, 2008), while analysis of the genetic variation in pathogen populations is used to 'type' and define strains associated with resistance, virulence, or other features of interest. Recently these have been starting to come together with population genetic analyses of genetic (and increasingly genomic) variation that can tell us about the history of the sampled sequences. This has been termed 'phylodynamics' as a result of the combination of phylogenetic methods with the study of disease dynamics (Grenfell *et al.*, 2004).

Epidemiology is a population science that studies the patterns of disease incidence, attempting to infer causes and consequences. Classical epidemiology, for instance, might seek to identify risk factors for a given condition, which might be environmental or genetic. This allows us to identify interventions to minimize the risk of disease. In the case of a transmissible disease, the situation is somewhat different. The spread of infectious disease is a dynamic process in which the increasing numbers of cases increase the risk to the rest of the population. Similarly, as people recover they may become immune (or more pessimistically they may succumb to disease – it makes little difference to the infecting organism) and be removed from the system. Hence the numbers of hosts available to be infected change over time. To describe the changing state of the population, infectious disease epidemiology makes use of mathematical models comprising sets of differential equations, or statistical models that are defined using a probabilistic framework. These models can then be used to explore the impact of vaccination or other interventions.

The Reproductive Number

A crucial parameter in infectious disease epidemiology is R: how many successful transmission events and new infections result on average from one infection. With an unlimited pool of susceptible individuals, this is equivalent to R_0 or the basic reproductive number. It is easy to intuitively relate these numbers to the course of an outbreak. If the reproductive number is >1, the expected number of new cases will increase, whereas if it is <1, then the numbers will fall. It is important to distinguish the incidence from the prevalence. Incidence is the number of new cases per unit time, whereas prevalence is the overall frequency of the disease in the population. The incidence can be falling but the prevalence can continue to

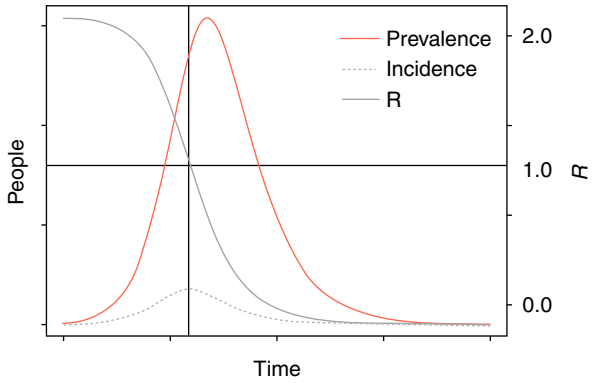

Figure 1 An idealized epidemic curve showing how the prevalence, incidence, and reproductive number R vary over the course of an outbreak. The illustrated case is the result of an SIR model, in which recovered hosts become entirely resistant to infection and as a result the prevalence returns to zero. The point at which the decline in the availability of susceptible hosts means each case causes on average just one onward infection is that where $R=1$, and is indicated. Note that this is coincident with the peak incidence, which precedes peak prevalence as described in the text.

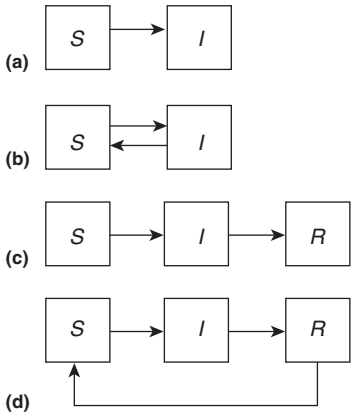

Figure 2 Four simple compartmental model structures referred to in the text: (a) shows an SI model in which hosts become infected and never recover; (b) shows an SIS model in which recovery is possible as shown by the additional arrow; (c) shows an SIR model in which a recovered population is resistant to infection; and (d) a SIRS model incorporating waning immunity. The rates with which hosts transition between compartments can then be described using differential equations.

rise, albeit at a slower rate. This is illustrated in Figure 1, which shows an idealized epidemic curve, and how R changes over the course of the outbreak. While in the illustrated case the epidemic burns out after the pathogen has run out of hosts to infect, if sufficient susceptible hosts are continually introduced (e.g., by birth or waning immunity) the disease can become endemic.

Although the expected final size of the outbreak falls rapidly as R_0 decreases, it is possible for outbreaks to occur in the case where R_0 is less than 1. To understand how this can happen we have to remember that R_0 is an average value, and by chance initial cases may result in an above-average number of onward infections. The effects of this can be probed using a stochastic approach, in which events are modeled as randomly sampled realizations from a probability distribution. While such models aim to capture the typical contact and transmission dynamics in a population, they can be confounded by rare cases of extreme behavior. For instance, a so-called 'superspreader' event may by chance infect many new hosts, starting an outbreak that persists until all the transmission chains descending from it die out (Lloyd-Smith *et al.*, 2005; Garske and Rhodes, 2008). For a real example of such a superspreader event, consider the early stages in the 2003 SARS (severe acute respiratory syndrome) outbreak in Hong Kong, when a single infected person infected at least 13 and possibly as many as 20 others staying at the same hotel (Braden *et al.*, 2013). The estimated R_0 for SARS is much lower than this unusual event would lead you to suspect (Riley *et al.*, 2003).

We can construct a simple transmission model by dividing the population into compartments, and then writing down equations for the rate of movement of individuals from one compartment to another in each time step. The simplest models contain just two compartments: susceptible and infected (Figure 2(a) and 2(b)). The results are a so-called SI or SIS model. In the first example, hosts move from being susceptible to being infected, and in the second, to infected hosts can clear the infection and become susceptible again. The slightly more complicated SIR model adds a 'resistant' compartment (Figure 2(c)), reflecting immunity that prevents infection while waning immunity can be incorporated in a SIRS framework where after a period of time hosts in the resistant compartment move back to S (Figure 2(d)). This flexible approach can be extended to include important features such as vaccination (McLean, 1995) (in the simplest case of a 100% effective vaccine, each vaccinated susceptible would move to the resistant compartment), and host population structure. This is important in multiple contexts; for instance the child–child contact rate is very high in daycare settings and hence the transmission rate too, which can have important consequences for model results (Schenzle, 1984). Similarly, humans vary greatly in the number and sort of sexual contacts they make, which is important to modeling sexually transmitted infections. In the case of vector-borne diseases, whether the vector is a biting insect or a health care worker (as in nosocomial infections), they can and should be incorporated into the model. It is easy to see intuitively how the endemic prevalence of disease depends on the supply of new susceptibles, either from birth or waning immunity.

Transmission Routes and the Target of Selection

Infectious agents can be categorized by how they get from one host to another, and whether this involves any intermediate hosts or environments. Some pathogens spread without spending significant time in the environment, examples being influenza or sexually transmitted infections. Vector-borne diseases in contrast rely on an additional host for transmission, often a biting insect. A considerable burden of disease also results from pathogens that transmit between humans rarely if at all. These infections arise from environmental exposure to the pathogen, and can include zoonoses – infections acquired from other species. In each case it is useful to consider which traits of the pathogen will be scrutinized by selection.

In the case of directly transmitted pathogens it is of paramount importance to colonize new hosts; in other words, maximize the reproductive number. Note that this does not necessarily mean causing disease, merely transmission, although some features of disease such as sneezing may be adaptive traits. As immunity builds in the population the effective reproductive number will fall, and one possible means of increasing it is immune escape, and there is typically evidence of strong diversifying selection on those antigens that can yield protective immunity (Li *et al.*, 1995). Vector-borne pathogens must successfully survive in and transmit between the host and the vector species, and *vice versa*. Again, we expect and observe strong diversifying selection on those antigens targeted by the immune system.

One area of particular interest is the evolution of virulence. It is commonly stated that virulence reflects an adaptive mismatch between the host and the pathogen: it is to the advantage of both parties that virulence be minimal, such that the host can continue to transmit for a long period of time without limits on the numbers of contacts they make. Empirical evidence for this comes from the example of myxomatosis in Australia, where the myxoma virus was introduced to control rabbit populations. With extraordinary vision, Frank Fenner of the Australia National University collected and stored isolates of virus collected from wild rabbits over decades, and showed evidence for both gradual attenuation of the virus, and adaptation of the rabbit population to become more resistant (Fenner, 1956).

While this argument sounds persuasive, it is also regarded with suspicion as an example of group selection. Surely more rapidly growing parasite variants should be selected during infection and, if this is linked to virulence, produce disease (Levin and Bull, 1994)? The tension between the two selective pressures – for growth within the host, and for transmission within the host population – is dependent on the link between virulence and transmission (Lipsitch and Moxon, 1997; Vale *et al.*, 2011). If by causing disease, a pathogen transmits to more new hosts than it would do otherwise, then virulence will be increased even if the result is the death of the host or the clearance of the infection (Anderson and May, 1982). This explains virulence in terms of the theory of life-history tradeoffs. A crucial factor is the availability of new hosts, because if opportunities for transmission are rare it will select for variants that do not kill their hosts before they can transmit (Lipsitch and Nowak, 1995; Lenski and May, 1994). In an outbreak situation, virulence can be temporarily selected even if the virulent strain infects fewer hosts per infection than its competitors, provided it does so more quickly. Selection for more rapidly growing variants is also termed Malthusian fitness (Orr, 2009).

The question of whether virulence is adaptive is not of merely academic interest. HIV-1 infection includes a long asymptomatic period in which the viral load (the amount of virus particles in peripheral blood) fluctuates around a value known as the 'set point' (Mellors *et al.*, 1996). It has been proposed that the set point viral load has been the object of selection to maximize transmission, the result of a trade off between the effect of set point viral load on infectiousness and the duration of the asymptomatic period (Fraser *et al.*, 2007). If set point viral load is too high, then infected individuals have fewer opportunities to transmit before developing AIDS whereas if it is too low, they are not infectious enough to efficiently transmit the virus. For natural selection to act on set point viral load, it must be a heritable property. That is to say, the set point viral load of the infector and infected must be correlated. If it is not, then there is no heritable variation on which natural selection can work. Subsequent work has produced evidence that set point viral load is indeed heritable, and correlated with viral genotype (Hollingsworth *et al.*, 2010; Alizon *et al.*, 2010).

Virulence has also been suggested to be adaptive in malaria transmission. Here, the greater the numbers of parasites in peripheral blood (higher parasitemia), the greater the chance that a mosquito becomes infected while feeding. Parasitemia is also correlated with severity of disease. If disease can limit onward transmission, 'imperfect' or partially effective vaccines have been proposed to select for strains capable of growing to a higher titer in both hosts receiving the therapy and those who are not (Gandon *et al.*, 2001; Barclay *et al.*, 2012). This could lead to an increase in virulence in the absence of vaccination – and given known inequities in access to health care, such a perverse outcome could have a major impact on some patient populations. However, unpicking which factors contribute to virulence is extremely difficult and the goal of virulence management remains an aspiration rather than an actuality (Dieckmann and International Institute for Applied Systems Analysis, 2005).

Molecular Epidemiology: The Short and Long Term

It is often important to be able to link individual cases of disease on the basis of the similarity of the infecting pathogen, such as in defining an outbreak or identifying drug resistant lineages. These are examples respectively of short-term and long-term epidemiology. To address such questions, the variation in the pathogen population must be assayed in some way, and the results compared. This is now almost always done using molecular variation, i.e., nucleic acid sequences and the proteins they encode. How much variation is present in the population, and of what sort, is the result of evolution, and how data from molecular epidemiology studies are interpreted is a question of phylogenetics. Increasingly, populations of viruses and bacteria are characterized using genomic methods, and it is likely that in the near future whole genomes will become the standard for molecular epidemiology (Croucher *et al.*, 2013).

Molecular epidemiology catalogs the variation in the pathogen population as different 'types' that can be distinguished. Historically these have often been defined using antibodies to distinguish between different variants of cell surface markers and divide the population into serotypes. Pathogens of all kinds have been distinguished using this approach: viruses, bacteria, and protozoa. When we speak of a case of influenza caused by 'H5N1,' we are referring to the serotypes of the surface proteins hemagglutinin and neuraminidase, which in this case are those associated with 'avian flu.' In *Escherichia coli* and *Salmonella*, the 'O' antigens (oligosaccharides) are combined with the 'H' antigens (flagellar proteins) to provide a discriminating serotyping scheme.

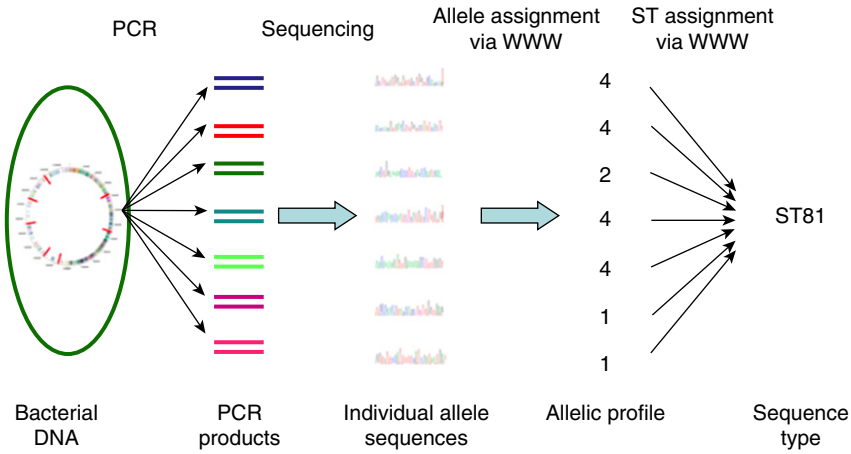

Figure 3 Illustration of the Multi-Locus Sequence Typing approach. The sequences of multiple housekeeping loci are determined, and then allele numbers assigned via an internet database, which together make up the allelic profile. This in turn determines the Sequence Type or ST, also assigned by interrogating an internet database. The example shown is for ST 81 in the *Streptococcus pneumoniae* typing scheme. While the illustration shows sequences determined by Sanger sequencing, increasingly data from genomic approaches are accepted.

Salmonella enterica alone can be divided into more than 2500 serovars (Grimont and Weill, 2007). The O:1 and O:139 serotypes of *Vibrio cholerae* produce a phage-borne toxin, cholera toxin, which causes the disease that bears its name (Finkelstein, 1996). More than 200 serotypes of *V. cholerae* are known that do not produce cholera toxin or cause cholera (Shimada *et al.*, 1994).

Serotyping and other typing methods that assay phenotypes suffer from limited discrimination: they use a tiny fraction of the diversity associated with a strain, and the variation they assay is frequently produced by intense diversifying selection from the immune system. The ideal data for typing are unambiguous and easily portable between labs. We also want to be able to describe how the types we identify are related. We can group serologically related strains as serovars or serogroups, but the relationships within and between these cannot be ascertained in detail. A single serological type can contain many different genotypes, and any horizontal transfer of the locus determining serotype can lead to distantly related lineages being indistinguishable by this method.

Outbreak analysis is a short-term question requiring highly discriminating methods. The response is to assay multiple, rapidly changing regions in the entire genome. Examples of such regions are restriction sites, sites at which PCR primers can bind, or regions of repeat sequences, where the numbers of repeats can change rapidly. Pulsed Field Gel Electrophoresis (PFGE), which uses restriction sites, is an excellent example. Genotypes are distinguished by banding patterns on a gel resulting from changes in the position and numbers of restriction sites. PFGE remains a commonly used method in this context (e.g., Choi *et al.*, 2014).

PFGE and related methods are less useful for evolutionary or population genetics. The selective impacts of the changes are unknown, because we do not know where the restriction sites lie in the genome. While we can identify closely related banding patterns, beyond very closely related strains relationships become hard or impossible to discern. Practically it is difficult to compare results between laboratories. As sequencing has become easier and more accessible, it has become standard to use nucleic acids as the source of assayed variation. In bacteria, a popular approach is to sequence multiple loci scattered around the genome that encode core metabolic or 'housekeeping' functions. This allows the analysis of synonymous SNPs that are unlikely subjects for diversifying selection. The approach, first applied to *Neisseria meningitidis*, is termed multi-locus sequence typing (MLST) (Maiden *et al.*, 1998), and is illustrated in **Figure 3**.

Unlike bands on gels, the sequence data used by MLST are unambiguous. This means that the effort of collecting data on the allelic variants found in the community can be distributed to researchers worldwide. Individual labs in remote locations can determine the sequence of the loci used in the MLST scheme, and then compare them with an online database (mlst.net and pubmlst.net). If the allele is novel, it may be added to the database for future users. Each isolate is defined by the combination of alleles at the MLST loci. Each unique allele is identified by an integer, and the combination of these makes up the allelic profile and the sequence type (ST). MLST has been applied to numerous organisms. One of the side effects of the data collected by epidemiologists has been in the study of homologous recombination (reviewed in this volume by Feil). The wealth of discriminating data collected for MLST has shown that in many named species, recombination is a more frequent source of change at the MLST loci than mutation.

Genomic Epidemiology

The genomic revolution promises to have a profound effect on molecular epidemiology. While in the past, whole genome analyses were the preserve of virologists, it is now economical and, more importantly, easy to obtain quality data on a far higher proportion of the bacterial (or protozoal) genome than afforded by previous methods. Genomic methods have been applied to outbreaks of diseases including tuberculosis (Gardy *et al.*, 2011; Walker *et al.*, 2013), *E. coli* infections (Grad *et al.*, 2012; Mellmann *et al.*, 2011) and cholera (Katz *et al.*, 2013;

Eppinger *et al.*, 2014), and are becoming folded into the routine epidemiologic work of public health authorities.

The pace of change in this field is such that any detailed discussion of technology will be shortly superseded. Nevertheless, important principles can be defined. At present multiple technologies for sequencing exist, and moreover, multiple approaches for putting raw data together to make a genome. The great majority of studies are more properly called 'genomic' rather than 'whole genome' methods, because in hardly any cases have genomes been completely sequenced. Instead, a very high proportion of the genome is determined. While high-quality draft genomes can be used for many interesting things, they are not finished: we do not know the sequence of the chromosome all the way round from the origin of replication.

Which parts of the genome get missed? This depends on the sequencing platform and the methods of assembly, but typically highly unstable regions such as repeats are problematic. These are of course the regions that are most useful for short-term questions in epidemiology. In their absence, we can look at single nucleotide polymorphisms elsewhere in the genome. In comparing very closely related isolates that may differ at a handful of SNPs, the possibility of false positives becomes acute, so we must deal with the fact that different technologies and analytic approaches have different error rates (Croucher *et al.*, 2013).

Taken together, the profusion of genomic methods means that rather than removing ambiguity in the comparison of closely related isolates, new sources of ambiguity have been discovered. At the level of resolving more distantly related isolates into major lineages, equivalent to MLST, genomics has been highly successful and revealed both considerable variation within closely related STs, and perhaps surprisingly shown that MLST in most cases effectively identified the major lineages. The potential of methods that can sequence through unstable repeat regions with high fidelity is real, but has not been conclusively shown at the time of writing. This is likely to change, but for the benefit to be felt the technology will have to be cheap enough for many labs to use.

Phylodynamics and Using DNA Sequence to Study Transmission

The most exciting recent development at the interface of evolutionary biology and epidemiology has come about from the proliferation of sequence data combined with methods capable of making inferences about the history of a sample of sequences from the structure of the genealogy underlying them. A key concept is the coalescent (Kingman, 1982), which describes the genealogy of a sample of sequences in terms of how often their lineages 'coalesce' or come together to form an internal node in the tree. Combined with a molecular clock that relates the accumulation of sequence divergence to time, this allows us to infer events that have happened in the history of the sequences, most notably and relevant for epidemiology, changes in population size. The basic rationale is readily grasped, and accessible software is available to implement the methods (Drummond *et al.*, 2012; Bouckaert *et al.*, 2014).

As stated above we describe the structure of a genealogy by looking at how often the lineages coalesce, relative to the branch length, which in the case of a molecular clock is a proxy for time. The most valuable data are sequences sampled over time, providing 'measurably evolving populations' that can be used to estimate the clock rate (Ewing *et al.*, 2004). An alternative approach is to model transmission as a birth death process, as discussed in Boskova *et al.* (2014).

The rate of coalescence over the tree is simply related to population size by reflecting that in a smaller population individuals are more likely to share a parent in the previous generation by chance. In fact the probability two individuals share the same parent in the previous generation is the reciprocal of population size. As a result the rate of coalescence is higher in smaller populations. This allows inference as to whether the population is expanding, has experienced a bottleneck, or any of multiple other demographic and other processes.

The ability to study changing population size from sequence alone can be used, with an estimate of the serial interval or time between infection and transmission, to estimate the reproductive number. Analysis of sequence variation has been used to study pathogens including Influenza (Hedge *et al.*, 2013), *V. cholerae* (Katz *et al.*, 2013), MERS (Cauchemez *et al.*, 2014) and the recent Ebola outbreak (Gire *et al.*, 2014). This work has shown the potential of the approach. While these analyses are readily approached using the BEAST suite of programs (Bouckaert *et al.*, 2014; Drummond *et al.*, 2012), as usual it should not be assumed that default parameters are appropriate.

Using sequence data to infer demographic history is distinct from using it to infer recent transmission. At the extremely local level it is hoped that we might be able to define infector and infected using high-resolution sequence data alone. Indeed, highly resolving methods that stop short of the whole genome have been used as a valuable complement to classical contact tracing for the control of gonococcal disease (Bilek *et al.*, 2007). At a higher level we might ask whether cases of disease occurring some distance apart are due to closely related pathogens, which might imply transmission over greater distances – for an example, involving wind-borne transmission of avian influenza see Ypma *et al.* (2013b). The overall principle is that the transmission tree is considered closely related to the phylogenetic tree (Ypma *et al.*, 2013c). To be useful this need not be the precise resolution of who infected whom; different epidemiological events like superspreading (Ypma *et al.*, 2013a) may leave distinct signatures in the resulting tree structure (Colijn and Gardy, 2014). Software packages are becoming available that implement multiple methods to probe sequence data to provide the detailed history of an outbreak (Jombart *et al.*, 2014a,b). However, concerns exist over the potential for within-host evolution to produce sequence diversity that can obscure true transmission networks (Didelot *et al.*, 2014; Worby *et al.*, 2014). The amount of diversification that occurs within the host is not known in most cases, but may be large (Nasser *et al.*, 2014). Coupled with the uncertainty arising from imperfect sampling and other missing data, it is likely that the most valuable uses of sequence data will be as a complement to traditional epidemiologic approaches (Ypma *et al.*, 2012).

Conclusion

Infectious disease epidemiology is a practical science, concerned with minimizing the impact of pathogens on public health. As both pathogens and their hosts have evolved, evolutionary biology is relevant to understanding the nature of their interactions for fitness, and also in resolving the history of pathogen transmission. Mathematical models can explore the consequences of different selective scenarios, and molecular data can define strains and the genetic variation that is the raw material on which natural selection acts. Recent advances, especially in the rapid determination of sequence data, are bringing evolutionary biology ever closer to the clinic.

See also: Evolutionary Medicine IV. Evolution and Emergence of Novel Pathogens. Recombination in Bacterial Populations

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Pest Management, Evolution and

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Glossary

Biological control The use of other organisms to control a pest species is called biological control.

Mutagenic chain reaction (MCR) A technique that uses CRISPR/cas9 genome-editing technology to convert heterozygotes into homozygotes in both the soma and the germline.

Niche explosion A hypothesis to account for some highly abundant and highly polyphagous species. It posits a positive feedback loop between niche breadth and population size. Many putative niche explosion species are pests.

Pest species Organisms that threaten harm to human health, agriculture, the economy, or ecosystems.

Selfish genetic elements (SGE) Stretches of DNA that enhance their own transmission at the expense of the fitness of the organism.

Overview

Pest management is the applied science that attempts to suppress pest species and/or mitigate the damage they cause. Pests are generally defined as organisms that threaten harm to human health, agriculture, the economy, or ecosystems. In this entry, pests will be limited to animal pests, though plants can often be pests, which are usually called weeds.

Pests are often associated with invasive species, but there is a difference between the two categories. By definition, invasive species are non-natives, but native species can become pests. Still, considerable overlap exists between pest management and the control of invasive species.

Retarding Resistance

Pest management, especially for that of insect pests, often involves the use of chemical pesticides. Although pesticides have been very successful in treating some pests, they have been less so with others. One reason that pests are difficult to contain via insecticides is that the pests evolve resistance to the insecticide (Georghiou, 1972; Mallet and Porter, 1992).

The problem of insects evolving resistance is not new; and principles from evolutionary biology have long been applied to address and possibly retard the evolution of resistance. Writing in 1972, the entomologist George Georghiou (1972, p. 158) concluded 'The accumulated evidence from intensive world-wide chemical control of insects during the past quarter of a century indicates that resistance to the selecting insecticide almost always develops.' As Georghiou (1972) summarized, early work in the evolution of resistance found the resistance is more likely to evolve and will evolve more rapidly if the selection is very intense, provided the population has sufficient genetic variation. Although the evolution of resistance to insecticides is nearly universal, the rate of evolutionary response varies depending on the type of insecticide used, presumably due to different responses by the insect pest. Georghiou (1972) recommended very intense selection is the best way to create a strain of insect highly resistant to the chemical dieldrin because resistance is due primarily to a single genetic factor. So, moderate chronic exposure is less likely to be conducive for evolving resistance, and thus, would be more useful for management. In contrast, resistance to DDT and carbamates is multifactorial and often involves recessive alleles. For these insecticides, resistance is most likely to evolve under a moderate chronic exposure (Georghiou, 1972).

Limiting the evolution of resistance is particularly useful when pesticides have been engineered by genetic modification into transgenic crops. Herein, the crops contain the toxins that kill pests. Various strategies to decrease the rate of evolution of resistance are used including the use of mixtures wherein plants with the toxin are planted with plants without the toxin, and rotations wherein the toxins are used at different times and places (Hoy, 1998). One mode of rotation is the use of refugia, regions where there are no toxins for the pests to adapt to. These methods all attempt to limit the evolution of resistance by reducing the amount of selection.

Mallet and Porter (1992) modeled the evolution of resistance under a variety of the conditions assuming a two-stage insect life cycle, similar to what Colorado potato beetles have, and found that refugia always performed better than mixtures in retarding the evolution of resistance. Indeed, if insects can readily move from one plant to another, then in some cases, the use of mixtures can paradoxically lead to faster rates of evolution of resistance than the absence of mixtures. These conditions include very intense selection, low (but non zero) dominance of the resistance allele, and relatively high rates of movement (Mallet and Porter, 1992).

Biological Control

Biological control uses other organisms to control the population of the pest species. Typically, the natural enemies (predators, parasites, pathogens) are used in this effort, though the use of a competitor is also feasible (Roderick and Navajas, 2003).

Evolution is central to biological control as both the target pests and their natural enemies are evolving. Indeed, evolution is both an advantage and a disadvantage of biological control. Because the natural enemy is evolving along with the pest,

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it should be able to counter the evolution of resistance by the pest. Evolution of the control agent, however, leads to the prospect of unintended consequences.

The classic case of biological control is the use of the *Myxoma* virus to control rabbits in Australia (Futuyma, 1998; Roderick and Navajas, 2003). Introduced to Australia in the middle of the nineteenth century, rabbits soon became a serious pest on that continent. In 1950, the *Myxoma* virus was introduced, and it soon greatly reduced the population size of the rabbits. The surviving rabbits evolved resistance, but the virus evolved counter-measures. However, the virus also evolved to become somewhat less virulent. This case shows the reciprocal interplay between evolutionary biology and applied pest control. Evolutionary principles were used in the control of the rabbits but the co-evolution of the virus and rabbits has also stimulated much work in the evolution of resistance (Futuyma, 1998; Roderick and Navajas, 2003). Another case of the use of pathogens for biological control is the fungus *Entomophaga maimaiga* that has been used to control gypsy moths in the northeastern United States (Hajek *et al.*, 1996; Roderick and Navajas, 2003).

Evolutionary change of microorganisms such as viruses and bacteria used in pest control is very well documented. The extent to which agents of biological control that are macroorganisms (arthropods, for example) evolve during the control is not clear (Roderick and Navajas, 2003).

Population genetic techniques that can identify sudden and severe population crashes are particularly useful because they can be used to assess the effectiveness of eradication programs, especially when direct censuses are difficult or impossible to obtain (Rollins *et al.*, 2006). Roderick and Navajas (2003) also note that traditional population genetic approaches that are used to assess dispersal and gene flow in the control agents may be flawed. These traditional methods assume that the population is at migration-drift equilibrium, which is unlikely for recently colonized populations. Additional non-equilibrium methods will be required in these cases (Roderick and Navajas, 2003).

Wolbachia

One agent that is promising for biological control is *Wolbachia*, a bacterial endosymbiont. It has captured the attention of many evolutionary biologists because it is extremely common (found in more than half of arthropod species) and because it has a wide array of effects on its hosts (Werren *et al.*, 2008). These properties make this bacterium a promising vehicle for insect pest control.

Wolbachia reproductive manipulation effects vary from strain to strain (and host species), and fall into the general categories of cytoplasmic incompatibility, parthenogenesis-inducing, feminization, and male killing (see Werren *et al.*, 2008, for a review). Cytoplasmic incompatibility appears to be the most common effect. Herein, *Wolbachia* modifies the sperm of infected males. In crosses between males from infected strains and females that are either uninflected or have *Wolbachia* from a different and incompatible strain, the modification that came from the sperm leads to embryonic mortality. The eggs from females of the same strain (or other compatible strains) have also been modified so as to rescue the toxic effect of the modified sperm. Thus, viability is unaffected in offspring between crosses of males and females from the same *Wolbachia* strain or compatible strains (Werren *et al.*, 2008).

Wolbachia has been suggested as a possible control agent for pest species, particularly for insects. Pilot studies have found that in some cases, the bacterium could help control pest species by increasing the extent of cytoplasmic incompatibility, reducing egg hatch rates and thus population sizes. Xi *et al.* (2005) have provided proof of principle of this application, showing it is feasible in laboratory populations of the mosquito disease vector, *Aedes aegypti*.

A particular strain of *Wolbachia* may be useful in combating dengue fever through the control of its pest vector *A. aegypti* (Schraiber *et al.*, 2012). Dengue fever is a viral disease that affects millions of people each year, and whose prevalence is increasing both with respect to numbers of new cases and geographic regions affected (Kyle and Harris, 2008). The disease has interesting dynamics in infection that in humans it requires two weeks or more of incubation of the virus in the mosquito (Schraiber *et al.*, 2012). For this reason, shortening the lifespan of the mosquito should reduce the transmission rate of the virus, potentially mitigating or even eradicating dengue epidemics. Fortunately, there is a strain *Wolbachia* that reduces lifespan in flies. This strain has been engineered into *A. aegypti* and reduces its lifespan in laboratory populations (McMeniman *et al.*, 2009).

The question is whether this longevity-reducing *Wolbachia* could spread in real mosquito populations. Modeling approaches to this question are complicated by the need to consider the effects of cytoplasmic incompatibility, the fitness effects from longevity reduction, and spatial dynamics of the spread. A recent modeling effort from Schraiber *et al.* (2012) is rather discouraging. They (Schraiber *et al.*, 2012, p. 31) conclude, 'Our results paint a somewhat grim picture: model predictions based on laboratory data suggest that lifespan-shortening *Wolbachia* either may not spread at all, or would take over a century to spread through a large city.'

Selfish Genetic Elements

In recent decades, the use of agents for biological control has expanded beyond whole organisms. Biologists are now using genetic elements within the pest organism for its control.

Selfish genetic elements (SGE), which are stretches of DNA that enhance their own transmission at the expense of the fitness of the organism, have received increasing attention by evolutionary biologists in recent decades (Burt and Trivers, 2006). These elements may have practical applications in managing pest species.

Several different kinds of SGE exist and some of these have been proposed or even are being used to control pest species (Burt, 2003, 2014; Sinkins and Gould, 2006). The use of different types of SGE will be considered below, but there are some commonalities. Typically, elements with desired properties are engineered into the pest species in laboratories, and then these SGE-carrying pests are introduced into the wild populations, typically with the aim of reducing population

size. In some respects, these SGE techniques are extensions of the traditional sterile male technique, which involved releasing large numbers of sterile males in order to reduce the population sizes of pests and which led to the elimination of the cattle-infesting screwworm fly in 1966 (Gould, 2008).

Burt (2014) makes the distinction between self-limiting and self-sustaining introductions. Self-limiting introductions are those where the element-containing pests are not expected to sustain themselves in the population. Such introductions have the disadvantage of usually requiring multiple treatments but these are less likely to have negative consequences. In contrast, self-sustaining introductions are designed to spread through the target population and reach high and sustained frequencies in those populations (Burt, 2014).

Population genetic models are typically used to address various questions about the dynamics of introductions of SGE into wild populations, especially for self-sustaining introductions. These questions include: Will the elements increase in frequency when rare, and under what conditions? Another question is; Will the elements reach internal (not zero, not one) equilibrium? Under some conditions, a minimal frequency is needed for element to spread to higher frequency. If this minimal frequency is sufficiency high, a large number of pest individuals with engineered element need to be released.

The elements that have been proposed to for pest control applications include meiotic drive elements, MEDEA, transposable elements, and homing elements. Very recently, genomic editing techniques have been used to develop the mutagenic chain reaction.

MEDEA

First discovered in *Tribolium* flour beetles, the MEDEA (maternal-effect dominant embryonic arrest) selfish genetic element has an unusual phenotype, causing lethality at the embryonic stage in individuals who lack the element but whose mother had the element (Beeman *et al.*, 1992; Wade and Beeman, 1994). Synthetic MEDEA elements have been created, and this bodes well for their use as a control agent (Chen *et al.*, 2007).

Because it kills off individuals without the element, it can spread through populations (Wade and Beeman, 1994; Ward *et al.*, 2011). In fact, if there is no fitness cost to MEDEA, it will spread to fixation, though rate of extinction of the non-MEDEA element can be very slow. If there is a fitness cost, an internal equilibrium is possible, depending on the extent of the cost and its dominance. Moreover, within family (soft) selection can enhance the spread of the element (Wade and Beeman, 1994; Ward *et al.*, 2011). Ward *et al.* (2011) note that the initial rate of increase of MEDEA is very slow unless the initial frequencies of the element are rather high. In most cases, to drive MEDEA to near fixation in 10–20 generations requires introducing large numbers of MEDEA-carrying insects.

Homing Elements

One class of SGE that have attracted interest as a means of pest management is Homing elements, which are also known as endonuclease genes (HEGs) (Burt and Trivers, 2006; Sinkins and Gould, 2006). These encode endonucleases that cut the copy of chromosomes that lacks the HEG. They can do this because the endonucleases recognize specific sequences flanking the HEG, but do not cut when the flanking sequence is interrupted by the HEG itself (Sinkins and Gould, 2006).

The Mutagenic Chain Reaction

The Mutagenic Chain Reaction (MCR) is a powerful technique that uses CRISPR/cas9 genome-editing technology to convert heterozygotes into homozygotes in both the soma and the germline (Gantz and Bier, 2015). As a consequence, allele frequency can rapidly increase toward fixation in populations, and gene replacement at the population level is made practical for a wide range of species (Gantz and Bier, 2015; Unckless *et al.*, 2015).

At least under laboratory conditions, the MCR is very powerful. In fact, it is so powerful, that there are concerns about accidental exposures and other consequences. Unckless *et al.* (2015) modeled the population genetic consequences of the MCR technique. They discuss how the MCR system can be designed so that it should reach fixation if above a threshold frequency, but go extinct if accidentally released at a low frequency.

The Evolution of Generalist Pests and the Niche Explosion

Evolutionary biology principles and studies can also address why some species become pests. Many pests have become pests due to changes in the environment, often due to human activity. Some species, however, may be evolutionary disposed to becoming pests. In addition to having very large population sizes, some pest species such as some species of scale insects and bagworm moths have extraordinarily broad niches and are able to feed on an extraordinary diversity of host plants (such as species from dozens of different plant families). Normark and Johnson (2011) noted several characteristics associated with this syndrome of highly abundant and highly polyphagous insects including flightless adults, passively dispersing early larvae, feeding on woody plants, and obligate parthenogenesis. Normark and Johnson (2011) proposed the 'niche explosion' hypothesis to explain this syndrome. This hypothesis poses a positive feedback loop between population size and host range. Species that acquire sufficiently high population sizes and sufficiently wide host plant use can become caught in this loop with each factor increasing the other. Although this feedback loop has not been directly tested, its principles appear sound. Demographic and population genetic processes should support population size leading to increased niche breadth. Demographic factors also support increased host range resulting in increased population size (Normark and Johnson, 2011). Parthenogenesis is likely a consequence, and not necessarily a cause, of the very large population size of niche explosion species. As the advantages of sexual reproduction over parthenogenesis decline with large population size (Otto, 2009), the disadvantages of sex would be expected to dominate in these extraordinarily abundant

species. An implicit assumption of the niche hypothesis model is that tradeoffs between performance on different hosts are sufficiently rare (Normark and Johnson, 2011). If tradeoffs are commonplace, they would be expected to constrain the evolution of niches. The existence of tradeoffs is a long-standing controversy in evolutionary ecology (Futuyma and Moreno, 1988; Futuyma *et al.*, 1995; Jaenike, 1990; Forister *et al.*, 2012). Peterson *et al.* (2015) systematically addressed performance tradeoffs in armored scale insects, a group that includes several extremely abundant and extremely polyphagous pest species. They developed a phylogenetic model of host plant evolution and applied that model to extensive collection records (23 810 individual specimen records) of host plant use from armored scale insects. In most cases, the model found positive pairwise correlations between evolutionary changes in scale insect presence on different host plants. This result suggests that adaptation to one host plant facilitates adaptation to other host plants, and stands in strong contrast to the pattern expected if tradeoffs were pervasive (Peterson *et al.*, 2015).

See also: Invasion Biogeography. Invasive Species, Evolution and. Sex and Selfish Genetic Elements

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Philosophy, Evolutionary Biology and

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Introduction: Philosophy and Evolutionary Biology

The interaction between philosophy and evolutionary biology has been particularly a fruitful one. Evolutionary biology has generated a considerable number of conceptual puzzles that have attracted interest from both biologists and philosophers. And evolutionary biology has generated a considerable number of controversies, both internally (arguments among practicing biologists) and in public discourse surrounding the discipline; in both cases, philosophers have participated in these debates. Finally, evolutionary biology has sometimes been thought to have implications for some traditional philosophical problems, making some long-standing positions in philosophy either more or less plausible.

In this article, some of the key issues and positions in each of these broad areas will be briefly sketched. First, the role that philosophy has played in conceptual analysis within evolutionary biology will be explored. In many fields, questions arise that are not strictly speaking about the subject area, as they involve primarily conceptual issues rather than empirical questions. In evolutionary biology, questions regularly arise that cannot be answered merely by gathering more data. They are, for example, questions about the meanings of terms, or the best perspectives from which to view certain phenomenon. Empirical evidence is often relevant to answering these questions – indeed, since some of the questions turn on the fruitfulness of particular perspectives, whether in fact a perspective is to be preferred will depend in part on whether it will really lead to more fruitful future research than alternative perspectives. But no particular empirical findings or experimental results are dispositive in these cases. Next, the role of philosophy in controversies surrounding particular aspects of evolutionary biology and the relationship between evolutionary biology and public discourse will be addressed. Philosophy has often weighed in on particular debates within evolutionary biology, suggesting that in particular areas reasonable evidentiary standards are not being upheld, or suggesting that standards of evidence differ unreasonably between different areas. And philosophy has played a role in the analysis arguments surrounding, for example, the teaching of evolutionary biology in public schools. Finally, some of the ways in which it has been suggested that evolutionary biology might bear on traditional philosophical problems will be noted. The fact that humans are the product of biological evolution, it has been suggested, at least makes some more or less traditional philosophical positions less plausible, and makes others more plausible, and may play a part in settling some philosophical disputes.

Before getting into these arenas, it is worth noting that the philosophy of biology has been, with only a relatively few exceptions, the philosophy of 'evolutionary' biology; the central questions and challenges that philosophers have taken up have emerged primarily (though not entirely) from evolutionary biology, broadly construed. This sometimes results in the philosophy of biology giving a skewed impression of the state of biological research, suggesting that most biological research is deeply wrapped up in evolutionary analyses. While Dobzhansky's (1973) dictum that "nothing in biology makes sense except in the light of evolution" may well be true, it is worth remembering that in practice, many research agendas in biology do not make continual or robust use of evolutionary biology in their day-to-day work. Philosophy of biology's focus on evolution to the exclusion of other domains, and its habit of seeing other domains through the lens of the questions most relevant to evolutionary biology, may in some cases be misleading.

Philosophy and Conceptual Analysis

There are questions in evolutionary biology that are not merely biological in nature, but rather centrally involve conceptual issues. The distinction is not always clear in practice, but in principle, it is the distinction between questions that can be answered by further research into the biological phenomena in question and questions that are better viewed as being about how to interpret the results of research, or how to think about an area of research. So for the most part, questions regarding when, say, some particular trait arose in a particular lineage are biological questions – if we want to know when, for example, swords arose in a particular lineage of swordtailed *Xiphophorus* fish, the question may be difficult to answer definitively, but it is generally agreed what kinds of evidence are relevant to answering it (see, e.g., Marcus and McCune, 1999; Basolo, 1990). Contrast this with the ongoing debates surrounding the nature of species (the 'species problem'). Hey (2001) lists some two dozen species concepts, and notes that these are, with a few exceptions, generally viewed as "competitors for the single best meaning" (p. 326). But it should be obvious, by now, that the inability of biologists to settle on a single best species concept is not because of a lack of biologically relevant data; indeed, one cannot easily imagine what new biological discoveries even in principle could determine, once and for all, that one of these competing concepts was the correct or the best one. Rather, any solution proposed must try to make sense of the different work that species concepts do in biology, and (perhaps) recognize that no single concept can do everything that one might have wished a species concept could do (see, e.g., Hey, 2001, 2006; Dupré, 2001). Similarly, arguments surrounding the ontological status of species – whether species are best thought of as individuals (Ghiselin, 1974; Hull, 1978), or as sets (see Kitcher, 1984), or kinds of some sort (see Boyd, 1999) – is not a question that more biological data on the behavior of individual organisms or of populations would permit us to settle.

It is of course not always clear whether a question is primarily a (more or less) straightforward biological question, or whether it involves important arguments over conceptual

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issues. Consider, for example, the current debates surrounding the necessity and/or the advisability of an 'extended evolutionary synthesis' (e.g., Pigliucci, 2007; Pigliucci and Müller, 2010), and the related arguments surrounding 'Developmental Systems Theory' (e.g., Oyama *et al.*, 2001) and 'Devo-Evo' (e.g., Hall, 2000). There are, obviously, a number of more or less straightforward biological questions that are relevant to arguments surrounding the advisability (and/or necessity) of these projects (though that doesn't, of course, mean that the questions are easy to answer). For example, how often is selection on heritable but nongenetic variation in fact relevant to a population's evolution? Are changes in heritable but nongenetic developmental resources in fact implicated in any major evolutionary transitions? (see, e.g., West-Eberhard, 2003; Jablonka and Lamb, 2005; for a more skeptical view, see, e.g., Hoekstra and Coyne, 2007). But there are also deep conceptual issues – for example, insofar as some problematic concepts of 'information' (understood as both a developmental and heritable resource) became part of the standard stories surrounding accounts of evolution in the modern synthesis understanding of the relationship between evolution and development, more rigorous analyses of the meanings and uses of information in biology can be relevant to understanding some of the concerns to which proponents of 'extended synthesis' and related views see themselves as responding (see, e.g., Moss, 2004). Or more generally, while arguments surrounding whether it makes sense to think in terms of some privileged class of 'replicators' that can be meaningfully distinguished from 'interactors' in evolution make use of empirical facts about development and evolution, they involve, critically, disagreements about conceptual issues, as well (see, e.g., Stereln and Griffiths, 1999 and cites therein).

The broad areas of research noted above – the species concept, the ontology of species, and extensions or modifications to the so-called modern synthesis – represent a few of the areas where the philosophy of biology has engaged with issues that emerge from the conceptual foundations of evolutionary biology. Below, several additional places where this kind of conceptual analysis seems important, and where philosophers and biologists have engaged in this kind of conceptual analysis, are sketched. This is not meant to be a complete list, but rather representative of the sort of work that the philosophy of biology is involved with.

Levels of Selection/Multilevel Selection Theory

Williams (1966) provided a strong critique of sloppy group-selectionist thinking, and this work is often credited with introducing gene-selectionism into evolutionary biology (Dawkins, 1976 popularized this approach). Gene-selectionism, narrowly understood, never attracted the full support of more than a minority of practicing evolutionary biologists, but the position was nevertheless quite influential. More generally, the questions raised by this work – What is it that gets selected? At what levels can selection operate to produce adaptations? What is it that is adapted? Is there an important distinction between replicators and interactors, and if so, what work does that distinction do? – remain active areas of both empirical research and contention regarding the proper interpretation of both the questions and the results of the research.

So, for example, consider the question of what should count as 'group selection.' Most would agree that group selection is the proper account of a situation in which the groups themselves (1) survive and reproduce as wholes and (2) where the features of the group that lead to fitness differences between groups are not the same features as the features that make the individuals making up the groups more or less fit (see **Figure 1(b)**). But what of the case where the fitness of individual organisms depends upon the group (the context) to which they belong – Is that also an example, or is it merely a perfectly ordinary case of individual fitness depending upon the environment in which the organism finds itself (**Figure 1**; see, e.g., Okasha, 2006; Okasha and Paternotte, 2012)?

Some of the questions in this area are fairly technical, but no less conceptually loaded for all that. Okasha's (2006) work on the different formal approaches to multilevel selection (the Price equation, Contextual Analysis, etc.) reveals both the deep mathematical compatibilities of what are sometimes interpreted to be competitors for the best approach (see also Kerr and Godfrey-Smith, 2002), as well as the places where, while each approach makes the correct predictions, only one approach seems to provide a causally satisfying explanatory story. What is the proper role of multilevel selection theory within evolutionary biology – (merely) proper accounting of evolutionary change, or providing accounts that not only get the right answer, but seem causally compelling? If there is often only one account that is correct, what kinds of biological facts determine which account is the correct one?

The Organism and Individuality

Organisms are usually thought to be of central importance to evolutionary biology, and in any event would seem to be a central ontological category for biology more generally. But individuating organisms – determining where particular individual organisms begin and end – has long been recognized as a challenge. Complex vertebrates would seem to be paradigmatic cases of individual organisms ('pace,' perhaps, the near ubiquity of commensal bacteria in vertebrates). On the other hand, the eukaryotic cells that makes up vertebrate bodies clearly seem not to be individual organisms, but rather parts of a more complex whole. But what to make of, for example, eubacteria that engage in obligate mutualism within particular environments (see, e.g., Dupré and O'Malley, 2009)? And are the colonies of eusocial organisms best thought of as collections of individual organisms (only some of which reproduced) or as some kind of organism-like entity in their own right (e.g., Hamilton *et al.*, 2009)? Note that some have suggested that this problem is closely related to issues involving multilevel selection – the move to having to think in terms of 'collectives' in order to make sense of the evolution of a population is at least part of what it means for those 'collectives' to count as biological individuals (Korb and Heinze, 2004; Okasha, 2006). Answers to one set of conceptual questions will sometimes influence the way other related questions are viewed.

Niche Construction

Lewontin (1983, 1985) noted that a standard view of adaptation was that of populations of organisms adapting to the

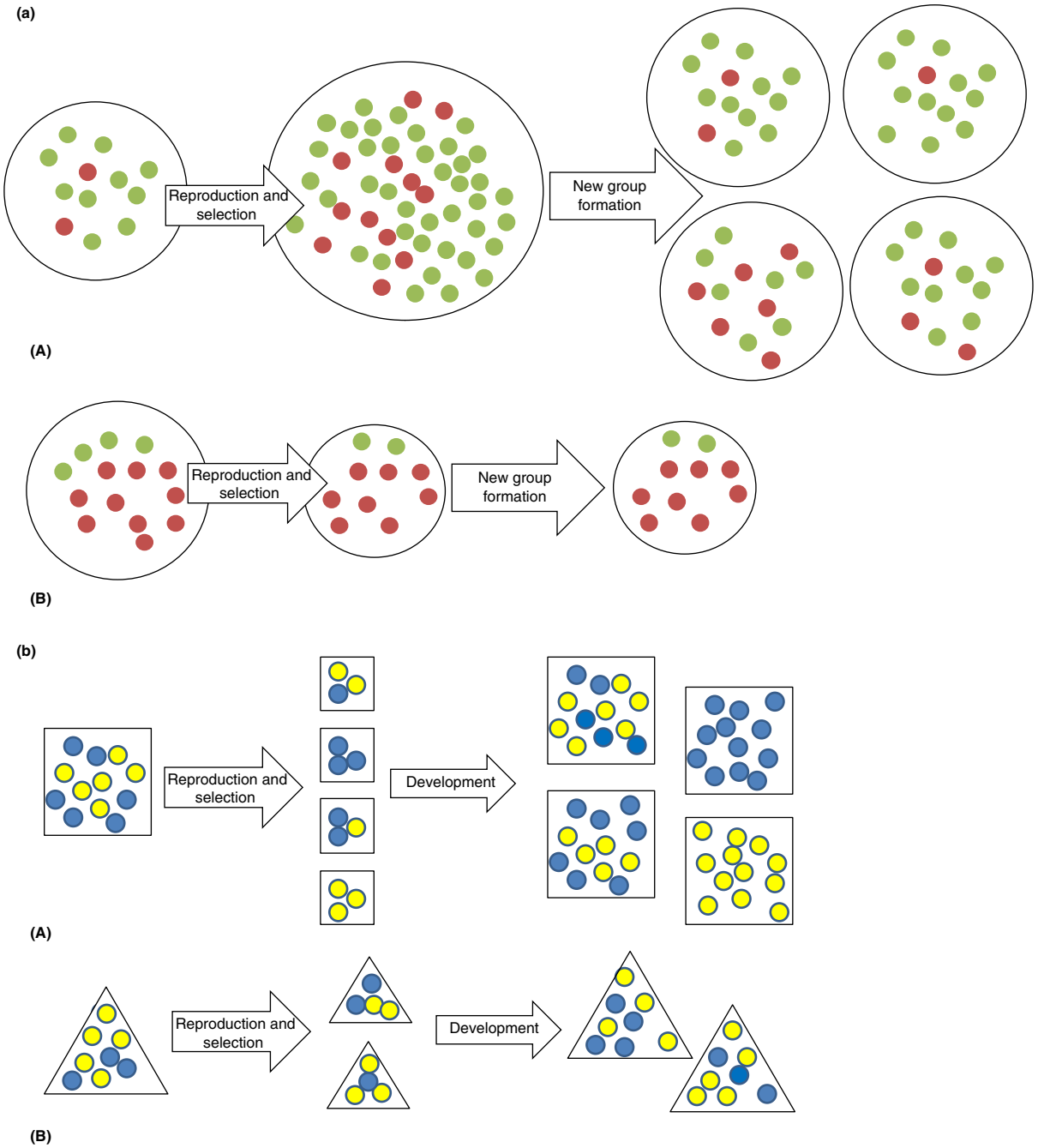

Figure 1 (a) Within each population, red (selfish) individuals are fitter than green (altruistic) individuals. However, individuals of both kinds in groups with a higher proportion of altruistic individuals (A) have higher fitness than individuals in groups with a higher proportion of selfish individuals (B). Given certain assumptions about how new groups form, under various conditions the proportion of groups comprised mostly of altruists can increase, despite being at a selective disadvantage in both kinds of groups. Note that the success of a group is simply a function of the success of the individuals that make the group up, and that the properties of the group are determined only by the fraction of individuals with particular traits that make up the group. In multilevel selection theory, this is known as MLS1 (multilevel selection 1); see Damuth and Heisler, 1988. (Adapted from Okasha, S., 2006. Evolution and the Levels of Selection, vol. 16. Oxford: Clarendon Press.) (b) Collectives (populations) of type 'A' are fitter than collectives of type 'B,' because of a feature of the collective itself; the feature that makes the collective fitter is not a feature that influences the fitness of the individuals ('particles') in the collective directly, and one cannot derive the fitness of the collective from the fitness of the individuals that make it up. The fitness of the collective is determined by how many decedent 'collectives' it leaves, not the number or fitness of the individuals within the collective. Selection at the lower levels (the particles within a collective) is at least partially decoupled from the fitness of the collectives. In multilevel selection theory, this is known as MLS2 (multilevel selection 2); see Damuth and Heisler, 1988. (Adapted from Okasha, S., 2006. Evolution and the Levels of Selection, vol. 16. Oxford: Clarendon Press.)

external environment, where changes in the external environment were conceived primarily as exogenous – "the environment proposes, natural selection disposes" (Gould, 2002, p. 31). But this view, he argued, was fundamentally mistaken. There was not, according to Lewontin, an eternal environment independent of the organisms that made it up, at least not in any meaningful sense. This critique had at least three distinguishable threads. First, the niche of an organism, Lewontin argued, could only be determined by observing the actions of the organisms itself. There were no 'preexisting' niches to be filled, there was no privileged way of diving up the world. Second, and closely related, what counted as a feature of the environment was determined not by the external world per se, but rather by how the organisms in fact lived in it. For example, what kind of environment 'water' is depends critically on the size of the organism trying to live in it; ways of moving through water that are very effective for large ocean predators (e.g., sharks and dolphins) would be hopeless for microorganisms (for whom water behaves less like the low-viscosity liquid most people are familiar with, and more like a very high-viscosity material such as warm tar – see, e.g., Yates, 1986). Finally, Lewontin suggested that the fact that organisms actively modified the environments in which they lived, through their actions in the world, had important implications for correctly understanding the ways that fitness differences emerged from the reciprocal relationship between organisms and their environments.

One research path articulating some of the implications of these observations is known as 'niche construction' (Odling-Smee *et al.*, 2003, 2013). It is uncontroversial that for some organisms, proper development requires environments deliberately formed by their parents or other conspecifics – nests, for example, are an important part of the developmental environment of many organisms. And the environment in which most beavers, say, are born is very different from what it would be like without previous generations of beavers creating and maintaining those environments (see, e.g., Naiman *et al.*, 1988). But what to make of these facts is controversial – does niche construction fundamentally change how evolution should be understood, or is niche construction rather a small wrinkle that does not require any profound changes to the usual understanding of evolution and the environment?

In a somewhat different context, Dawkins argued that the effects of genes should sometimes be seen as extending beyond the organisms that housed them, such that features of the environment could be thought of as part of the gene's 'extended phenotype' (see Dawkins, 1982); Dawkins does not, however, wish to endorse the whole niche-construction package, which he views as too detached from genetic differences that can influence fitness more or less directly (Dawkins, 2004). For Dawkins, part of the value of gene-selectionism is that it permits one to track the effects that genes have on the external environment (nest-building behaviors, parasites subverting their hosts' behaviors, etc.) in the same way as genes that have their effects in the 'usual' way – on the phenotypes of the organisms that house them (see, e.g., Hughes, 2013 for a review of the extended phenotype in parasitism). For Dawkins, while it is ultimately the genes that are the beneficiaries of selection, selection can act on genes through the external environments associated with those genes in just the same way it can act on genes through organismal phenotypes so-associated. In this way, questions about the role of the organism (or the genes of organisms) in producing environments gets tied to questions about the levels at which selection operates, and the nature of selection more generally.

The Nature of Selection, Fitness, and Drift

There have been a number of positions defended on the nature of selection, of fitness, and drift. It is sometimes claimed, usually by people ignorant of the basics of evolutionary theory, that evolution by natural selection rests on a tautology, because fitness is defined in terms of actual reproductive success; if the fit are simply those that survive, differential fitness cannot explain evolutionary change (see, e.g., Bethell, 1976). But this mistake is revealing – expressing precisely what fitness is, and what natural selection and drift are, is more difficult than it is sometimes imagined.

Probably the dominant view today is some variant of a propensity view – fitter organisms are those that, all else being equal, have a propensity to leave more offspring (or grandoffspring) than their less-fit counterparts in the range of environments in which organisms of that type are found (see, e.g., Mills and Beatty, 1979). Natural selection is responsible for those propensities, and drift is the main reason that actual outcomes fail to match those propensities (see, e.g., Richardson and Burian, 1992). One can understand evolution, on a closely related view, by thinking of selection and drift as separable forces, each of which has a distinct kind of impact on population dynamics (see, e.g., Sober, 1984). But more recently, some have argued that the natures of selection and fitness have been fundamentally misunderstood. According to defenders of the 'statisticalist' position, neither selection, nor drift, are properly thought of as processes nor as causes of evolution per se; rather, they are best thought of as ways of characterizing particular kinds of outcomes (e.g., Matthen and Ariew, 2002). Others have argued that defenders of these views move too quickly from statistical interpretations to rejecting casual efficacy, and suggest that a causal interpretation of fitness and drift can still be defended (e.g., Millstein, 2006, 2009).

This debate may seem quite distant from the concerns of practicing biologists. But one might reasonably ask what is being measured when biologists attempt to detect selection and measure fitness differences in the wild, and whether what is being measured in those cases fits in reasonably well with the notions of fitness that emerge from formal work in, for example, population genetics (see, e.g., Pigliucci and Kaplan, 2006). The debates over the proper interpretations of fitness highlight some of these conceptual difficulties.

A different kind of issue related to the nature of selection emerges from consideration of the in-principle minimum requirements for evolution by natural selection to occur. Under what conditions, in other words, can we legitimately expect cumulative selection to result in adaptations? Evolutionary biology is concerned primary with the actual state of the world, and the features that living things in fact have that permit evolutionary change. But explorations of the more general question of what abstract features of systems make evolution by

natural selection possible can shed light on important features of actual world (see, e.g., Godfrey-Smith, 2009).

Adaptations and Functions

What is it for a trait to be an adaptation, and how does being an adaptation differ from being adaptive? Below, arguments surrounding adaptationism are considered, from the standpoint of the project of critique – philosophy has played a role in arguments surrounding the appropriate evidentiary standards for adaptive hypotheses. But before one can argue about the evidence required to support the claim that some particular trait is an adaptation for performing some particular function (or set of functions), one needs to know what it means to claim that a trait is an adaptation. (Godfrey-Smith (2001) provides a good summary of the ways in which the debates surrounding adaptationism has often been ambiguous between empirical, explanatory, and methodological projects.)

Here, biologists themselves provided what many regarded as the definitive solutions, with Williams' (1966) notes regarding the 'onerous' nature of adaptive explanations (p. 41), and Lorenz's (1966) claim that "Unless selection is at work, the question 'What for?' cannot receive an answer with any real meaning." (p. 11) point toward what the clearly dominant view is. For a trait to be an adaptation for performing some function, the explanation for its current form and prevalence in the population in question must be due to past selection on traits with variation in their ability to perform that function. It is worth noting, though, that this position is not universal; in his textbook *Evolution*, Futuyma (2009) notes that some biologists still defend an ahistorical definition of adaptations (p. 284), though this is clearly a minority position. Gould and Vrba (1982) introduced a complication with their term 'exaptation' for traits that are now used by an organism in a way that increases fitness, but which did not arise in the population because of their association with that use; however, neither the terminology nor the concept ever caught on. Indeed, Godfrey-Smith's (1994) 'Modern History' view of functions entails that for a trait to be functional, and hence to be an adaptation, it must have been subject to selection for that function in its relatively recent evolutionary history, whatever else it was or was not selected to do in the more distant evolutionary past.

The Nature of Populations

The concept of a population plays important roles in evolutionary biology, but, again, stating precisely what constitutes a population is more challenging than is often recognized. There is no consensus on necessary conditions for some collection of organisms to form a biologically meaningful population. Some authors have tried to identify how, in practice, working biologists in different fields in fact pick out populations (see, e.g., Winther and Kaplan, 2013) while others have defended stronger claims regarding what biologists ought to be picking out when they identify populations (e.g., Millstein, 2010). Some of the interest in this work comes from investigations of population structure using genomic data; especially when applied to human population structure, how to interpret the results of such research remains controversial (see, e.g., Weiss and Fullerton, 2005). But more generally, whether a collection of organisms is itself a population, rather than merely being part of a larger population, can have important implications for conservation efforts (including the application of the Endangered Species Act, in the United States) and for thinking about the evolution of the (putative) population (see, e.g., Allendorf and Luikart, 2009).

The Gene Concept

Stotz and Griffiths (2004) identify a number of different gene concepts in regular use in different domains in biology. Genes are sometimes conceived of as difference-makers – relatively abstract entities associated with phenotypic differences (this gene concept is very roughly what Moss dubs the 'Gene-P' concept; see Moss, 2004). It is this concept of the gene that is most like Mendelian genes, and also most like the genes referred to in much of population genetics. But genes are also regularly thought of as particular physical strands of DNA – physical molecular entities. These physical stretches of nucleic material are used in various ways during development, and Moss dubs the gene thought of in this way the 'Gene-D' concept (Moss, 2004).

Even the most cursory investigations reveals the difficulty with reconciling genes as difference makers related to phenotypic traits (roughly, Mendelian genes) with molecular genes (stretches of DNA that involved in protein synthesis and its regulation) (see, e.g., Sterelny and Griffiths, 1999, Chapter 6 'Mendel and Molecules'). There is no one-to-one relationship between stretches of DNA and gene-products; a given stretch of DNA can produce multiple different proteins (e.g., alternative splicing) and a particular protein may be produced by the interactions of multiple different stretches of DNA that are transcribed separately (Stotz and Griffiths, 2004). Researchers working in different traditions identify different kinds of things as genes, and hence even those who work primarily on molecular genes can count them differently – some, for example, focus on the total number of discrete gene-products (proteins), and others on the number of distinct functional elements, etc. (Griffiths and Stotz, 2006).

Indeed, the arguments surrounding the claims emerging from ENCODE regarding the functionality of so-called junk DNA emerge in part from arguments surrounding what will count as a gene, as well of course from arguments surrounding the nature of biological functions (see Graur *et al.*, 2013). Is having a sequence similar to sequences that are actively used in development enough to call something a gene, or need there be evidence that the sequence is actually used – that, for example, when transcribed the transcription products are in fact put to biological use? Does a sequence have to be used in some way during development, such that the details of the sequence itself matters, or does the fact that some sequence (rather than none) is needed (to serve as a 'spacer' perhaps) suffice to identify something as a gene (Graur *et al.* (2013) call the latter sequences 'indifferent DNA,' pp. 586–587)?

Philosophy and Evidentiary Questions

Above, it was noted that one issue raised by the debates surrounding adaptationism was just what, precisely, was required in

order for a trait to be an adaptation. But even after one has settled upon the definition of adaptation, there may still be arguments regarding the minimum evidence required in order to appropriately claim that a particular trait is in fact an adaptation for performing such and such a function. Gould and Lewontin's (1979) famous 'Spandrels' paper criticized the so-called adaptationist program in part by claiming that too many biologists were willing to argue that a trait was an adaptation on the basis of far too weak evidence; indeed, they claimed that often the mere qualitative agreement between a plausible-sounding selective story and contemporary behavior was regarded as sufficient for accepting the selective story (587ff). Contrast this, for example, with the work done to defend the claim that the sword-tails of male sword-tailed *Xiphophorus* were an adaptation to a preference that female *Xiphophorus* had for males with sword-tails. Here, observational work established that females really were more likely to mate with males that had longer sword-tails. Phenotypic manipulations were used to determine if it really was the tails that were doing the work, and phylogenetic analyses supported the claim that the female preference for males with sword-tails predated the appearance of males with sword-tails. The hypothesis that the swords arose in response to female preference, and that what explains the presence of the sword-tails is past selection based on female preference, seems, in this case, rather well supported, albeit not without some complications (see Basolo, 1990; Marcus and McCune, 1999). On one reading, one of Gould and Lewontin's complaints in 'Spandrels' was that this level of careful investigation and testing was too rarely exercised (for a summary of some methods available for testing adaptive hypotheses, see, e.g., Rose and Lauder, 1996; Pigliucci and Kaplan, 2000).

Claims about human psychological adaptations were seen by Gould and Lewontin as particularly sloppy and ill-supported, and most philosophers who waded into the debates surrounding human sociobiology tended to agree. Kitcher's (1987) *Vaulting Ambition* was a book-length critique of what Kitcher argued were the poor evidentiary standards being deployed by so-called sociobiologists. More recently, one can see David Buller's extended critique of 'evolutionary psychology' (see **Box 1**), in *Adapting Minds* (2005), as fitting into the same framework – Buller argues that evolutionary psychologists have been too quick to draw (often quite sweeping) conclusions based on too little (and often fragmentary) data. Lloyd's (2005) work on the human female orgasm takes a similar critical tack – that in the human case, particular hypotheses involving traits as adaptations are too often made on the basis of poor evidence. Part of the problem is that it is very difficult to rigorously test hypotheses regarding adaptations in humans (especially for psychological or behavioral traits) (see **Table 1**; see, e.g., Kaplan, 2002).

While most philosophical discussions of issues surrounding arguments for and against the need for an extended synthesis, or DST as a plausible research program, etc., have focused on the conceptual issues engendered by these arguments, some philosophers have taken on the interpretations of the empirical data, arguing, for example, that a careful review of the evidence makes certain positions untenable (see, e.g., Oyama *et al.*, 2001; Moss, 2004). Here, the results of biological research are deployed in order to support particular research programs, and to make particular analyses seem more plausible.

Box 1 Evolutionary Psychology

As suggested in the main text, philosophers of biology have been interested in evolutionary psychology for a number of reasons. But what is the object of that interest? Buller (2005) distinguishes between **E**volutionary **P**sychology (capitalized) and **e**volutionary **p**sychology (while useful, this distinction is not universally used). The former is a particular research tradition associated with a particular set of claims and methodological practices, and the latter is any attempt to use evolutionary biology in the service of understanding human behaviors (and it is worth remembering that researchers engaged in evolutionary psychology will sometimes endorse some but not all of the basic claims and methodological practices of Evolutionary Psychology, so the lines are not as crisp as this might make them seem; see, for example, Confer *et al.*, 2010). The latter, evolutionary psychology, is at least in principle uncontroversial – since the human brain is the product of biological evolution, and since its larger size and greater complexity are metabolically expensive and of relatively recent origin, the idea that the brain is an adaptation for some kinds of psychological abilities or other is fairly straightforwardly correct. Even here, though, Lewontin (1998), for example, charts a very pessimistic course regarding what, precisely, we might learn about the evolution of human cognition, given the limitations of evidence available (see **Table 1** for some of these). But the former, Evolutionary Psychology, is associated with much more controversial claims. In its more or less canonical form, these include:

1. 'Massive modularity' – The human mind is made up of a very large number of 'modules,' each of which evolved to 'solve' some particular adaptive problem.
2. 'Human universals' – The adaptations that make up the human mind are universal in the human species.
3. 'Stone age minds' – The particular problems the human mind evolved modules to solve are those that were most important to our ancestors in the so-called 'environment of evolutionary adaptedness'; for many traits, especially those that are supposed to be uniquely human, this is assumed by EP practitioners to be the Pleistocene (2.6 million to 11 700 years ago).
4. 'Adaptive thinking' – Reflecting on the problems faced by our ancestors, and considering what solutions would have been both biologically possible and adaptive, is a useful technique for generating hypotheses regarding the existence of mental modules. One can then test for these hypothesized adaptations by thinking through the cognitive consequences of their existence, and making predictions about how people will behave on that basis.

(See, e.g., Cosmides and Tooby, 1997; Downes, 2014; Tooby and Cosmides, 1990).

Different sorts of objections have been raised to these basic claims, such as: the empirical evidence for modularity of the sort proposed in (1) is weak, and the arguments that the mind must be modular in that way problematic; there is no reason to think that the mental problems faced by different human populations were universal as in (2) (consider: the evolution of lactase persistence in some but not all human populations); it is at least plausible that the problems faced during human evolution were not stable (3), but rather the result of living in cultures that changed with the changes in the people making them up, so there never was an environment of evolutionary adaptedness, even in aggregate; the suggested methodology (4) is not the primary methodology deployed in the study of nonhuman adaptations, including behavioral adaptations in nonhuman animals (see, e.g., Buller, 2005; Downes, 2014; Lewontin, 1998; Stereln and Griffiths, 1999). In addition, as the main text makes clear, there are objections to the many of the specific hypotheses put forward by practitioners of EP.

Table 1 Human adaptations: Why testing hypotheses regarding adaptations is so difficult for traits in humans

Technique	*Evidence generated*	*The trouble with humans*
Phenotypic manipulation (laboratory or field)	Fitness consequences of the traits in question, causal mechanisms associated with traits and fitness consequences	Ethical constraints + no controls in natural cases
Transplant studies	Fitness consequences, hypotheses re: selective pressures, hypotheses re: local adaptations	Ethical constraints + few ways to control for confounding variables in natural cases
Laboratory evolution	Robustness of pathways, strength of constrains	Ethical constraints + very poor model organism
Optimization analyses	Quantitative plausibility of qualitative assessments, sensitivity, path-dependence	Little knowledge of relevant selective history or specific functions for many of the traits of interest
Phylogenetic analyses	History of trait, homology (shared-derived trait) versus homoplasy (independent derivation of trait)	Sparse clade; very few reasonably close extant relatives
Comparative method/regression analyses	Relationship between trait and environmental variables, strength of relationship, relationship between trait and fitness	Very little known about environment/trait relationships, little known about trait/fitness relationships, little known systematic variation between populations within species

Notes: For more on testing hypotheses involving putative adaptations, see, for example, Rose and Lauder (1996) and Pigliucci and Kaplan (2000). For some of the specific difficulties in the case of testing adaptive hypotheses involving human traits (especially human psychological or behavioral traits), see, for example Kaplan (2002).

In a somewhat similar vein, arguments surrounding adaptive and fitness landscapes rely on the results of empirical research to support particular positions regarding the advisability (or coherence) of particular models (and metaphors). Here, some philosophers have argued that relatively recent advances in modeling make certain uses of fitness landscapes problematic (see, e.g., Pigliucci and Kaplan, 2006; Kaplan, 2008; Pigliucci, 2012); others have argued that these objections are at least overstated, and at most apply to only some potential uses of fitness landscapes (see Skipper and Dietrich, 2012; Svensson and Calsbeek, 2012).

Finally, philosophy has played some role in the debates surrounding the teaching of evolution in public schools in the US context; Michael Ruse, for example, was called as an expert witness in the famous 'McLean v. Arkansas Board of Education' (1981) case regarding the teaching of creation science and several philosophers testified at the 'Kitzmiller v. Dover Area School District' (2005) trial regarding so-called Intelligent Design. And more recently, Kampourakis (2014) has written on how educators might work to overcome what he regards as conceptual barriers to students' properly understanding evolutionary biology.

The Implications of Evolutionary Biology on Philosophical Problems

This article has so far mainly focused on the ways that philosophers of biology, and biologists working on conceptual issues in a philosophical way, have approached issues arising in evolutionary biology. But some have argued that evolutionary biology might have implications for traditional philosophical problems. On one view, this might be part of the expected progression of what were thought to be philosophical problems succumbing to scientific investigation – the claim that as more and more fields become empirically firmly grounded, less is left for philosophy. Some questions about basic ontology are now, for example, generally thought to be best addressed by physics, and not by philosophical investigation. And insofar as one thinks that design arguments of the sort defended by Paley (1802) (someone not generally considered a philosopher) and criticized by Hume (1779) (a canonical philosopher) were part of a philosophical tradition, evolutionary biology of course completely undermines them as reasonable philosophical questions (contemporary defenders of intelligent design theses notwithstanding). But whether evolutionary biology will result in similar usurpations of what are now thought of as traditional philosophical questions remains a live issue.

For example, one question that has received a fair bit of attention is the extent to which evolutionary biology might provide insights relevant to moral philosophy ('evolutionary ethics'). Certainly, insofar as our abilities to engage in moral reasoning require sophisticated cognitive systems, the fact that our ability to so-engage is the result of our recent evolutionary history must be at least relevant to understanding how moral reasoning is possible. But how it is relevant remains opaque. If our ability to engage in moral reasoning was the result merely of our ability to engage in particular general sorts of abstract reasoning (a view that would seem in line with, for example, Kant's interpretation of morality and its relationship to our moral abilities), then evolution would explain nothing in particular about our moral lives, but merely how we became the sorts of being capable in engaging in that kind of reasoning (compare: it is only because of the sorts of being we evolved to become that, for example, modern set-theory is possible, but the details of our evolutionary history are generally believed to be broadly mute on the issues considered interesting by set-theorists). On the other hand, if our moral lives emerged from earlier pro-social behaviors, and if selection acted on our abilities to engage successfully in those kinds of behavioral repertoires, then the details of our evolutionary history might

explain, for example, some common intuitions or the reasons that certain ways of organizing our moral lives are more common than others. There is, with such work, often the concern that the 'naturalistic fallacy' is lurking nearby – in this case, for example, one might worry that even a complete explanation of how particular tendencies related to our moral abilities evolved would not reveal anything about how we ought to behave (but merely about how we in fact tend to actually behave) (see, e.g., FitzPatrick, 2014; Ruse, 1986).

Other research areas that might be thought to impact on some traditional areas of philosophical enquiry include at least the following. Work on the evolution of language and communication more generally might have some implications for some issues in the philosophy of language (e.g., Ruse, 1986; Skyrms, 1996). Evolutionary epistemology – the study of evolution's role in the formation of beliefs about the world – may impact some approaches to epistemology in philosophy; it has in any event inspired some new approaches (see, e.g., Millikan, 1984; Godfrey-Smith, 1996). Some people have made claims about the implications of evolutionary biology for our understanding of the genesis and maintenance of religious beliefs and practices (Norenzayan and Shariff, 2008); while not precisely a philosophical issue, it seems related. Some philosophers have turned to evolutionary thinking in their work on esthetics (e.g., Voland and Grammer, 2003). And, of course, some of the kinds of claims sometimes associated with some (particularly problematic) articulations of sociobiology (and later, evolutionary psychology) were thought to have implications, for example, the range of plausible political positions, the possibility of eliminating particular kinds of sexism or racism, etc.; though these positions never got much traction either in philosophy or biology properly speaking, they still attract some support.

Even where evolutionary (or biological) reasoning and empirical work are not the last word, it is worth noting that philosophical positions that are wildly at odds with broadly accepted biological theories are liable to be criticized harshly, especially if they fail to confront the empirical research that would seem to undermine them. For example, in her devastating review of McGinn's *The Meaning of Disgust*, Strohminger (2014) notes that McGinn fails to even mention "the most widely accepted theory of disgust today," namely that it is an adaptive behavioral extension of the immune system for pathogen avoidance (p. 214). While biology might only rarely settle a philosophical question, it can at least make certain avenues far less promising. At the very least, philosophers interested in explaining the etiology of particular human behaviors need to be aware of the contemporary biological work in the fields that they address.

Final Thoughts

There is, and has been for many years, a rich and ongoing exchange between philosophy and evolutionary biology; indeed evolutionary biology has been involved with philosophical questions from its very founding. Biologists interested in the conceptual foundations of their research areas have been forced to engage in conceptual analysis – forced, in other words, to tackle philosophical questions. Indeed, the line between the philosophy of biology and biology proper has often been blurred by both biologists and philosophers. Is Gould and Lewontin's famous 'Spandrels' paper a biology paper, or a paper in the philosophy of biology that just happens to have been written by two practicing biologists? Is Okasha's (2006) *Evolution and the Levels of Selection* a philosophical work that just happens to be valuable to practicing biologists and hence regularly cited in primary research articles in straightforwardly biological journals, or is it (also) a contribution to biology itself? And the philosophy of biology has involved, and continues to involve, many fruitful collaborations between practicing research biologists and philosophers (Eliot Sober, one of the founders of the philosophy of biology as a separate discipline, has published with R.C. Lewontin, with D.S. Wilson, and with S. Orzack; Elizabeth Lloyd, has published with Lewontin, with M. Feldman, with C.G. Anderson, and with Gould; Peter Godfrey-Smith has published with Kerr, as well as with Bergstrom; etc.).

The relationship between evolutionary biology and philosophy has been valuable for both fields. Philosophy has brought a kind of conceptual rigor to bear on some foundational issues in evolutionary biology, and has, at times, played an important role in helping to clarify the complex role that evidentiary standards played in the acceptance (or rejection) of particular hypotheses. Evolutionary biology has opened new avenues of exploration in a number of traditional philosophical domains, and, in providing answers to some questions traditionally thought of as philosophical, has solved (or perhaps dissolved) some philosophical problems. This article merely sketches the broad outlines of some of these areas.

See also: Adaptation, History of. Adaptive Landscapes. Animal: What Is an Animal?. Epigenetic Inheritance. Maternal Effects. Natural Selection, Measuring. Quantitative Genetics in Natural Populations. Social Effects. Sociobiology, History of. Speciation Continuum. Waddington's Epigenetic Landscape, History of

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Phylogenetic Approach to Studying Developmental Evolution: A Model Clade Approach

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Glossary

Blastoderm A stage in the early development of animals in which a layer of cells lines the periphery of the embryo.
***cis*-regulatory element** A sequence of DNA that is located in the vicinity of a gene coding region and which is responsible for its transcriptional regulation (see also, Enhancer).
Clade (monophyletic group) An ancestor and all of its descendants. Can be used to describe organism or gene phylogenies.
Core eudicot A monophyletic clade comprising over 70% of flowering plants.
Corolla The petals of a flower, collectively.
Enhancer A short, usually locally acting, region of DNA near a gene locus that is bound by proteins acting to modulate the transcription of the gene in a spatially and temporally specific fashion.
Labrum One of the mouthparts of the arthropod head, a flap-like structure covering the mandibles.
MicroRNA A short noncoding RNA molecule that functions in posttranscriptional gene regulation.
Ortholog Genes found in different species that are both derived from the same gene in an ancestral species.
Pair-rule gene A suite of genes expressed in seven discrete bands in the early insect embryo perpendicular to the anterior–posterior axis.
Paralog Genes found within a species that are derived from an ancestral gene duplication.
Petaloid bracts Modified leaves that resemble petals.
Pollination syndrome A suite of floral characteristics (color, morphology, etc.) providing adaptation for pollination by a specific pollinator type (bee vs. moth vs. hummingbird, etc.).
Synapomorphy Shared derived character, an evolutionary transition that happened in the ancestor of that clade.
Transcription factor A protein that binds to DNA in a sequence-specific fashion and alters the rate of DNA transcription.
***trans*-regulatory element** Genes that modify or regulate the expression of genes at a distance (see also, transcription factor).
VIGS Virus-induced gene silencing, a RNAi method for gene knockdown.

Toward the Use of Phylogenetics in Evo-Devo

Comparing ontogeny across species to examine similarities and differences among organisms is an old discipline. In the late twentieth century there was a resurgence of this field, Evolution of Development (Evo-Devo), with the growing numbers of molecular and developmental tools becoming available in key model species (Gould, 1977; Hall, 2012). The discovery of shared major developmental genes, such as HOX and MADS-Box genes (Averof, 1997; Chanderbali *et al.*, 2010; Coen and Meyerowitz, 1991; Gehring, 1985,1998; Janssen *et al.*, 2014; Soltis *et al.*, 2006), further fueled interest in comparing shifts in the molecular basis of traits across disparate species. The research was initially very broad, with researchers comparing the growing gene expression and functional data in model systems, aiming to find common ground across all animals or plants. Insights have been gleaned by the observation that many important signaling and transcription factor families are similarly utilized or co-opted for the developmental growth of certain traits across disparately related model organisms (Boyle *et al.*, 2014; Gerstein *et al.*, 2014). This research was hindered by the fact that genomic information and gene expression and functional tools were only available in model species. The next step was to study fine scale differences among different varieties, sister species, or a small species complex encompassing model systems. The most notable group was within the small Drosophilid species complex including the fruit fly, *Drosophila melanogaster* (Green II and Extavour, 2012; Jeong *et al.*, 2006; Prud'homme *et al.*, 2006).

All of this information led us into the twenty-first century with a growing awareness that large amounts of the genetic basis of development are similar across broad phylogenetic groups and that many of the differences among species were largely due to gene regulation and likely shifts in the timing or location of gene expression with or without gene duplication (Abzhanov *et al.*, 2008). Comparison of gene expression and function in model organisms, especially in organisms with morphologically similar but nonhomologous structures, has highlighted the frequent genome tinkering of similar genetic pathways to result in similar structures (Carroll, 2008; Gehring and Ikeo, 1999; Olson, 2006; Panganiban *et al.*, 1997). Examples of traits co-opting the same gene regulatory network to independently generate a similar structure continue to grow. Two compelling examples are the separate evolution of sight in animals (Arendt, 2003; Gehring and Ikeo, 1999) and the independent evolution of bilateral symmetry in flowers (Hileman, 2014; Specht and Howarth, 2015), which both independently co-opt the same gene regulatory network repeatedly. Likely due to the fact that most comparative studies examining the genetic basis of trait changes were comparing closely related sister species complexes, it was initially hypothesized that most evolutionary change has been due to *cis*-regulatory mutations (Carroll, 2008). However, growing

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evidence indicates that changes in the patterns of gene expression, either through *cis*- or *trans*-regulation, is a more thorough explanation (Hoekstra and Coyne, 2007; Specht and Howarth, 2015). Examining gene expression across multiple species could shed significant light on these hypotheses.

With the growing availability of techniques to assay gene expression and function in non-model systems, researchers have been increasingly branching out into emerging model species (Abzhanov *et al.*, 2008). In fact, a significant portion of current Evo-Devo research revolves around examining a gene that has a known function in model organisms and examining it in a distantly related non-model organism. These comparisons are often determining if changes in gene expression and/or function are correlated with a trait change. For instance, Janssen *et al.* (2014) examined HOX gene expression in a single species of a small ecdysozoa phylum, Onychophoran, and compared it to known arthropod expression, concluding that discrete spatial expression in different HOX gene paralogs predates the specialization of arthropod segments. These kinds of comparisons have been highly informative and provide clues about a candidate gene's role in developmental patterning that we may not have concluded from model systems alone. That said, they are still a snapshot of a single organism. In the last few years modern techniques for assaying gene expression and function have become much cheaper and more approachable across multiple species, and we are starting to see research across clades. These gene expression and function studies across multiple species at once, which often encompass multiple trait gains, losses, or modifications, utilize a more 'model clade' approach. Utilizing not just model species, but also model clades, could more rapidly provide clues about the genetic basis of evolutionary change.

Model Clade Approach

In recent years it has become easier to assay gene expression and function in nontraditional model organisms. Techniques such as qPCR and RNA-seq have become potentially useful tools in any organism to examine fine scale differences in the level of expression temporally, spatially, or across species (Boyle *et al.*, 2014; Gerstein *et al.*, 2014; Morrison *et al.*, 1998; Pantalacci and Sémon, 2015; Pfaffl, 2001; Roux *et al.*, 2015). Additionally with RNAi technologies, functional studies of non-model organisms have become more reachable (Dinesh-Kumar *et al.*, 2003; Fire *et al.*, 1998). With these new techniques, many researchers have branched out into new emerging model species. These species have been chosen with several key characteristics in mind, such as having large numbers of individuals available either in the lab or the wild, having reliable tools for expression analysis, and having at least one functional tool available (Abzhanov *et al.*, 2008). Additionally, many new model species are chosen because they either represent an undersampled part of the tree of life, they have a breadth of traditional genetics and/or morphological research already to build on (such as *Heliconius* butterflies; Joron *et al.*, 2006), or they are closely related to current model species but vary in key traits. All of these attributes are important things to consider when working on new model species.

With improved research technologies, however, we can search out not just good potential model species, but also potential 'model clades.' A model clade would be a group of species within a defined clade that could be targeted for gene expression and/or functional studies. These species do not need to be sister species, necessarily, but could be chosen to sample across each of smaller groups across a clade. The benefits of a clade-based approach include a number of factors that give us more power to interpret gene expression and function and how it plays a role in morphology. For instance, a model clade could include not just a single shift in a trait of interest (like comparing a model to a non-model, for instance), but multiple similar shifts. This gives us more than one data point, and therefore the potential to uncover patterns in gene utilization or regulation. Perhaps even more informative, a model clade can include early transitional forms of the trait. Most research logically focuses on model species with highly derived (i.e., strong phenotypes) complex traits, usually in the crown group of the clade. In other words, we examine species with strongly bilaterally symmetrical flowers (Hileman, 2014; Luo *et al.*, 1996) rather than species that have nearly radially symmetrical flowers. When we are uncovering novel genes, it is often necessary to examine species that have a highly derived form of the trait so that we can more easily assay morphological changes. However, once we have an hypothesis of the gene's role, examining it across a clade that includes potential steps in increasing complexity can add a significant amount of understanding to how the gene evolved and is utilized by organisms.

A model clade would have a number of factors that would make it useful: (1) A well-supported species phylogeny. This allows for a simpler comparison of gene trees to a backbone species tree to uncover gene duplication or loss events or other genomic changes. (2) Character traits that are mapped across the phylogeny. Ideally a model clade would have either interesting transitional forms or multiple similar morphological shifts in the trait of interest. (3) Finally, a model clade should have multiple species spread throughout the clade that are easy to obtain and amenable to gene expression and/or functional techniques.

Dipsacales, an Example of a Model Clade for Floral Symmetry

We have used Dipsacales as a model clade to examine the evolution of the developmental pathway specifying floral symmetry. By choosing several species across the Dipsacales, a diverse clade of plants with multiple different patterns of floral symmetry, we are able to examine and track multiple character transitions across a large group of closely related organisms. In addition to identifying single character transitions among sister species, using a broad clade approach allows us to identify distinct morphological shifts among entire lineages of species, thereby examining the developmental context of species diversification.

The Dipsacales provides a group of plants with (1) a well-resolved species phylogeny to track trait changes (Backlund and Donoghue, 1996; Bell *et al.*, 2001; Bell, 2007; Judd *et al.*, 1994; Pyck, 2001; Winkworth *et al.*, 2008a; Zhang *et al.*, 2003),

(2) tremendous morphological diversity, especially in many floral characteristics and floral symmetry which have been mapped across the phylogeny (Donoghue *et al.*, 2003), and (3) a number of sample species which are horticulturally viable and can potentially be manipulated with modern developmental and genetic tools (Berger *et al.*, 2015; Boyden *et al.*, 2012; Carlson *et al.*, 2011; Howarth and Donoghue, 2009, 2005; Howarth *et al.*, 2011). Having a robust Dipsacales phylogeny provides a solid basis for inferring the location of evolutionary changes in flower characters (Donoghue *et al.*, 2003), as well as a secure framework within which to infer the evolution and duplication of known floral symmetry genes and their relation to morphological changes. A variety of floral forms are found in Dipsacales (**Figure 1**), including radially symmetrical, pseudo-radially symmetrical, bilaterally symmetrical, and asymmetric flowers as well as stepwise losses of stamens (Donoghue *et al.*, 2003). It is likely that Dipsacales was ancestrally radially symmetrical and therefore that bilaterally symmetrical flowers originated independently within this lineage (Donoghue *et al.*, 2003, 1998; Ree and Donoghue, 1999; Winkworth *et al.*, 2008b).

We have previously sequenced candidate genes that are part of the floral symmetry gene regulatory network, with the most focus on the ECE clade of TCP transcription factors similar to *CYCLOIDEA* (*CYC*). These genes were initially characterized in *Antirrhinum majus* and highlighted as dorsal identity genes, being expressed in the dorsal petals and aborted stamen (Luo *et al.*, 1996, 1999). Our analyses have allowed us to pinpoint gene duplication events that correlate with shifts in floral shape. For instance, the shift to bilateral symmetry correlates with mirrored duplications in two different *CYC*-like genes (Howarth and Donoghue, 2005). Other researchers have now confirmed a trend in an increase in *CYC*-like genes in plants with more complex floral symmetries (Carlson *et al.*, 2011; Chapman *et al.*, 2008; Tähtiharju *et al.*, 2012). Using a model clade approach, and comparing gene copies from across a clade allowed us to pinpoint 3 *CYC*-like gene clades. Adding data from other published groups outside of Dipsacales showed that these gene duplications spanned the core eudicots (~70% of flowering plants), uncovering two gene copies that had not yet been examined in other groups (Howarth and Donoghue, 2006). We were only able to pinpoint these gene clades because of a large phylogenetic dataset in Dipsacales (Howarth and Donoghue, 2006).

Using a clade approach in Dipsacales has also allowed us to examine how changes in gene expression correlate with stepwise progressions in bilaterally symmetrical flowers (**Figure 1**). We used semi-quantitative PCR to determine gene expression through the early diverging lineages of bilaterally symmetrical Caprifoliaceae (a sub-clade of Dipsacales) (Howarth *et al.*, 2011). These studies indicated that shifts in ubiquitous expression of CYC2 genes in radially symmetrical sister groups became stepwise more dorsally restricted in each more nested clade of increasing dorsoventral specialization (**Figure 1**). An initial duplication of CYC2 genes in the ancestor of Caprifoliaceae was followed first by the dorsal restriction of both paralogs and then by the uncoupling of their expression domains, resulting in one paralog being more dorsally restricted than the other (Howarth *et al.*, 2011). These kinds of studies indicate, for instance, that *CYC* is not simply a dorsal identity gene, being restricted to only dorsal petals in highly bilaterally symmetrical species, but instead the level of restriction of CYC2 on the dorsoventral axis correlates with petal divergence along that axis, a hypothesis impossible to uncover through comparisons of individual CYC2 paralogs to *Antirrhinum CYC* alone.

Phylogenetic Evo-Devo Studies

An increasing number of studies are incorporating a clade approach in examining Evo-Devo questions. Thanks to newer and more versatile techniques, we are able to compare gene expression or function using similar methodologies across a larger sampling of species in a clade. For instance, even in species with little previous molecular research, tissues can now be easily assayed for gene expression with semi-quantitative rtPCR or qPCR (Howarth *et al.*, 2011; Zhang *et al.*, 2012, 2013b). Additionally, because of better-understood species phylogenies and high-throughput sequencing techniques, it is now easier than ever to determine gene orthology across a group (Kellogg, 2006). While many of these studies are still using a candidate gene approach, an increasing amount of phylogenomic data is also being used to uncover and implicate novel genes, suites of genes, and microRNAs as well. We here highlight a sampling of recent studies examining Evo-Devo in a phylogenetic context across a clade.

Gene Expression across Model Clades

In plants, the buttercup family (Ranunculaceae) has been highlighted as a useful clade for Evo-Devo studies as it is sister to the rest of eudicots and it encompasses much of the floral form diversity that makes up angiosperms, especially in petal morphology diversity (Sharma *et al.*, 2014a; Zhang *et al.*, 2013a). Loss of petals occurs in many groups in the Ranunculaceae and a more recent understanding of the phylogenetic relationships suggests that the ancestral character state was petalous and that petals have been lost independently in at least seven different lineages (Damerval and Nadot, 2007; Wang *et al.*, 2009; Zhang *et al.*, 2013a). Zhang *et al.* (2013a) dissected floral tissue from five of these seven transitions and quantified gene expression of the three Ranunculaceae paralogs of the petal identity MADS-box gene *APETALA3 – AP3-1*, *AP3-2*, *AP3-3*. Using qPCR these researchers show that each loss of petals correlates with loss of expression of *AP3-3* (Zhang *et al.*, 2013a). These data more strongly suggest a similar function of this paralog across the family than a hypothesis formed from the observation of a single gene loss in one species. A similar study of B class MADS-box genes in *Cornus* (dogwood) determined that three paralogs had overlapping, yet differing expression patterns in the inflorescences of three separate species. The researchers utilized phylogenetic character mapping analyses to trace the expression of each gene on the *Cornus* phylogeny. They used these analyses to show that the two species with petaloid bracts are likely independently derived, which differs from the previous hypothesis (Feng *et al.*, 2012).

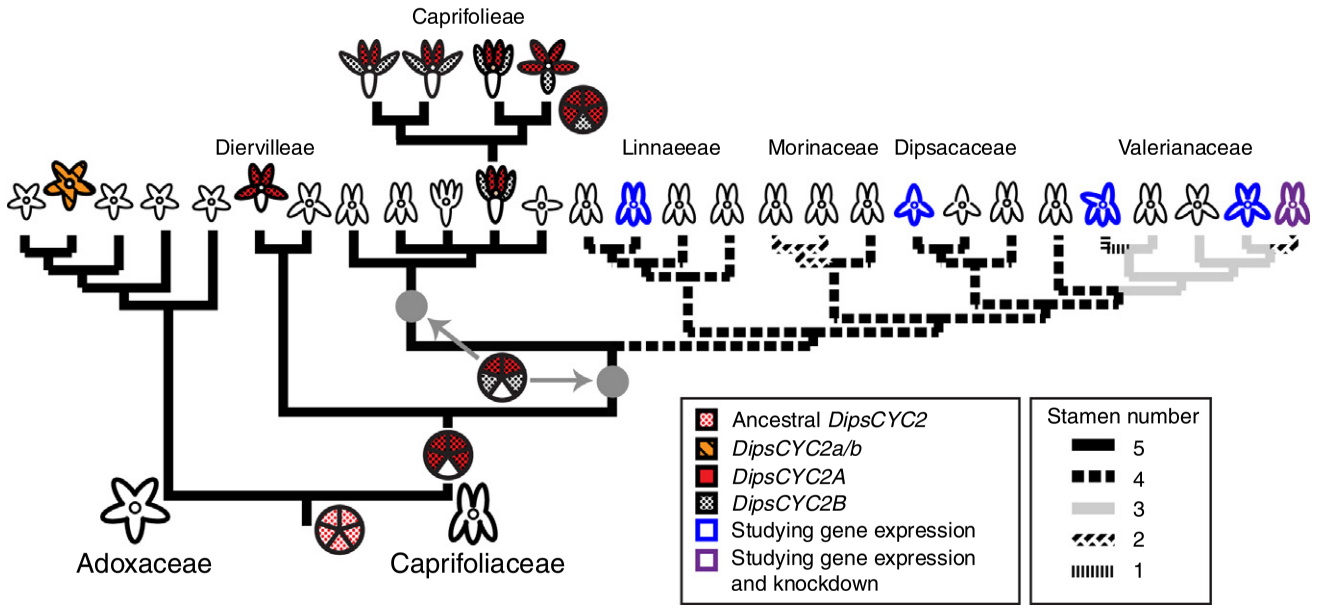

Figure 1 Illustration of Dipsacales as a model clade for studying the evolution of floral symmetry. We are studying the ontogeny and molecular basis of shifts in floral shape across species from each of the major clades. Floral diagrams in color indicate targeted groups. Red, orange, dotted, and striped shading indicates expression of *CYC* paralogs from the CYC2 clade (*DipsCYC2A*, *DipsCYC2a*, *DipsCYC2B*, *DipsCYC2b*, respectively). Separate independent duplications in Adoxaceae and Caprifoliaceae led to the a/b paralogs and the A/B paralogs. Five-parted circles labeling nodes indicate hypothesized ancestral expression of the duplicated paralogs across the five petals. Gray circles indicate that the transition could have happened at either node. Work is currently being continued on gene expression in groups with floral diagrams in blue. The floral diagram in purple indicates a group being studied with VIGS to assay loss-of-function morphology. The data and image are modified from Howarth, D.G., Donoghue, M.J., 2005. Duplications in *CYC*-like genes from Dipsacales correlate with floral form. International Journal of Plant Sciences 166, 357–370; and Howarth, D.G., Martins, T., Chimney, E., Donoghue, M.J., 2011. Diversification of *CYCLOIDEA* expression in the evolution of bilateral flower symmetry in Caprifoliaceae and *Lonicera* (Dipsacales). Annals of Botany 107, 1521–1532.

An increasing number of studies are examining floral symmetry candidate genes across various angiosperms and correlating expression with floral shifts (see Hileman, 2014; Specht and Howarth, 2015). Similar to previously discussed Dipsacales, three recent papers in Malpighiales have examined the expression of CYC2 gene clade members across multiple species, targeting groups with differing floral symmetries (Zhang *et al.*, 2010, 2012, 2013b). These studies, using semi-quantitative and qPCR, suggest that radially symmetrical flowers have uniform CYC2 expression that then shifts to dorsally restricted CYC2 expression only in bilaterally symmetrical groups (Zhang *et al.*, 2010). Four independent reversals to radial symmetry are correlated with either absent or ubiquitous CYC2 expression across the corolla (Zhang *et al.*, 2013b). Our lab group is similarly working on CYC2 clade expression across the Core Goodeniaceae clade, a group of plants with a well-resolved phylogeny that has multiple gains of fan flowers, in which all the petals are oriented ventrally (Han *et al.*, 2013; Jabaily *et al.*, 2012).

Other model clade approaches that have been used to map expression across a clade include examining floral development genes in Poaceae (grasses) (Malcomber and Kellogg, 2004; Preston *et al.*, 2012; Reinheimer and Kellogg, 2009), Zingiberales (gingers and bananas) (Yockteng *et al.*, 2013), and *Aquilegia* (columbines) (Whittall *et al.*, 2006). In grasses, for example, Malcomber and Kellogg (2006) showed that variation in gene expression of *TASSELSEED2* (*TS2*) across three species indicated that this gene likely plays a role in more morphological shifts across grasses than simply causing gynoecium abortion (resulting in the male flowered corn tassel) in maize. A separate model clade that has been used to assay expression is *Aquilegia* (columbines), especially for examining the evolution of developmental traits in an adaptive radiation (Abzhanov *et al.*, 2008). Examining seven independent losses of floral anthocyanins, the researchers found that, in a genetic pathway of six known genes, most of the losses were caused by changes in the downstream-most members of the pathway, suggesting convergence in loss of gene expression in floral color (Whittall *et al.*, 2006).

Among animals, there have long been calls to use mammals in a similar way that we outline here, especially in regards to studying the morphological and functional variations of the tetrapod limb (Ross *et al.*, 2013; Sears *et al.*, 2007; Sears, 2011, 2014; Stopper and Wagner, 2005). Despite strong underlying constraints in bone structure, including a distal autopod (hand/foot) with predominantly five digits (fingers/toes), mammalian limbs exhibit striking variation not just across species (i.e., human arms, bat wings, cetacean flippers, hoofed ungulates), but also from the forelimbs to hindlimbs of a given species (i.e., bat wings vs. feet). In addition to the variation in limb morphology, there are multiple well-mapped reductions in digit number throughout the clade, matching the criteria described above for a useful model clade. For example, by comparing multiple species throughout the clade, Cooper *et al.* (2014) determined that at least two different mechanisms were independently utilized to accomplish the reduction. They found that in jerboas, horses, and camels, increased apoptosis led to the reduced digit number, while a shift in anterior-posterior (thumb to pinky) patterning was behind digit reduction in the pig hoof, a mechanism recently also found in cows (Lopez-Rios *et al.*, 2014).

Perhaps one of the most well-studied clades of phenotypic variation among closely related species is the Galapagos finches. Using a candidate gene approach, Abzhanov *et al.* (2004) took advantage of the well-known group in an attempt to identify the genetics underlying the differences in beak size and shape among six species of *Geospiza* (ground finches). The expression of multiple candidate genes with roles in craniofacial patterning known from the chicken were examined for correlation with changes in beak morphology across *Geospiza*. Because they were able to examine several closely related species with specific known morphological variation, they found via in situ hybridization that the expression of one of their candidate genes, *BMP4*, varied in concert with beak depth and breadth across the clade. Species that exhibited *BMP4* expression in the beak primordium mesenchyme earlier and at higher levels developed broader and deeper beaks than those species in the clade with lower levels of expression. They corroborated this finding by misexpressing *BMP4* in the chicken beak mesenchyme, which induced similarly larger beaks. Therefore, Abzhanov *et al.* (2004) discovered a novel role in patterning beak depth for *BMP4* by examining gene expression across a clade. While it may seem obvious that studying a model clade can inform our understanding of evolution, this is a case where the use of a model clade provided a significant insight into developmental processes.

While the Drosophilid species complex was an early target of fine scale model clade studies (Jeong *et al.*, 2006), other studies have broadened the scope of the clade used to include a wider diversity of insects. Yoder and Carroll (2006) used Diptera to understand the causes of the reduction in both size and number of abdominal segments specific to the higher Dipterans such as *Drosophila*. Comparison of timing and localization of *Abd-B* gene expression between *Drosophila* and two other disparately related insect species (a locust and a firebrat) (Kelsh *et al.*, 1993; Peterson *et al.*, 1999) suggested that *Drosophila* gene expression shifts and the presence of a splice-variant protein isoform may correlate with abdominal segment loss. Using five species across the Dipteran clade, they aimed to evolutionarily pinpoint more precisely the increase in *Abd-B* gene expression and alternative splicing seen in *D. melanogaster*. However, they found that the increase in the *Abd-B* gene expression domain occurs across all Diptera and the alternative splice variant was found only *D. melanogaster*, and therefore neither correlates with the shift in abdominal segment loss that occurs specifically across higher Diptera. Using this model clade approach, the authors suggest that changes in *Abd-B* downstream target genes may be responsible for this morphological shift (Yoder and Carroll, 2006).

Using the Arthropod clade, Sharma *et al.* (2014b) examined gene expression of *cap-n-collar* (*cnc*), a transcription factor important in mandible development, in three arthropods. They found that while *cnc* is specific to segments containing the mandibles and labrum in Hexapoda, Crustacea, and Myriapoda (collectively, the Mandibulata), its expression is not localized in non-mandible-containing groups, such as the Chelicerata. This correlates hypothesized gene function (patterning development of mandibles) with the synapomorphy of having mandibles in Mandibulata (Sharma *et al.*, 2014b). All of these clade-based expression studies collectively demonstrate how much more complex the relationship between

gene expression and function is across groups than examining distantly related individual models would imply.

Gene Function or Regulation across Model Clades

Growing numbers of functional tools are becoming available, particularly for non-model species. In plants, virus-induced gene silencing (VIGS) uses the natural immune response of an RNA-mediated posttranscriptional gene silencing to knock down genes (Dinesh-Kumar *et al.*, 2003). This method does not require transformation and can potentially be used across many plant species. For instance, *Thalictrum* is being examined as a model clade for Evo-Devo in pollination syndrome shifts. VIGS has been successfully used to knock down reporter genes in three species that encompass the major pollination diversity in the group: inconspicuous flowers, showy petaloid flowers, and petaloid stamens. Gene function can be analyzed in all three species with a similar protocol, providing information about function in different morphological characteristics (Di Stilio *et al.*, 2010). A separate study published an effective VIGS protocol that can be similarly used across four Solanaceae species (Yan *et al.*, 2012). Gene function is more complex than gene expression to assay across a clade; however, methods such as VIGS provide a tool to determine how loss-of-function phenotypes of orthologous genes may differ among related wild-type morphologies.

Pinpointing the specific regulatory changes that result in trait variation is currently of great interest in Evo-Devo and addresses fundamental questions about how adaptive evolution occurs (Carroll, 2008; Hoekstra and Coyne, 2007; Specht and Howarth, 2015). Prud'homme *et al.* (2006) examined regulatory changes across the Drosophilid clade, examining two independent losses and two independent gains of wing coloration. The authors found that the two losses target the parallel inactivation of the same *cis*-regulatory element. The two gains, on the other hand, resulted from modifications of different *cis*-regulatory elements. Similarly in the plant clade Ranunculaceae, *AP3-3* expression loss (described above) is correlated with petal loss, however in each of the five studied cases, the loss occurred through different mechanisms to silence or down-regulate the ortholog (Zhang *et al.*, 2013a). These studies suggest that even when there is co-option of the same gene to build (or lose) the same trait, the gene can be differentially regulated.

Martinez *et al.* (2014) also used the Drosophilid clade, in this case to examine the evolution of the *eve* stripe 2 element (S2E), which drives the stripe 2 expression of the pair-rule gene *even-skipped* (*eve*) in the *Drosophila* blastoderm (see Borok *et al.*, 2010). While the expression of *eve* is conserved across the clade, the sequence of the S2E, a 1.7 kb region, is essentially unalignable. Seeking to understand how this region maintained conserved expression over 100 million years of sequence divergence, the authors were able to work from previous theoretical models built in their lab to model *eve* gene expression based on changes in enhancer sequence throughout the clade. Working from the phylogeny and their model, they were able to 'walk back' putative ancestral states of the enhancer to nodes of species divergence and demonstrated conserved expression of a reporter driven by their putative ancestral enhancers.

Phylogenomics

Where phylogenetics is particularly necessary and informative is determining gene orthology across multiple groups. With the growing number of published genomes, transcriptomes, and EST databases, there is a need for thorough phylogenetic analyses of various gene groups, and innumerable research projects examining molecular evolution of these genes (Becker *et al.*, 2011). An additional powerful use of phylogenomics involves analyzing genomic data across clades to uncover novel genes and microRNAs (Drosophila 12 Genomes Consortium, 2007; Stark *et al.*, 2007). The sequencing of 12 *Drosophila* genomes has provided powerful phylogenomic tools, even in providing higher-quality assemblies by using closely related species as guides. As an example, these genomes have been compared to find genes that are specific to certain clades such as 44 protein-coding genes specific to the six sampled species in the melanogaster clade, and 4 genes specific to *Drosophila melanogaster* (Drosophila 12 Genomes Consortium, 2007). Researchers further used these genomes to predict at least 41 novel microRNAs in *Drosophila*. They show that as they increase species number and divergence, they are able to find increasingly more microRNAs (Stark *et al.*, 2007), further highlighting the power of a clade-based approach.

Genomic and microarray data have also been used to uncover novel gene function in two bird clades. Using Galapagos finches, Abzhanov *et al.* (2006) examined cDNA microarrays derived from the developing beak of five finch species, comparing these data with beak length. From the microarrays, the Ca^{2+}-binding protein *calmodulin* (*CaM*) emerged as the most promising candidate, with expression of CaM being higher in species with longer beaks. As in their previous study (Abzhanov *et al.*, 2004, see above), expression levels were compared across species via *in situ* hybridization, and misexpression in the chicken corroborated the observed relationship between gene expression and phenotype. As *CaM* was not previously known to be involved in beak development, this is an example of the discovery power of the model clade approach. In a separate example, Greenwold *et al.* (2014) used genomes from 48 different bird species to compare α- and β-keratin gene evolution, indicating that the diversity among feathers may be due in part to a large expansion in β-keratin genes.

Conclusion

The growing use of a phylogenetic- or clade-based approach to Evo-Devo is suggesting that much of trait variation, and therefore evolutionary change, is due to shifts in location, timing, or intensity of gene expression. However, there is considerable variability among studies as to the genes that are shifted and the mechanisms that have resulted in the shifts. Based on the data outlined here, it appears to be much more common for the same gene to be targeted in independent morphological shifts, although this can happen through different mechanisms, such as changes within or across enhancers, or the transcription factor binding to those enhancers. It remains to be seen whether there are general patterns in how gene expression is modulated to lead to trait gains and losses, particularly whether the same molecular site or mechanism

gets utilized repeatedly across a clade. Data from model clade studies should shed light on the genetic tinkering that shapes morphological change.

Evolution has resulted in endless numbers of novel and modified morphological traits. Using a phylogenetic context allows us to pinpoint the location of trait changes among the natural biological variation of organisms, therefore, it provides the functional experiment already completed in many different diverse ways. The ability to use a model clade approach, placing morphological and genetic change in a phylogenetic context, creates the backbone of the complex evolutionary puzzle that we are aiming to put together. This context allows us to connect the remarkable group of randomly shaped and colored puzzle pieces that Evo-Devo has provided into a clearer picture of how developmental processes and evolutionary tinkering have led to diversification.

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See also: Model Systems: The Key Roles of Traditional and New Models in Evolutionary Developmental Biology. Regulatory and Coding Changes in Developmental Evolution, Roles of

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Phylogenetic Comparative Method

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Glossary

Brownian motion Motion of a particle being bombarded by other particles, often used as a model for continuous trait evolution.

Independent contrasts Another term for the comparative method described in this article.

Phylogeny Branching history representing evolutionary relationships.

All life on earth shares traits due to common ancestry: use of DNA for information storage, use of proteins for molecular machinery, and much more. Within smaller subgroups consisting of species all sharing a common ancestor (such a grouping is known as a clade), there are even more similarities: all mammal species are warm-blooded, have some fur or hair, and produce milk, for example. Species are not independent of each other: it may be that hair and milk production are advantageous in combination, but this has arisen once in history, not independently in thousands of mammal species.

Many statistical methods rely upon independence of data points. For example, hemophilia is more common in European royalty than in the general population. It could be that something about being a member of royalty carries this risk, but the close genetic relationships within many royal families suggests that instead this is due to sharing the gene for hemophilia in common: the frequency is due to shared ancestry rather than some causal association between traits. There are many questions relating to traits of species and correlations that seem to violate this assumption of independence. For example, primates have larger brains for their body size than other mammals, and they are also more intelligent on various measures than most other mammals. It could be that large relative brain size leads to increased intelligence, but primates share many other traits as well: binocular vision, grasping forelimbs, and more: it could be that brain size increased once in the lineage leading to modern primates due to some other reason.

Fortunately, Felsenstein (1985) developed an approach to deal with the nonindependence of species. This has become known as independent contrasts or the comparative method. The basic idea underlying this approach is that while species are not independent, the changes happening along branches are. Rather than examine the raw values at the tips of the tree, independent contrasts infers changes along pairs branches and standardizes these by time to provide transformed data points that meet the assumptions of most statistical methods. It is roughly equivalent to doing a twin study in medicine, except that rather than looking at pairs of siblings to see how a change in trait X correlates with a change in trait Y it uses pairs of branches descended from the same common ancestor.

Figure 1 shows an example of this. It has six species which share a common ancestor. The raw species values show a positive correlation between Trait X and Trait Y. However, this is largely due to an increase in Trait X in the branch leading to from the common ancestor to the ancestor of taxa D, E, F and a decrease in Trait Y on the branch leading from the common ancestor to the ancestor of taxa A, B, C. These changes happened on two different parts of evolutionary history but by using the raw species values one would think that there are six points that support this pattern. The comparative method, instead, compares along a pair of edges (shown by blue arrows). There are five such comparisons; of this, only one, the contrast between the ancestor of A, B, C and the ancestor of D, E, F, shows a strong positive correlation.

Contrasts between a pair of sister taxa, such as between D and E or A and B, just requires computing the difference between observed values. However, other contrasts must use reconstructions of states at internal nodes. In most methods for doing reconstruction at ancestral states, information from all taxa on the tree is relevant and used. In independent contrasts, only information from descendant nodes of the node of interest is used (these descendant nodes could be other nodes on the tree or tip taxa). This allows for true independence: information about C does not affect the reconstruction of the ancestor of A and B. For such reconstructions, a model must be used. This approach canonically uses Brownian motion. Brownian motion

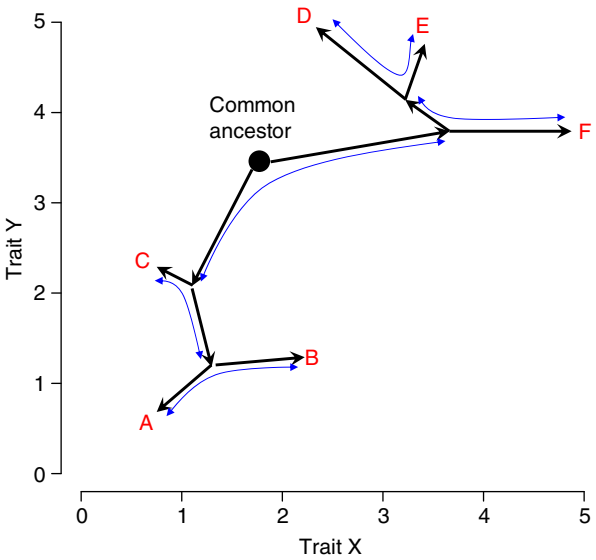

Figure 1 Black lines show the phylogeny, with end points placed corresponding to the values of Trait X and Trait Y. Blue arrows show the comparisons between nodes that are done in independent contrasts.

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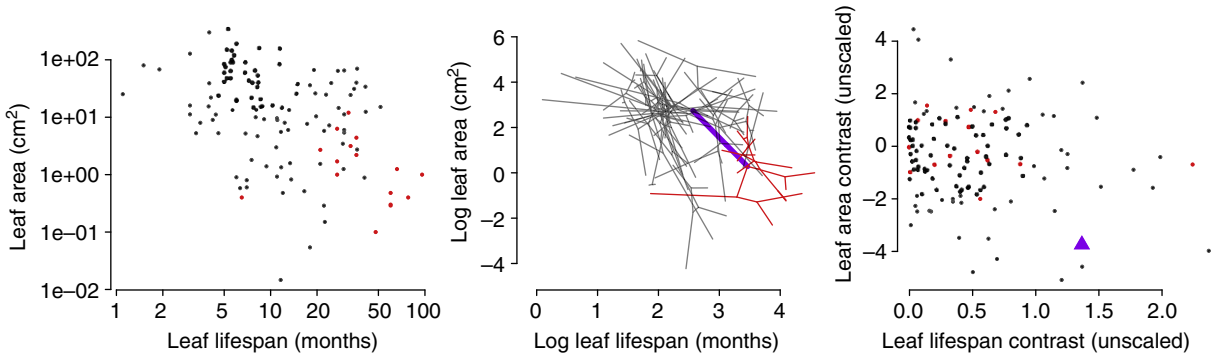

Figure 2 The left hand plot shows the raw trait values for a variety of plant species. The center plot shows the tree with nodes placed at the trait values (on a log scale). The right hand plot shows the independent contrasts. In all plots, gray or black corresponds to angiosperms, red to acrogymnosperms, and purple to the branch leading from the acrogymnosperm part of the tree to the angiosperm clade.

is a general process describing the movement of a particle being shifted due to addition of multiple other forces. Its power and generality comes from the central limit theorem: a particle being moved by the additive combination of a set of independent and identically distributed impulses will have its net displacement follow a normal distribution. For example, a species following an adaptive optimum that shifts across a landscape undergoes Brownian motion, as does a species where the trait phenotype is set by genetic drift (Hansen and Martins, 1996). One assumption of Brownian motion is that expected magnitude of a movement is the same regardless of the current trait value. This may not work for many traits: an elephant species is more likely to lose one kilogram of mass over a million years than a mouse species is (especially given that the mouse species starts out at less than a kilogram and mass has to be positive). A usual approach to deal with this is to work with log-transformed characters instead of the original characters: an elephant and a mouse species maybe equally likely to lose 5% of body mass over a million years. Contrasts down the tree are usually standardized to have unit variance; this scaling is based on the branch length which is usually in units of time, though number of generations or other metrics may be used depending on the evolutionary process. While the comparative method is implemented in many software programs (Martins, 2004; Paradis *et al.*, 2004; Felsenstein, 2005), making it straightforward to use, application of the comparative method does benefit from careful procedures to make sure its use is valid: does the data meet the assumptions, are the appropriate branch lengths being used, and so forth. Garland *et al.* (1992) provide an overview of some of the procedures which are typically followed.

A compelling example of the need for and use of the comparative method comes from Ackerly and Reich (1999). They reanalyzed published data for various seed plant traits using the comparative method. For many correlations, results were unchanged with and without adopting this approach. One counterintuitive result from using raw species values was a strong negative correlation of -0.424 between leaf area and leaf life span: plants with larger leaves held on to them for shorter periods of time. However, this is due to a single evolutionary change: flowering plants like oaks and blueberries have large, relatively short-lived leaves while non-flowering seed plants (acrogymnosperms) like pines have small leaves that they hold on to for long periods of time. Raw species values overcount this: it changed on one branch, but there is then pseudoreplication by treating each species as an independent data point. If the comparative method is used instead, that change is still present but it represents just one contrast; the larger set of all contrasts indicates that there is no correlation between an increase in leaf life span and a decrease in leaf size. **Figure 2** demonstrates this using data from Price *et al.* (2014) and a phylogeny from Zanne *et al.* (2014). The left panel shows the raw data, which suggest a negative correlation. However, plotting flowering plants in black and acrogymnosperms in red it is easy to see that much of this comes from some long-lived, small gymnosperm leaves (such as pine needles) in contrast to angiosperm leaves. The center panel shows the same information, but with the points connected by their evolutionary relationships. This is a phylogenetic tree, but with the tips placed based on the two trait values and the internal nodes placed based on reconstructed ancestral states. The crossing lines suggest that during the evolution of seed plants, the same regions of trait space were invaded multiple times. One thing to note is the purple line representing the branches connecting flowering plants to acrogymnosperms: it shows a definite displacement and may lead to much of the pattern. The right panel shows the raw contrasts (unscaled for variance) generated by the comparative method. The purple contrast represents the difference between flowering plants and acrogymnosperms and is clearly an outlier; the points in general do not show a clear pattern of increases in life span correlating with a decrease in size, suggesting that just a few changes on the branches were driving the pattern.

The comparative method has been used in thousands of empirical studies; the original Felsenstein (1985) paper has been cited over 4000 times, only recently levelling off at over 250 citations per year for each of the past 7 years. It has been appropriately used for morphological, behavioral, and physiological data for species ranging from fungi to mammals, bacteria to conifers.

There are criticisms of the method, as well. First, it imposes a requirement of getting a tree with branch lengths proportional to amount of expected change. Phylogenetic trees are becoming more available through repositories like TreeBase or Open Tree of Life, and making of phylogenies for one's group of interest has become more accessible through GenBank and other repositories of relevant data and wide availability of phylogeny

inference software, but the process is still nontrivial and sometimes extremely difficult in understudied groups. Another question is whether this is worth doing. Price (1997) compared correlations generated with raw species values with those from independent contrasts and found a correlation of 0.86; Ricklefs and Starck (1996) performed a similar analysis with the same basic outcome. This suggests that in some, but clearly not all, cases, one could ignore the nonindependence of raw species values and have the same basic results about correlations. Another concern is the introduction of error by dint of using an uncertain phylogeny, though independent contrasts has been shown to be robust, or at least conservative, to many errors (Diaz-Uriarte and Garland, 1998; Symonds, 2002). There are also concerns about its appropriateness at increasingly large scales, when rates and correlations likely vary across taxa (Felsenstein, personal communication; Losos, 2011).

Overall, however, while there are numerous other methods for investigating correlations between traits (i.e., Hansen, 1997; Martins and Hansen, 1997; Paradis and Claude, 2002; Revell and Collar, 2009; Felsenstein, 2012), the comparative method remains a staple of evolutionary analysis. It provides a robust method for returning statistically well-behaved data that can be used with standard statistical techniques to investigate trait correlation, and it is an essential part of an evolutionary biologist's skill set.

See also: Ancestral Reconstruction: Theory and Practice. Maximum Likelihood Phylogenetic Inference. Molecular Evolution, Models of. Phylogenetic Approach to Studying Developmental Evolution: A Model Clade Approach. Phylogenetic Tree Distances

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Phylogenetic Invariants

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Introduction

'Phylogenetic invariants' as a possible method for phylogenetic inference were introduced by Cavender and Felsenstein (1987) and Lake (1987). The method was intended to provide a new way for tree estimation that is capable of overcoming the usual limitations and issues of neighbor-joining or maximum-likelihood methods. Instead of estimating branch lengths and substitution parameters, and searching in tree space, phylogenetic invariants have the potential to predict whether specific observed frequencies of patterns in the real data belong to a particular tree topology. Phylogenetic invariants are equations in the expected frequencies of site patterns that must be satisfied on any probability distribution under a particular Markov model and tree.

Although invariants had attractive statistical properties, they did not perform well on simulated data as was demonstrated by Huelsenbeck (1995) and as a result doubt about their usefulness had settled among some researchers. However, since that time a lot of progress has been made in our understanding of phylogenetic models using techniques from algebraic geometry. Many useful constructions and more complicated invariants have emerged, which led to the development of several inference methods based on specific invariants associated with the bipartitions induced by an internal edge of the tree (Eriksson, 2005; Fernández-Sánchez and Casanellas, 2014; Chifman and Kubatko, 2014). Our understanding of the structure of invariants for some complicated models is not complete but it is clear that they have a lot of potential not only for theoretical analysis but also for data analysis and inference.

In this article we provide very brief introduction to phylogenetic invariants and concentrate primarily on those that have led to methods for tree estimation. We omit algebraic interpretation of phylogenetic models as the language of algebraic geometry is beyond the scope of this article, but we hope this article supplies enough flavor and demonstrates the complexity and beauty of phylogenetic invariants that will encourage the reader for further investigations. We suggest the book *Inferring Phylogenies* by Felsenstein (2004, Chapter 22) as an initial introduction to invariants. For a gentle introduction from the algebraic view point, see *Reconstructing Evolution: New Mathematical and Computational Advances*, Chapter 4, written by Allman and Rhodes (2007).

Phylogenetic Models on Gene and Species Trees

Phylogenetic models on gene trees have been introduced in earlier articles, hence in this section we just recall a few ideas for notational purposes for the general Markov model. Additionally, we provide a brief introduction to phylogenetic model under the coalescent for a species tree (for a concrete introduction to this model see Chifman and Kubatko (2015)).

Phylogenetic Models on Gene Trees

For convenience we will assume that all trees are binary, that is, all internal nodes have degree three, except possibly for a root node, which is of degree two. Let T be an n-leaf, binary, gene tree and consider the general κ-state Markov model, $GM(\kappa)$, of character evolution, with $\kappa=4$ corresponding to DNA sequences, i.e., possible states are A, C, G, and T. Taxa labeling the leaves of the tree T are identified with an integer in the set $\{1,2,...,n\}$ and the root is identified with some existing node. The probability distribution of the states at the root is represented by the vector $\pi=(\pi_1, \pi_2,\ldots, \pi_\kappa)$, with the constraints that

$$\pi_j>0 \text{ for } j\in\{1,2,\ldots,\kappa\} \text{ and } \sum_{j=1}^{\kappa}\pi_j=1$$

For each edge e of the tree T (e directed away from the root), a $\kappa\times\kappa$ Markov matrix M_e describes transition probabilities. Each (i, j)-entry of the matrix M_e, denoted by $P(j|i, e)$, gives the conditional probability that the character is in state j at the end of edge e given that it was in state i at the start. From the definition of M_e one can see that

$$P(j|i,e)>0 \text{ and } \sum_{j=1}^{\kappa}P(j|i,e)=1$$

When the process of evolutionary change is modeled by a continuous-time Markov process (e.g., general time-reversible model), we specify a rate matrix Q and edge lengths, t_e. Matrix Q has nonnegative off-diagonal entries and the diagonal entries are set so that the rows sum to zero. In this case, the Markov matrix is given by $M_e(t_e)=\exp(QT_e)$ that describes the mutation along an edge e, which represents a time of length t_e.

Computation of site pattern probabilities on gene trees is straightforward once the gene tree and the Markov model have been specified. Since the states are not observed at the internal nodes of the tree, we must sum over all possible states for these nodes. In addition we assume that the mutation process proceeds independently along each branch and thus probabilities are multiplied together along each edge e.

Example 1: Consider a gene tree as labeled in Figure 1. Let X_ℓ be the state observed for lineage ℓ, $\ell=1,2,3$ and consider

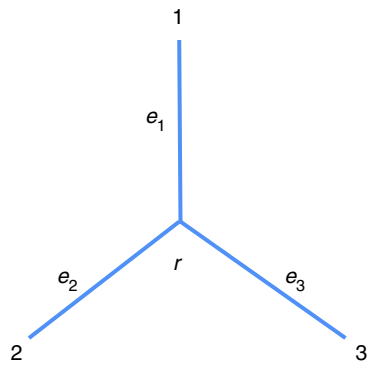

Figure 1 Three-leaf tree.

the tree to be rooted at r. Also, identify states with four bases $\{A, G, C, T\}$, that is, we let $\kappa=4$. The probability distribution of the states at the root is represented by $\pi=(\pi_A, \pi_G, \pi_C, \pi_T)$. The 4×4 Markov matrix on each edge e_i will be of the following form:

$$M_{e_i}=\begin{pmatrix} P(A|A,e_i) & P(G|A,e_i) & P(C|A,e_i) & P(T|A,e_i)\\ P(A|G,e_i) & P(G|G,e_i) & P(C|G,e_i) & P(T|G,e_i)\\ P(A|C,e_i) & P(G|C,e_i) & P(C|C,e_i) & P(T|C,e_i)\\ P(A|T,e_i) & P(G|T,e_i) & P(C|T,e_i) & P(T|T,e_i)\end{pmatrix}$$

The site pattern probability on this three-taxon gene tree for a particular observation, say AGG, at the tips of the tree is computed as follows:

$$\begin{aligned} p_{AGG} &= P(X_1=A,X_2=G,X_3=G)\\ &= \pi_A P(A|A,e_1)P(G|A,e_2)P(G|A,e_3)\\ &+\pi_G P(A|G,e_1)P(G|G,e_2)P(G|G,e_3)\\ &+\pi_C P(A|C,e_1)P(G|C,e_2)P(G|C,e_3)\\ &+\pi_T P(A|T,e_1)P(G|T,e_2)P(G|T,e_3)\end{aligned}$$

For a three-taxon tree with $\kappa=4$ states we will have $\kappa^3=64$ possible site pattern probabilities.

Let P denote the probability distribution on all possible site patterns for the n-leaf tree T under the specified Markov model. The probability distribution P is the n-dimensional $\kappa\times\cdots\times\kappa$ array with entries:

$$p_{i_1 i_2 \ldots i_n} := P(X_1=i_1,X_2=i_2,\ldots,X_n=i_n)$$

Site Pattern Probabilities for Species Trees under the Coalescent

Organismal evolution occurs at two distinct levels; at the level of the individual genes, and at the level of the species or populations as a whole. Both levels must be considered, since the evolutionary history of the species constrains the histories for the individual genes. Each individual gene has its own phylogeny, which may not agree with the species tree. One of the causes for the incongruence is incomplete lineage sorting (ILS), in which gene lineages share a most recent common ancestor much further back in time than the immediately ancestral population.

Multispecies coalescent theory is primarily used to model ILS. In particular, it models probabilities of rooted gene tree topologies within a given rooted species tree topology and branch lengths. Figure 2 is an example of all possible gene trees (blue lines) nested within the three-leaf tree representing the evolutionary history of the species (black lines that 'outline' a tree). In this figure the embedded gene trees within the species tree represent the phylogenetic histories of the lineages sampled from species a, b, and c, denoted by A, B, and C, respectively. The dotted black horizontal lines in this figure represent speciation events, times when one ancestral species becomes two distinct species, and are denoted by τ_i. The times $0<\tau_i<\infty$ are measured backward from the present time. Internal nodes in the gene tree represent gene divergence events, times when multiple gene copies arise in the ancestral population. Dotted blue lines are coalescent events that occur in the gene trees. In particular, the coalescent time $0<t_i<\infty$ is the time from the jth coalescent event to the next speciation event (looking forward in time). Highlighted portions of the tree between speciation events, denoted by P_i, are the ancestral populations. One can clearly see that the gene tree topology does not match the species tree topology in two instances (the bottom two images

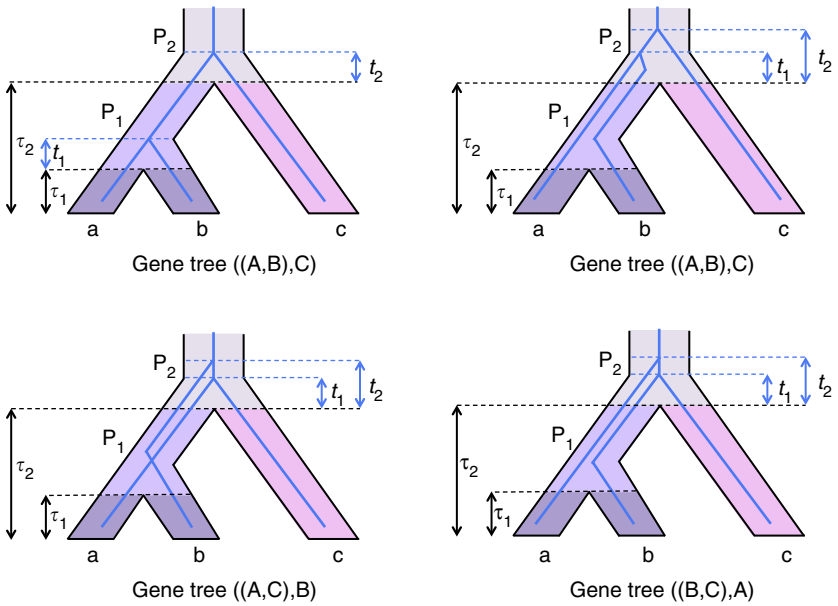

Figure 2 All possible relationships between the species tree (black, outline tree) and the gene tree (blue lines embedded within the species tree) for a three-leaf tree.

in Figure 2). This figure provides an example of ILS and highlights the crucial observation that gene trees and species trees need not agree with one another (Pamilo and Nei, 1988; Maddison, 1997; Nichols, 2001).

As in the case of gene trees it is possible to compute the probability distribution on all possible site patterns for n-taxon species trees under the multispecies coalescent model. However, the computation is rather involved: the model in Figure 2 implies that DNA sequence data evolve along gene trees, but the site patterns observed at the tips of the species tree may arise from more than one gene tree. Thus, the computation of site pattern probability at the tips of a species tree involves integrating over gene trees weighted by their probabilities under the coalescent model and then summing over all possible gene trees consistent with the given species tree. We omit the details here; the interested reader is encouraged to consult Chifman and Kubatko (2015). Let (S, τ) represent rooted, binary, n-leaf species tree endowed with a vector of speciation times τ. We will denote the probability distribution on all possible site patterns for a given species tree by the n-dimensional $\kappa \times \cdots \times \kappa$ array $P_{(S,\tau)}$ with entries $p_{i_1 \ldots i_n|(S,\tau)}$.

Phylogenetic Invariants

Consider a DNA Markov model $GM(4)$ on four bases $\{A, G, C, T\}$ for two three-taxon trees T_1 and T_2 as in Figure 3 under the molecular clock assumption, that is all leaves are equidistant from the root. Let AGA be a specific observation at the tips of T_1. Then, one can easily show that

$$p_{AGA} - p_{GAA} = 0 \qquad [1]$$

This linear function in the site pattern probabilities is an example of a linear invariant. This invariant holds for any distribution P arising from DNA Markov model $GM(4)$ on the tree T_1. On the other hand, the invariant (eqn [1]) does not hold on the tree T_2. Instead, a linear relation that holds on T_2 but not T_1 is

$$p_{AGA} - p_{AAG} = 0 \qquad [2]$$

Now, suppose we choose a submodel of $GM(4)$ on the same trees T_1 and T_2. Let the model of base change be the Jukes–Cantor DNA model, i.e., the equilibrium distribution among the four states is given by $\pi = (0.25, 0.25, 0.25, 0.25)$ and it has equal mutation rates. Under this model for both trees T_1 and T_2 the expected frequency of the pattern AGA will be the same as frequency of the GTG, that is,

$$p_{AGA} - p_{GTG} = 0 \qquad [3]$$

This is an example of a symmetry invariant, and these types of invariants are simply a consequence of a symmetry of the model. Symmetry invariants depend on the chosen model and not on the tree topology.

In general, an 'invariant' is a real-valued continuous function (usually a polynomial expression) in the site pattern probabilities (expected frequencies) that evaluates to zero on any probability distribution arising from the model. Originally, Felsenstein (1991) defined 'phylogenetic invariants' as polynomials that evaluate to zero on the expected frequencies on one tree topology but do not evaluate to zero for at least one tree of a different topology. Some researchers emphasize a difference between (1) invariants that distinguish among different tree topologies and (2) all invariants.

Phylogenetic invariants for many models have been studied using techniques from algebraic geometry and in some special cases the structure of invariants is well understood. Algebraic geometry is the study of the zero sets of collections of polynomials and its language provides powerful tools for describing and addressing probabilistic models. The study of phylogenetic models is part of a growing discipline – algebraic statistics. The richness and complexity of this subject is beyond the scope of this article but we hope this will inspire curious readers for further investigation. For a concrete introduction to algebraic statistics see Pachter and Sturmfels (2005) and to algebraic geometry see Cox *et al.* (2008).

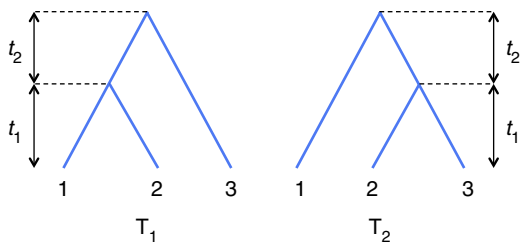

Figure 3 Three-leaf binary trees T_1 and T_2 satisfying molecular clock.

Splits and Flattenings

Phylogenetic invariants arising from flattenings of probability distributions are the most relevant and important among invariants. In this section we provide an overview of invariants that arise from flattenings induced by the single edge of the tree. Our choice for introducing these group of invariants (induced by an edge) lies in the fact that they play crucial role in the arguments concerning identifiability and estimation of the tree topology, and several algorithms have been implemented based on these invariants. For a comprehensive review and extensions to flattenings and invariants induced by a vertex we encourage the reader to consult Allman and Rhodes (2003, 2007, 2006, 2008a).

Let T be an n-leaf binary unrooted tree with leaves labeled by $L = \{1,2,\ldots,n\}$ and let $L_1|L_2$ be a split (bipartition) of L into two nonempty subsets L_1 and L_2. A split $L_1|L_2$ is 'valid' if the subtrees containing the taxa in L_1 and in L_2 do not intersect. We will say that a split is 'edge induced' if it is obtained by removing an edge of the tree T. Notice that a split is valid exactly when it is induced by an edge.

Example 2: Consider a four-taxon tree as in Figure 4 (top panel). This tree has a valid split 12|34 induced by an edge e_3. Splits 13|24 and 14|23 are not valid. One can see in this figure (bottom right image) that trees induced by the partition 13|24 intersect at the edge e_3 making this partition not valid.

Splits of a set of taxa provide a natural way to flatten (rearrange) an n-dimensional $\kappa \times \cdots \times \kappa$ probability distribution P into a matrix. Let $L_1|L_2$ be any split of a set of taxa L and denote

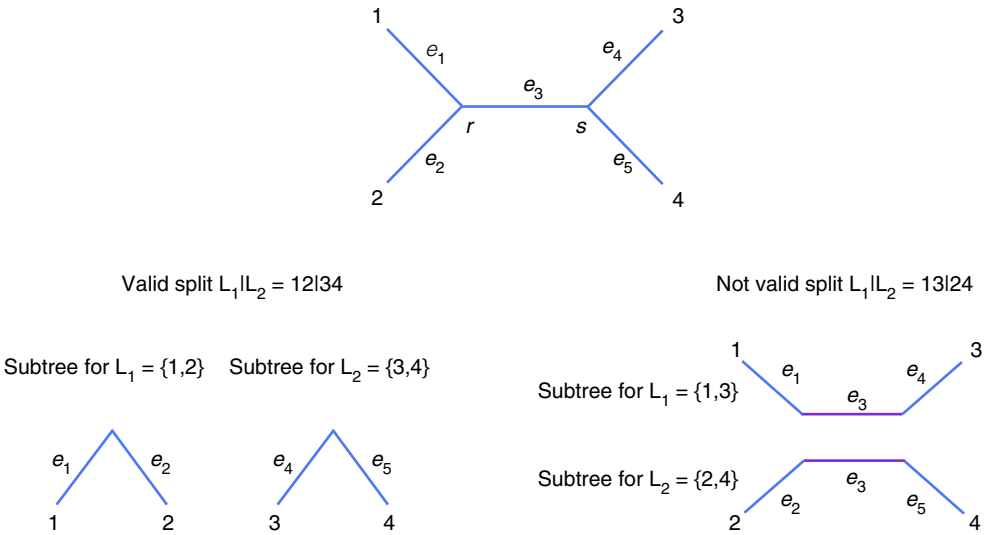

Figure 4 Four-leaf tree with an example of two partitions 12|34 and 13|24 and corresponding subtrees.

the cardinality of a set L_i (i.e., the number of elements) by $|L_i|$. A 'flattening' of the n-dimensional $\kappa \times \ldots \times \kappa$ array P, $\text{Flat}_{L_1|L_2}(P)$ is a $\kappa^{|L_1|} \times \kappa^{|L_2|}$ matrix, whose rows are indexed by possible states for the leaves in L_1 and columns by possible states in L_2. The entries of $\text{Flat}_{L_1|L_2}(P)$ correspond to the probability of the site pattern specified by the row and column indices.

Example 3: For a DNA Markov model $GM(4)$ on four bases $\{A, G, C, T\}$, let the four-taxon tree T be as in Figure 4 (top panel). Then the two flattenings of P for the splits 12|34 and 13|24 are the 16×16 matrices:

$$\text{Flat}_{12|34} = \begin{bmatrix} p_{AAAA} & p_{AAAG} & p_{AAAC} & p_{AAAT} & p_{AAGA} & \cdots & p_{AATT} \\ p_{AGAA} & p_{AGAG} & p_{AGAC} & p_{AGAT} & p_{AGGA} & \cdots & p_{AGTT} \\ p_{ACAA} & p_{ACAG} & p_{ACAC} & p_{ACAT} & p_{ACGA} & \cdots & p_{ACTT} \\ p_{ATAA} & p_{ATAG} & p_{ATAC} & p_{ATAT} & p_{ATGA} & \cdots & p_{ATTT} \\ p_{GAAA} & p_{GAAG} & p_{GAAC} & p_{GAAT} & p_{GAGA} & \cdots & p_{GATT} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ p_{TTAA} & p_{TTAG} & p_{TTAC} & p_{TTAT} & p_{TTGA} & \cdots & p_{TTTT} \end{bmatrix}$$

$$\text{Flat}_{13|24} = \begin{bmatrix} p_{AAAA} & p_{AAAG} & p_{AAAC} & p_{AAAT} & p_{AGAA} & \cdots & p_{ATAT} \\ p_{AAGA} & p_{AAGG} & p_{AAGC} & p_{AAGT} & p_{AGGA} & \cdots & p_{ATGT} \\ p_{AACA} & p_{AACG} & p_{AACC} & p_{AACT} & p_{AGCA} & \cdots & p_{ATCT} \\ p_{AATA} & p_{AATG} & p_{AATC} & p_{AATT} & p_{AGTA} & \cdots & p_{ATTT} \\ p_{GAAA} & p_{GAAG} & p_{GAAC} & p_{GAAT} & p_{GGAA} & \cdots & p_{GTAT} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ p_{TATA} & p_{TATG} & p_{TATC} & p_{TATT} & p_{TGTA} & \cdots & p_{TTTT} \end{bmatrix}$$

Edge invariants

To describe edge invariants for the models discussed in Phylogenetic Models on Gene and Species Trees, we first informally introduce a few necessary concepts from linear algebra. Consider three vectors $x_1 = (3,6,6)$, $x_2 = (1,0,2)$, and $x_3 = (1,3,2)$. Then $x_1 = x_2 + 2x_3 \rightarrow (3,6,6) = (1,0,2) + 2(1,3,2) = (1,0,2) + (2,6,4) = (1+2, 0+6, 2+4)$. Since we can write one of the vectors as a linear combination of the other vectors, we say that they are 'linearly dependent.' If none of the vectors is a linear combination of the others, then we say the set of vectors is 'linearly independent.' We can also talk about linear dependence (independence) if we replace vectors consisting of numbers with real-valued functions, polynomials, or site pattern probabilities.

Example 4: Let $\text{Flat}_{12|34}$ be as in Example 3 for the general Markov DNA model on a four-taxon tree T in Figure 4. Suppose that edges labeled by e_4 and e_5 are equal. Then, for example, for observation $AAAG$ we will get

$$p_{AAAG} = p_{AAGA}$$

Notice that p_{AAAG} is located in column 2 first entry and p_{AAGA} in column 5 first entry of the matrix $\text{Flat}_{12|34}$. The same will be true about second entry of columns 2 and 5, i.e.,

$$p_{AGAG} = p_{AGGA}$$

In particular, columns 2 and 5 of the matrix $\text{Flat}_{12|34}$ are equal and thus these two columns are linearly dependent. Note, there are other pairs of columns that are equal (the reader is encouraged to find them all).

One of the fundamental properties of a matrix is its rank. It is defined as the maximum number of linearly independent columns (rows) and is called column (row) rank. One of the basic facts is that row rank equals column rank, and thus we simply call it rank. Another important concept is that of a determinant of a square matrix, which is a value that can be computed from the entries of the matrix. Here we omit specific expressions for computing the determinant. An important fact about this value is that if columns/rows of a square matrix are linearly dependent then the determinant will be zero. Lastly, we would like to emphasize that if we have a square $m \times m$ matrix such that rows/columns of this matrix are linearly dependent then rank of the matrix will be strictly less than m, call this rank r. This means that any submatrix obtained from the original matrix by picking $r+1$ columns and $r+1$ rows will have determinant equal to zero. The determinant of such

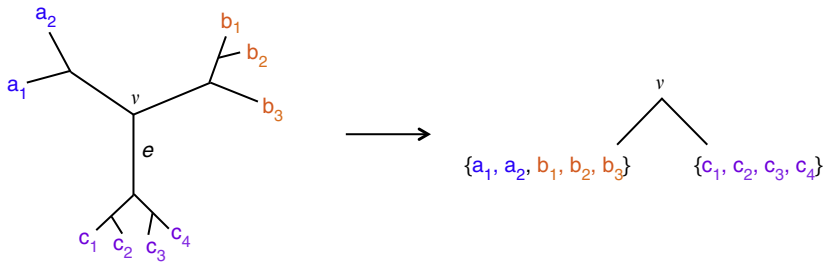

Figure 5 Flattening a model on an edge e.

submatrix is called $(r+1)\times(r+1)$ minor. We will denote the determinant of any matrix A by $det(A)$.

Flattenings based on valid splits, i.e., splits induced by an internal edge of the tree, have special properties. For the DNA Markov model $GM(4)$ on four bases $\{A, G, C, T\}$, let 9-taxon gene tree T be as labeled in Figure 5 (left panel). Then the internal edge e in this figure induces a split of the taxa $L_1|L_2$, where $L_1=\{a_1, a_2, b_1, b_2, b_3\}$ and $L_2=\{c_1, c_2, c_3, c_4\}$. Now we can think of a flattening of P as a probability distribution for a less complicated but related graphical model. In particular, by choosing the root to be one of the vertices of e, we view a new graphical model as the one that has one hidden random variable at the root with $\kappa=4$ states, and two descendent observed random variables with $\kappa^{|L_1|}=4^5$ and $\kappa^{|L_2|}=4^4$ states, respectively (see Figure 5 for a graphical depiction).

By interpreting a model in this way it can be shown that rank of the flattening $\text{Flat}_{L_1|L_2}(P)$ is at most 4. This implies from our discussion above that all 5×5 minors of $\text{Flat}_{L_1|L_2}(P)$ are zero, that is, they are phylogenetic invariants for the $GM(4)$ model on this tree T. Since these invariants are induced by the internal edge of the tree, they are called 'edge invariants.' We would like to note that edge invariants do not depend on the location of the root. These ideas can be extended to any κ-state general Markov model for any n-taxon gene tree T, and it can be shown that rank of the flattening for a valid split induced by any internal edge of the tree is at most κ. For a comprehensive overview of the above ideas see Allman and Rhodes (2007, 2006).

Example 5: Consider $\text{Flat}_{12|34}(P)$ from Example 3 for the $GM(4)$ model. This flattening is based on a valid split and thus this matrix has rank at most 4. This means that the determinant of any submatrix with 5 rows and 5 columns will be zero, i.e., edge invariant. As an example choose the first 5 rows and the first 5 columns from the matrix $\text{Flat}_{12|34}(P)$. The determinant of this submatrix is zero.

$$\det\begin{pmatrix}\begin{bmatrix} p_{AAAA} & p_{AAAG} & p_{AAAC} & p_{AAAT} & p_{AAGA}\\ p_{AGAA} & p_{AGAG} & p_{AGAC} & p_{AGAT} & p_{AGGA}\\ p_{ACAA} & p_{ACAG} & p_{ACAC} & p_{ACAT} & p_{ACGA}\\ p_{ATAA} & p_{ATAG} & p_{ATAC} & p_{ATAT} & p_{ATGA}\\ p_{GAAA} & p_{GAAG} & p_{GAAC} & p_{GAAT} & p_{GAGA}\end{bmatrix}\end{pmatrix}=0$$

We omit the expansion of this determinant (due to its length), which is just a polynomial in site pattern probabilities.

Now let S be a four-taxon binary species tree and let $P_{(S,\tau)}$ denote the probability distribution for the κ-state model under the coalescent as described in Section Site Pattern Probabilities for Species Trees under the Coalescent. It can be shown that for a valid split $L_1|L_2$ rank of $\text{Flat}_{L_1|L_2}(P_{(S,\tau)})$ is at most $\binom{\kappa+1}{2}$ (see Chifman and Kubatko (2015)). In the case of the DNA model under the coalescent, this rank is at most $\binom{4+1}{2}=\binom{5}{2}=10$ and thus 11×11 minors are all zero and are edge invariants. We would like to point out that rank condition exists for any n-taxon gene tree for $GM(\kappa)$ model, whereas for species tree under the coalescent model rank has been proven only for the four-taxon species tree.

Using Phylogenetic Invariants

This section provides a brief introduction to two important topics: identifiability of parameters and tree estimation from observed site pattern probabilities. There are other potential practical uses for invariants. For example, invariants can be used as a basis for a statistical test to differentiate between trees or to detect and quantify the extent of hybridization. Invariants and their structure are extremely appealing to many scientists and most likely they will find their way to be more useful for data analysis.

Identifiability

Many phylogenetic inference methods assume that the tree topology and other parameters can be identified from DNA sequence data at the leaves of the tree. Thus establishing as to what can be inferred under ideal conditions and proving that methods are statistically consistent and valid is of great importance.

The classical definition of identifiability can be formulated as follows: suppose that $\mathcal{M}(\Theta)=\{P_\theta:\theta\in\Theta\}$ defines a family of probability distributions with parameters θ. The model $\mathcal{M}(\Theta)$ is said to be 'identifiable' if the mapping $\theta\mapsto P_\theta$ is injective, that is, if for any $\theta_1\neq\theta_2\in\Theta$ we get that $P_{\theta_1}\neq P_{\theta_2}$. Unfortunately, for many models this map will not be injective. However, identifiability of parameters for finite space models is established by demonstrating that the set of parameters on which the model is non-identifiable is of measure zero within the parameter space. In this case we say that model parameters are 'generically identifiable.'

Techniques from algebraic geometry and invariants play an important role throughout proofs about identifiability. In the case of analytic models (e.g., covarion model or models under the coalescent), one can still use perspectives from algebraic geometry to prove generic identifiability (Chifman and Kubatko, 2015; Allman and Rhodes, 2006, 2009). Generic

identifiability for many evolutionary models on gene trees is well-studied and established. For a comprehensive introduction to identifiability using algebraic techniques we suggest a series of papers by Allman and Rhodes (2006, 2008a,b, 2009), Allman *et al.* (2008, 2011), and Rhodes and Sullivant (2012).

Species and Gene Tree Inference

Generally, identifiability of the tree topology is of prime interest. In this case edge invariants are used and are sufficient to identify the tree topology for generic parameters. Results in most cases are established using rank conditions on flattenings of the probability distribution. Moreover, it has been proved in Casanellas and Fernández-Sánchez (2011) that edge invariants are enough to reconstruct the phylogenetic tree for any number of species.

Using edge invariants for the estimation of the gene tree topology was initially proposed by Eriksson (2005). Eriksson's approach is similar to neighbor-joining; the tree is built by considering splits of varying sizes and iteratively joining taxa corresponding to the best split. Evaluating edge invariants for each flattening for various splits of taxa would be somewhat impractical, thus the main idea of the method is to compute a distance between each flattening and the nearest rank 4 matrix. However, the original method did not perform as well as neighbor-joining or maximum-likelihood and had a problem with short alignments and long branches.

Recently, the method has been revisited by Fernández-Sánchez and Casanellas (2014) for gene trees where they provide a procedure that supplies weights, which then can be used as inputs for quartet-based methods. For species trees under the coalescent, Chifman and Kubatko (2014) have also developed a method to infer relationships among quartets, and then use the quartets to estimate the overall tree. These methods performed well and showed high levels of accuracy. Below we provide a brief overview of the method for species tree estimation.

Suppose we have a set of n species and that there are D sites in the observed DNA sequences. First, we sample a set of four species $\{a, b, c, d\}$ from the n species and consider the three possible splits: $ab|cd$, $ac|bd$, and $ad|bc$. For each of these splits the flattening matrix $\text{Flat}_{L_1|L_2}(\hat{P})$ is estimated by replacing each entry with the observed site pattern frequency in the data. Next we assess which of these flattenings is closest to a rank 10 matrix. The number that we compute for each split is called an SVD score. The split yielding the smallest score is inferred to be the true split for taxa a, b, c, and d. Repeating this procedure for all sampled quartets leads to a collection of inferred splits for a set of quartets sampled from the overall set of n species. These quartet relationships can be used together to form an estimate of the overall tree. This is the well-studied problem of quartet assembly, for which numerous algorithms have been proposed (Strimmer and von Haeseler, 1996; Snir and Rao, 2012; Strimmer *et al.*, 1997). A species tree inference method under the coalescent model based on the results of Chifman and Kubatko (2014, 2015) has been implemented in the popular phylogenetic inference package PAUP* developed by Swofford (1998).

Conclusion

Introduction of phylogenetic invariants in 1987 led to a very reach area of research and many advances have been made since that time. Methods for the estimation of the tree topology based on phylogenetic invariants are now recognized as competitive and robust. Moreover, they have proved to be useful when addressing theoretical issues such as identifiability of the tree topology and other parameters. Invariants are definitely worth our attention and we hope that this overview, though very brief, will encourage many readers to continue their own investigations and possibly find new ways to use invariants for practical problems.

Acknowledgments

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See also: Maximum Likelihood Phylogenetic Inference. Molecular Evolution, Models of

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Phylogenetic Networks

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Glossary

Bipartition Division of the complete set of taxa into two nonoverlapping groups (sometimes also called a split).
Character A characteristic or trait being measured on a set of taxa for use in a phylogenetic analysis, which displays at least two mutually exclusive character states (e.g., present vs. absent).
Gene duplication The duplication of a block of genetic material, often involving a complete gene or even a whole chromosome.
Gene tree A generic term for a phylogenetic tree derived from data for a single non-recombining sequence block (sometimes loosely referred to as a locus).
Graph In phylogenetics, the model used is a graph consisting of nodes (representing taxa) connected by edges (representing their inferred relationships).
Horizontal gene transfer Movement of a small piece of a genetic material between unrelated organisms by means other than sexual reproduction.
Hybridization The merging of distinct population lineages to produce a new hybrid lineage, achieved by combining the genomes within each organism.
Incomplete lineage sorting Retention of allelic polymorphisms through one or more speciation events, followed by selective loss of some of the alleles (sometimes also called deep coalescence, or ancestral polymorphism).
Introgression Movement of a small piece of a genetic material between related organisms by means of sexual reproduction.
Most recent common ancestor The ancestor most recently shared between two or more taxa.
Recombination The formation of composite genetic material within an individual by the mixing of parental genes via processes such as crossing-over or re-assortment.
Sequence alignment An arrangement of DNA or protein sequences to indicate which nucleotides or amino acids are related by inheritance from a common ancestor, usually with the sequences running horizontally and the related molecules aligned vertically.
Taxon (plural taxa) A generic term for any level of the biological hierarchy (e.g., individual, population, species, genus, etc.)

Phylogenetic networks are graphical representations used to explore and model complex evolutionary relationships, most notably ones that contain reticulations, such as those caused by horizontal gene transfer (HGT) or hybridization (Huson *et al.*, 2011; Morrison, 2011). In this article, we describe the different classes of phylogenetic networks, their inference, and how they are to be interpreted.

Phylogenetic networks differ from the networks most commonly encountered in biology, in which the nodes represent observed entities (individuals, populations, and species) and their connecting edges represent observed relationships (e.g., food webs and protein interaction networks). Phylogenetic networks, on the other hand, connect nodes representing observed taxa via edges representing inferred historical relationships and extra nodes representing inferred ancestors.

The article is organized as follows. We begin by giving a brief overview of phylogenetic trees and their interpretation. We then discuss processes that motivate the need for phylogenetic networks. In the third part, we describe the types of data that are commonly used to reconstruct phylogenetic networks. In the last two parts, we describe data-display and evolutionary networks, with special emphasis on their interpretation.

Phylogenetic Trees First

A phylogenetic tree on a set of taxa is a tree whose leaves are labeled bijectively (i.e., every taxon labels exactly one leaf in the tree, and no leaf is unlabeled) by the taxa. There are two types of phylogenetic trees: unrooted trees (**Figure 1(a)**) and rooted trees (**Figure 1(b)**).

An unrooted phylogenetic tree provides a graphical representation of bipartitions, also known as splits, of the taxa that are pairwise compatible such that they could be uniquely combined into a single tree. That is, if the partitions are nested then the graph will be treelike, but if they overlap then the graph will show complex reticulation patterns.

For example, the unrooted phylogenetic tree in **Figure 1(a)** represents the bipartition where A and B form one partition and C and D form the other part. Given an unrooted tree, the set of all bipartitions that it represents can be obtained by 'cutting' the tree edges one at a time and, for each, recording the bipartition formed by the two sets of taxa separated by the removal of the edge. Indeed, inspecting the bipartitions displayed by an unrooted phylogenetic tree constitutes its main exploratory use by practitioners (i.e., it shows which bipartitions are best supported by the data).

A rooted phylogenetic tree provides a single hypothesis of the evolutionary history of a set of taxa from their most recent common ancestor. The interpretation of such a tree is based on the ancestor–descendant relationships that it captures. For example, the tree in **Figure 1(b)** captures three such relationships: A and B descended from a most recent common ancestor, say X; X and C descended from a most recent common ancestor, say Y; and, Y and D descended from the most recent common ancestor of all four taxa, which is the root node of

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the entire tree. Interpreting the tree the other way, the common ancestor splits into two descendants, one of which further splits into two descendants, and one of these splits again.

There is a straightforward relationship between unrooted and rooted phylogenetic trees. An unrooted tree can be rooted at any edge by introducing a new node into the edge and rooting the tree at the new node. A rooted tree can be turned into an unrooted tree by 'ignoring' the root node. These two operations are illustrated in Figure 2.

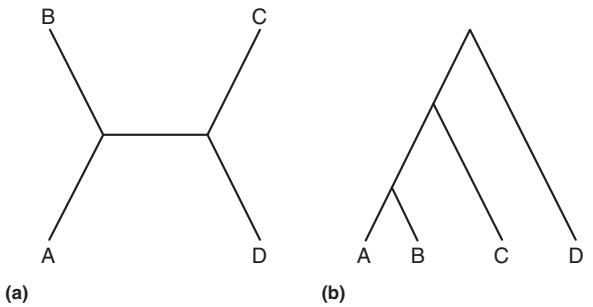

Figure 1 Phylogenetic trees. (a) An unrooted phylogenetic tree on four taxa: A, B, C, and D. This tree does not model ancestor–descendant relationships; rather, it provides a graphical representation of possible grouping of the taxa based on the data. In this case, A and B are grouped together, and C and D are grouped together. (b) A rooted phylogenetic tree on four taxa: A, B, C, and D. This tree models ancestor–descendant relationships: A and B descended from a common ancestor that, along with C, descended from a common ancestor that, along with D, descended from the most recent common ancestor of all four taxa.

Beyond a Single Tree

A phylogenetic tree is most commonly inferred, directly or indirectly, from a collection of characters and their states, most commonly a molecular sequence alignment, collected from the taxa under consideration. A main assumption underlying the inference of a phylogenetic tree is that all of the characters in the alignment have evolved down a single tree. When the sequence alignment consists of a single character, this assumption always holds. However, when an alignment contains two or more characters, different characters might have evolved down different trees (Nakhleh, 2010). It is important to note here that the different trees do not necessarily have to disagree topologically: Two different characters could have evolved down two trees whose topologies are identical, yet whose branch lengths are different. In situations where at least two characters have evolved down two different trees, a single phylogenetic tree is not sufficient to capture the evolutionary history of the whole sequence alignment.

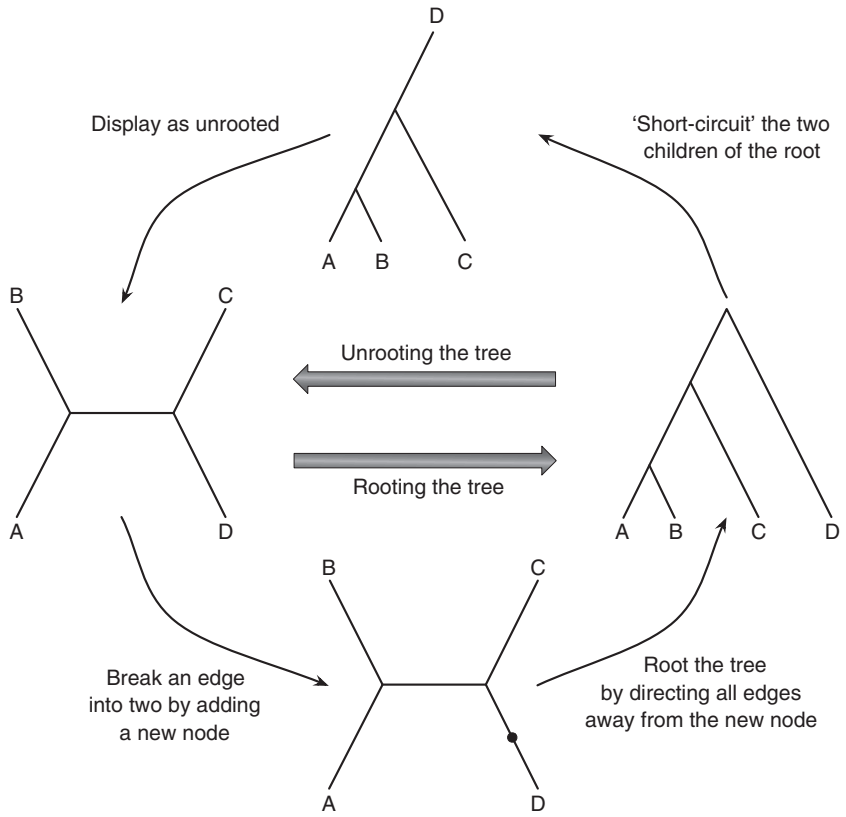

Figure 2 The relationship between unrooted and rooted phylogenetic trees. An unrooted tree can be rooted in many ways (one possible root for each edge). Here, the edge incident with the leaf labeled by taxon D is broken into two edges by the addition of a new node and then the tree is rooted at the new node by directing all edges away from it. A rooted tree is turned into an unrooted one in one way: the root node is removed, the two children of the root are connected, and all edge directions are ignored.

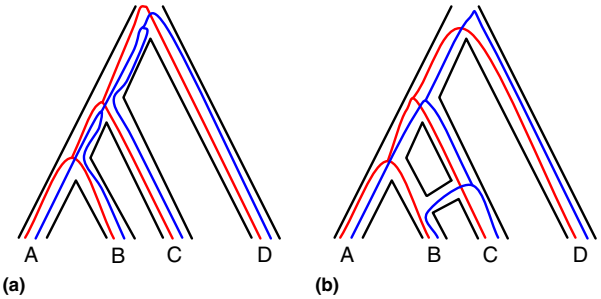

Figure 3 Beyond a single tree. (a) A 'trees within a tree' scenario. Here, different characters in the sequence alignment might have different evolutionary trees, while the evolutionary history of the whole alignment is still a tree. Processes that result in such a scenario include incomplete lineage sorting, heterogeneity in mutation rates across characters, and gene duplication and loss. (b) A 'trees within a network' scenario. Here, different characters in the sequence alignment might have different evolutionary trees, while the evolutionary history of the whole alignment is a phylogenetic network. Processes that result in such a scenario include recombination, horizontal gene transfer, and hybridization (including introgression).

Biological processes that result in scenarios where the evolutionary history of a sequence alignment cannot be captured by a single tree can be divided into two categories: treelike discord processes and reticulation processes. Treelike discord processes correspond to 'trees within a tree' scenarios (Figure 3(a)), in which the gene histories are different even though the species history is treelike. Processes such as incomplete lineage sorting (ILS), heterogeneity in mutation rates across characters, and gene duplication/loss belong to this category. Modeling evolutionary histories in the presence of these processes does not require networks, though these processes could be explored using data-display networks, as we discuss below.

Reticulation processes correspond to 'trees within a network' scenarios (Figure 3(b)), in which the species history itself is not treelike. Processes such as (meiotic) recombination, re-assortment, HGT, introgression, and hybridization belong to this category. When such processes occur, the evolutionary history of the sequence alignment takes the form of a phylogenetic network. Here, both the data-display and evolutionary networks could be used to explore and model the data (Morrison, 2005; Huson and Bryant, 2006).

It is important to note that phylogenetic networks can model both categories of processes, where phylogenetic trees cannot model reticulation processes. Therefore, 'network thinking' is a more encompassing and powerful paradigm than is 'tree thinking' (Bapteste *et al.*, 2013).

The Data

Various types of data have been used by the different methods to infer phylogenetic networks. These data types include:

- A single sequence alignment: In this case, each taxon is represented by a sequence of character states.
- Several sequence alignments: In this case, there is one sequence alignment per locus of interest, which are combined to infer the phylogenetic network.
- A set of distances: Differences are calculated pairwise between all of the taxa, based on one or more alignments, which provides a summary of their evolutionary relationships. It is these distances that are used to infer the phylogenetic network.
- A set of gene trees: Here, the data consist of a collection of gene trees inferred from individual sequence alignments of different loci. It is important to note that the use of 'gene tree' does not mean that the tree is necessarily inferred from the sequence alignment of a protein-coding gene. Rather, this naming is historical, and in the post-genomic era, these gene trees often include trees inferred from noncoding regions as well. Further, it is worth mentioning here that some methods make use of special types of trees, such as rooted triplets (rooted trees with three leaves each) or quartets (unrooted trees with four taxa each).
- A set of splits: Here, the data consist of splits, or bipartitions, of the set of taxa. Often, this set is obtained from sequence-alignment or gene-tree data. For example, given a collection of gene trees, the set of all their splits is computed (as described above), and this set is then used in the procedure for inferring the phylogenetic network.

Data-Display Networks

As we discussed above, the data for phylogenetic inference and analysis often consist of sequence alignments (when inferring networks from gene trees, these gene trees are estimated from the alignment data). Visual inspection of the sequence alignment provides a valuable way to understand the phylogenetic data. However, as the datasets become bigger, either in the number of sequences or number of characters or both, visual inspections of large tables of character states across sequences and characters become infeasible. Instead, a graph representation of the relationships that the sequence data encode among the taxa is a more natural and powerful tool (Morrison, 2010). Graphs where a subset of the nodes (leaves) are labeled by taxa are data-display networks; for example, Figure 4(a).

Consider two binary characters whose states for four taxa A, B, C, and D are

	Character 1	*Character 2*
A	0	0
B	0	1
C	1	1
D	1	0

This alignment gives two conflicting relationships among the taxa that cannot be represented simultaneously using a single tree. Character 1 groups taxa A and B together and taxa C and D together. Character 2, on the other hand, groups taxa A and D together and taxa B and C together. These binary relationships are incompatible from a tree perspective; that is, they do not fit a single tree. A data-display network provides a natural way to represent these relationships in a way that is amenable to visual inspection and exploration. Indeed, Figure 4(a) provides such a representation and describes how the relationships are embedded into and can be obtained from the network.

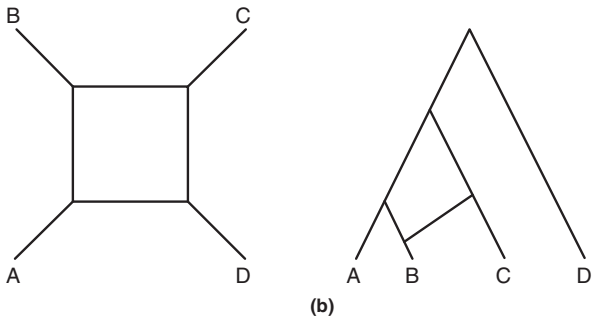

Figure 4 Phylogenetic networks. (a) A data-display network on four taxa: A, B, C, and D. Cutting the two horizontal parallel lines of the box displays the relationship '(A,B) vs. (C,D),' whereas cutting the two vertical parallel lines of the box displays the relationship '(A,D) vs. (B,C).' These two relationships cannot be simultaneously embedded in a single tree (using phylogenetics terminology, these two relationships are incompatible). (b) An evolutionary network on four taxa: A, B, C, and D. This network is interpreted as a rooted (the topmost node), directed (all edges are directed away from the root), acyclic (no directed cycle) graph that models ancestor–descendant relationships in the presence of reticulation. Here, parts of the genetic material of taxon B trace their ancestry to the most recent common ancestor of A and B, whereas other parts of B's genetic material trace their ancestry to the most recent common ancestor of B and C.

It is important to note here that the two characters in the example could very well have evolved down a single tree. For example, if each character represents a different site in the genomes of the four taxa, where 0 is A and 1 is C, then, the evolution of the sequence data can be explained by invoking multiple mutations at one or both of the characters. This is a central issue that underlies the construction, analysis, and interpretation of this type of network. The fact that the graph is not treelike does not mean reticulation has occurred during the evolution of the taxa; rather, the non-treelike components of the network call for a closer inspection of those parts to understand the conflicts in the data.

Algorithms are well developed for data-display networks, and computer programs are widely available. The most commonly used data-display networks are variants of what are known as splits graphs, which are explicitly designed to display bipartitions (Morrison, 2014). The variants differ in how they 'decide' which bipartitions to display under those circumstances where they cannot all be displayed simultaneously. Currently, the most commonly used variant for distance data is NeighborNet (Bryant and Moulton, 2002), and for character data it is the Median Network (Bandelt *et al.*, 2000). For gene trees there are Consensus Networks (Holland and Moulton, 2003) and SuperNetworks (Holland *et al.*, 2007). In population genetics, it is common to encounter the Reduced-median Network (Bandelt *et al.*, 1995) and the Median-joining Network (Bandelt *et al.*, 1999).

Evolutionary Networks

An evolutionary network (e.g., Nakhleh, 2010) gives an explicit model of the reticulate evolutionary history of the taxa under consideration. Formally, the topology of an evolutionary network is a leaf-labeled rooted, directed, acyclic graph with four types of nodes:

- The root node: This is a unique node that corresponds to the most recent common ancestor of all of the taxa. All edges in the networks are directed away from this node.
- The leaf nodes: These are the nodes that are bijectively labeled by the taxa. A leaf is characterized by having a single parent and no children.
- The tree nodes: These are those nodes each of which has at most one parent. Tree nodes are directly analogous to the nodes in a phylogenetic tree in that they represent events such as speciation and gene replication. The root is a tree node. Further, each leaf node that has a single parent is a tree node.
- The reticulation nodes: These are those nodes each of which has more than one parent. A leaf node that has more than one parent is a reticulation node. A reticulation node corresponds to a reticulation event involving the parents of the nodes, such as hybridization between two species or HGT from one organism to another.

Figure 4(b) shows an evolutionary network that has a single root node (the topmost node), four leaves (labeled by the taxa A, B, C, and D), eight tree nodes, and one reticulation node (the parent of the leaf labeled B).

While 'reticulation' is an umbrella term for all non-treelike evolutionary processes (e.g., recombination, HGT, and hybridization), evolutionary networks have appeared in the literature in many flavors, each emphasizing the biological process assumed to underlie the reticulation. Examples include hybridization networks and ancestral recombination graphs (ARGs). This classification notwithstanding, it is important to note that assigning a biological process to the inferred reticulation events in the network is best done a posteriori using knowledge of the data and organisms. In other words, from a mathematical and algorithmic perspective, methods that infer evolutionary networks cannot reliably distinguish meiotic recombination from hybridization, for example.

The three main flavors of evolutionary networks in the literature are:

- ARGs. This type of evolutionary network is mostly used for analyzing a sample of sequences obtained from a single population in the presence of mutation and recombination events. The reticulation nodes in ARGs correspond to recombination events, and are annotated with numbers that denote the physical location of (inferred) recombination

breakpoints. Under certain models of evolution, such as the infinite sites model, the edges of the ARG are annotated with the sites that mutate along those edges. The data used in ARG inference consist of either a sequence alignment or a set of single nucleotide polymorphisms. The evolutionary history of each non-recombining region in the sequence alignment is a tree embedded inside the ARG, which is obtained in a straightforward manner using the coordinates of the non-recombining region and the annotation of the reticulation nodes in the ARG. Gusfield (2014) is an excellent book that surveys the history as well as recent mathematical and algorithmic advances of ARG inference and use.

- HGT networks. This type of evolutionary network consists of a designated underlying tree and a set of horizontal edges connecting pairs of the tree edges. That is, HGT networks are used whenever the practitioner wants to emphasize the notion that the evolutionary history is mostly treelike, yet with some horizontally transferred elements. In most cases, the designated underlying tree is referred to as either the species tree or organismal tree. In terms of inference, HGT networks are inferred by reconciling the evolutionary histories of a set of genes with the designated underlying tree (which is assumed to be known). The genes can be given either by their sequences (e.g., Jin *et al.*, 2006, 2007) or their trees (surveyed in Doyon *et al.*, 2011). The evolutionary history of each gene is either identical to the designated underlying tree, in which case the gene's evolutionary history involves no HGT, or it is one of those trees inside the network that is obtained by using some or all of the horizontal edges (**Figure 5**).
- Hybridization networks. Unlike HGT networks, hybridization networks do not designate any underlying tree as the species or organismal tree. The data used in the inference of these networks consist of a set of genes given by their sequence alignments or gene-tree estimates. The inferred hybridization network can be viewed as an embedding of the gene evolutionary histories in a network under some criterion (**Figure 5**). That is, the network 'displays' a set of trees, obtained by cutting one of the parent edges of each reticulation node, and these trees represent the different gene histories involved in the hybridization. Introgression is included here as being a form of (partial) hybridization.

Methods for inference of evolutionary networks can be categorized based on the criterion they employ, as well as based on the processes they model (Nakhleh, 2013). In terms of criteria, most inference methods are based on the maximum parsimony principle: An evolutionary network with the fewest number of 'gene-level events,' including reticulations, is sought to model the evolutionary history of the data. For example, while an evolutionary network with three reticulation events could be constructed to embed the two trees in **Figure 5**, the network in **Figure 4(b)** is the desired one under the maximum parsimony criterion since it has the fewest number of reticulations needed to embed both trees (i.e., one). If more than solely reticulation is accounted for (e.g., reticulation plus gene duplication), then a weighted sum of the numbers of these events is minimized as the inference criterion. It is also possible to minimize related measures of reticulation complexity, such as the network level. More recently, methods for statistical inference of evolutionary networks have been introduced, based on likelihood. These methods make use of probabilistic models that incorporate reticulate evolutionary events (e.g., hybridization) in addition to vertical evolutionary events.

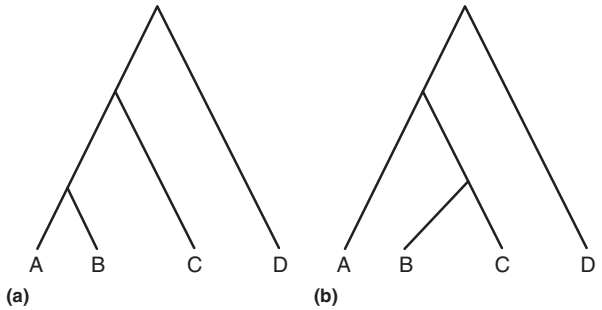

Figure 5 The two trees displayed by the phylogenetic network of **Figure 4(b)**. (a) The tree displayed by the phylogenetic network when the right edge coming into the reticulation node is removed. (b) The tree displayed by the phylogenetic network when the left edge coming into the reticulation node is removed. Assuming that the evolutionary history of a set of genes is the phylogenetic network of **Figure 4(b)**, and barring any processes such as gene duplication and incomplete lineage sorting, then every gene has an evolutionary history that is captured by one of these two trees.

While the majority of evolutionary network inference methods currently assume that reticulation is exclusively the cause of heterogeneity in evolutionary histories across genes, recently developed methods are beginning to incorporate more processes, and consequently should provide more accurate estimates of evolutionary histories. For example, Yu *et al.* (2013, 2014) provided maximum parsimony and maximum likelihood methods, respectively, for inference of hybridization networks in the presence of both reticulation and ILS.

Summary

Phylogenetic networks provide a powerful tool for exploring evolutionary relationships among taxa. While the use of tree methodologies in evolutionary analyses precludes, a priori, the potential for modeling reticulation, network methodologies allow simultaneously for treelike and non-treelike evolutionary relationships. Thus, networks go beyond what trees offer in terms of modeling power and expressiveness.

The input data for the network inference algorithms can be alignments, distances, or trees. In the latter case, the trees represent the assumed treelike evolutionary history of loci or non-recombining sequence blocks. The inferred networks can be solely multivariate summaries of the data (data-display networks) or they can represent hypotheses of phylogenetic history (evolutionary networks). In the former case, the objective is to display as many as possible of the bipartitions represented in the data. In the latter case, the optimization criteria are the same as for phylogenetic trees (parsimony- and likelihood-based).

As networks generalize the tree model, they fit the data at least as well as trees. This is why extra caution must be used when interpreting phylogenetic networks. A complex cobweb-like structure of an inferred data-display network is not necessarily an indication of many reticulations; rather, it indicates conflicting signals in the data about the relationships among the taxa, which merits closer inspection by the practitioner. Similarly, an evolutionary network with reticulation nodes indicates that the data do not fit a single tree well, and reticulations are inferred as potential hypotheses that should be pursued further.

See also: Genome Size and Structure, Bacterial. Hybrid Speciation. Phylogenetic Tree. Polyploid Speciation. Recombination in Bacterial Populations. Speciation-with-Gene-Flow. Species Trees, Inference of

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Who's Who in Phylogenetic Networks.

Phylogenetic Tree

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Glossary

Branch The portion of a phylogenetic tree that connects two nodes (internal branch) or one node and a tip (external branch).
Clade A monophyletic group. Part of a rooted tree that can be separated from the rest of the tree (which includes the root) by cutting a single branch.
Cladogenesis See lineage splitting.
Cladogram A tree diagram that communicates only the topology (branching pattern). A cladogram provides all the information relevant to determine the degree of relatedness between taxa.
Crown node The node that represents the last common ancestor of a clade of interest.
Hybrid speciation When members of two distinct lineages interbreed and form a new evolutionary lineage distinct from the parental lineages. See also lineage fusion.
Introgression When individuals from two distinct lineages produce hybrid offspring that subsequently interbreed with one of the parental lineages, causing genes from one lineage to be introduced into the other.
Lineage fusion When two distinct evolutionary lineages merge into a single, hybrid descendant lineage. See also hybrid speciation.
Lineage splitting The division of an ancestral lineage into two or more descendant lineages.
Monophyletic Composed of all the descendants of a particular ancestor. In phylogenetic trees, monopohyletic groups are clades.
Node A branching point in a phylogenetic tree.
Outgroup Taxa that are assumed a priori not to be within the ingroup and may therefore be used to root a phylogenetic tree.
Phylogeny The treelike evolutionary history of a group of organisms.
Plesiomorphy An ancestral character state.
Population lineage A group of populations united by gene flow.
Relatedness The recency of common ancestry, with more closely related organisms being those that share more recent common ancestors.
Reticulation Having a netlike population history with both lineage splitting and fusion.
Root The earliest node in a tree, representing the last common ancestor of all the tips.
Sib taxa Two taxa descended from a common ancestor.
Stem lineage The branch that subtends a clade of interest.
Stem node The node that represents the last common ancestor shared by a taxon of interest and any other organisms.
Synapomorphy A shared derived character state.
Taxon (plural = taxa) A formally named group of organisms. The groups of organisms represented by the tips of a phylogenetic tree.
Tip (= leaf) The entities (e.g., taxa, genes) whose relationships are depicted using a tree diagram.
Topology The history of lineage splitting depicted by a tree; a rooted tree's topology being defined by the list of all its clades.
Trait A heritable feature of a lineage; a character or character state.
Tree thinking The ability to read and interpret phylogenetic trees and use trees to accurately represent the evolutionary process.
Unrooted tree A tree without a root and, thus, without a specified time axis.

Introduction

Evolution is a central concept that enriches our understanding of the natural world and has very real societal impacts. For example, evolutionary principles have been harnessed for agricultural improvement and to develop protocols for the appropriate use of antibiotics. Given its broad significance, individuals at all levels of biological expertise, from the general public to the research biologist, need to be able to think clearly about evolution. Key among the topics that need to be mastered is the ability to correctly interpret evolutionary trees and use them as a substrate for visualizing evolutionary history.

Describing the relationships among organisms has been a challenge since well before the origin of evolutionary theory. In antiquity, biological diversity was classified and placed on the Great Chain of Being or Ladder of Life, the *Scala Naturae*. This perspective held that organisms could be arranged as the rungs on a ladder, with rocks and minerals at the bottom moving up through plants and 'simple' animals, with humans close to the pinnacle. However, we now understand that the organisms alive today did not evolve from other living species, but from shared ancestors. Furthermore, a ladder is a misleading metaphor because it implies that evolution is progressive and directed toward a particular endpoint, which it is not. Instead, evolution is more accurately described as an exploration, a freewheeling road trip in an all-terrain vehicle, perhaps. There is no predetermined destination, no prescribed route, and no need to stick to predictable roads. Unlike a road trip, however, evolution doesn't stop. Modern day species are not at destinations, but wherever they happened to be when we humans decided to map things out. But while we cannot know their future, we can (in principle) figure out the path that each species took to its current locale. This broad-scale

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history of evolutionary relationships is known as 'phylogeny.' So, if a ladder is not an appropriate metaphor, how can we picture phylogeny? No less a scientist than Charles Darwin provided an answer:

> The affinities of all the beings of the same class have sometimes been represented by a great tree. I believe this simile largely speaks the truth … the great Tree of Life … covers the earth with ever-branching and beautiful ramifications. (Darwin, 1859, pp. 131–32)

In the one hundred and fifty years since this passage was written, tree diagrams have come to be an indispensable tool for summarizing what is known about evolutionary history and guiding research in several fields, such as epidemiology, community ecology, and genomics. High school and university students also encounter phylogenetic trees in textbooks and in lectures as a means to organize knowledge of biological diversity. And trees are even found in popular media and museum displays, where they are designed to bring evolutionary discoveries to a general audience. As trees are so widespread and provide such a useful tool for representing evolutionary relationships, it is critical that we know how to read them and understand what they can and cannot tell us, a skill now commonly known as 'tree thinking' (O'Hara, 1992; Baum *et al.*, 2005; Baum and Smith, 2012).

Tree Terminology

Phylogenetic trees, by analogy to botanical trees, are made of leaves, nodes, and branches (Figure 1). Let us consider a tree from the canopy down to the trunk, or from the modern day to the past.

The leaves of a tree, also called tips, can be species, populations, individuals, or even genes. If the tips represent a formally named group, they are called taxa (singular: taxon). A 'taxon' is a group of organisms at any hierarchical rank, such as a family, genus, or species. The tips of a phylogenetic tree are most commonly living, but may also represent the ends of extinct lineages or fossils.

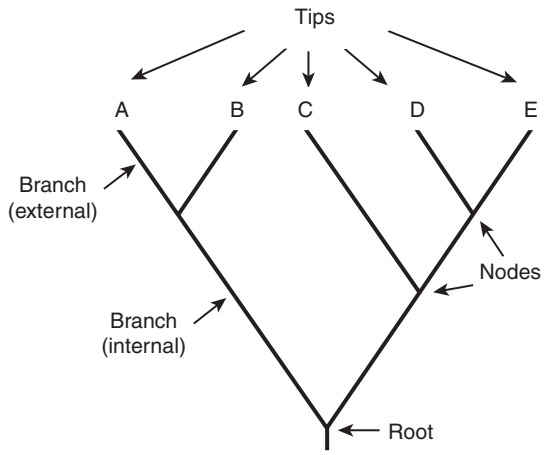

Figure 1 Components of a phylogenetic tree.

As in the trees you are already familiar with, tips or leaves are subtended by branches. A branch, which represents the persistence of a lineage through time, may subtend one or many leaves. Branches connect to other branches at nodes, which represents the last common ancestors of organisms at the tips of the descendant lineages. A branch connecting a tip to a node is called an external branch, whereas one connecting two nodes is called an internal branch (Figure 1).

Reading a tree from the past toward the present, a node indicates a point where an ancestral lineage (the branch below the node) split to give rise to two or more descendant lineages (the branches above the node). Branching on an evolutionary tree is also called 'cladogenesis' or 'lineage splitting.' After a lineage splits into two, evolution happens independently in these newly formed descendant lineages. The sequence of lineage splits in a tree creates its structure or 'topology.' Tree topology shows us the branching of lineages through time that gave rise to the tips.

'Clades' are groupings on a tree that include a node and all of the lineages descended from that node. The set of all the tips in a clade is defined as being 'monophyletic,' referring to the fact that it includes all the descendants of an ancestral lineage. In Figure 2, we could say that the tree supports monophyly of taxa C, D, and E or, put another way, C, D, and E together form a clade. Clades can be hierarchically nested within one another, as shown in Figure 2. A tree's topology can now be defined more precisely as the set of clades that the tree contains.

What a Tree Represents

Phylogenetic trees are a convenient way to represent millions or billions of years of evolution and the shared history of diverse organisms. So what are the branches and nodes really showing us?

Evolutionary trees are essentially about ancestors and descendants. You are probably familiar with pedigrees, which may be used to trace the ancestry of purebred dogs, tulip varieties, or royal families. Consider for a moment your own ancestry. Your line of ancestry includes your two parents, your four grandparents, and so on. You could map out your own ancestry in a family tree, and trace back your line of descent,

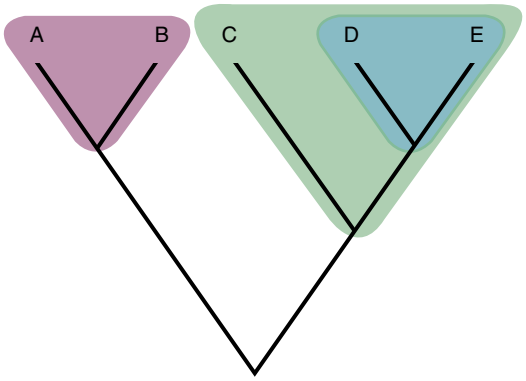

Figure 2 Clades are highlighted in a phylogenetic tree. Note clades can be hierarchically nested.

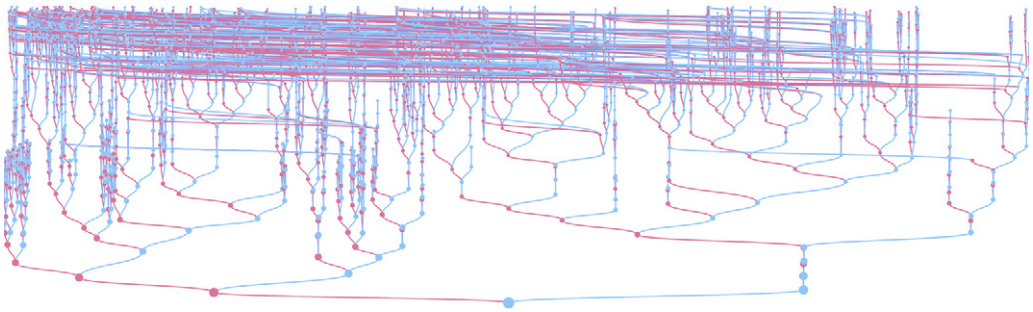

Figure 3 Image showing the ancestry of one person (Winston Churchill). Visualization tool by Bradford F. Lyon located at https://learnforeverlearn.com/ancestors/.

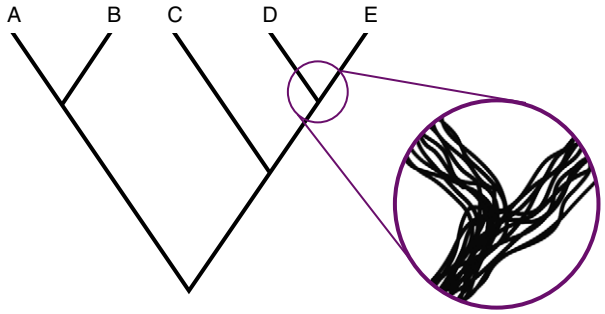

Figure 4 Close-up of lineage splitting on a tree. Lines of descent look sloppy up close, but are represented as clean lines when considered in evolutionary time.

but as you go farther back time, the number of individuals tends to balloon, at least initially (Figure 3). If you go far enough back in time or include many individuals besides yourself from the current generation it rapidly becomes difficult to map out all of the lines of descent neatly without crisscrossing. Now imagine expanding the pedigree to include every other member of our species. That would be messy and certainly not treelike! The problem is that we are thinking at the wrong timescale.

Evolution may be changed over time, but just how much time can be hard to grasp. In a short time frame, such as a human lifespan, the relationship among most organisms within a species is not treelike at all. However from a 'zoomed-out' view, we can consider the members of a species to be part of the same 'population lineage.' That is to say that members of a species share genes frequently enough that they evolve more or less as a single unit. The branches of a phylogenetic tree represent these population lineages, which are composed of many individuals over many generations (Figure 4). Every so often in evolution, a single population lineage splits into two (or more) descendant lineages. This happens when a species is split into two subsets, whose individuals do not exchange genes. When this occurs, the descendant lineages become free to accumulate differences and, if they don't come back together and fuse, will eventually give rise to very different organisms. A node represents such a lineage splitting event – the breaking of genetic connections that allowed the descendant lineages to accumulate differences and eventually give rise to distinct descendant clades.

It is worth noting that even from a zoomed-out perspective, there are cases where a phylogeny does not appear strictly treelike. Branches on the tree of life can sometimes grow together. Such a rendezvous between formerly distinct lineages is called 'reticulation.' Reticulation can be attributed to a few different biological processes (Figure 5). 'Introgression' happens when hybrids form between two distinct lineages and, through subsequent crossing, novel genetic material comes to be transferred from one species to another. In some cases, lineages can hybridize to form a new lineage that is distinct from either parental lineage – a process known as 'hybrid speciation.' Introgression of very few genes (whether by sexual reproduction or some other mechanism), called horizontal gene transfer, is usually best visualized as a treelike population history with a few genes having a discordant history. Indeed, so long as introgression and hybrid speciation are rare or limited to closely related tips, it is appropriate to represent evolutionary relationships using the tree metaphor. However, in extreme cases the tree metaphor may break down, meaning that evolutionary relationships are best represented as a network.

If evolution can be summarized as descent with modification, it makes sense that when we talk about evolution, we often do so in terms of those modifications (i.e., traits). 'Traits' are heritable characteristics of organisms. For example, flowers are a trait shared by all angiosperms, backbones are a trait shared by all vertebrates, and chitin cell walls are a trait shared by all fungi. Molecular characteristics, such as having the amino acid leucine at a certain position in a protein, should also be considered as traits. It is important to understand how traits evolve on trees, since traits serve as the basis of tree inference (see other articles within the Phylogenetic Methods section for theory and methods of phylogenetic inference).

Traits arise due to evolutionary changes within population lineages (see other articles within the Population Genetics section). Once a new trait arises and becomes fixed in a population lineage all descendant lineages are expected to have that trait, though due to subsequent evolution it might look quite different. All land vertebrates ('tetrapods'), for example, are descended from an ancestor with four limbs, though the form of their limbs differs greatly among species. Some land vertebrates did evolve a lack of limbs, but this occurred by further modifying the trait out of existence rather than by evolutionarily back-tracking to the precursor condition of having fins (Figure 6).

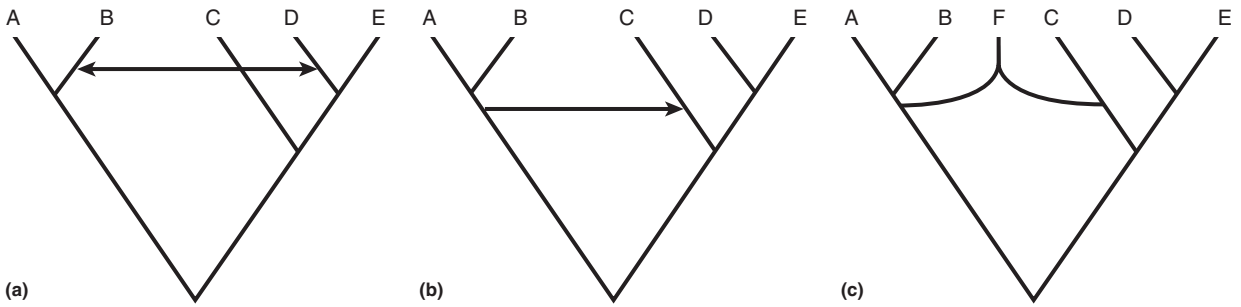

Figure 5 Processes leading to reticulation. (a) Bidirectional introgression into two parental lineages. (b) Unidirectional introgression. (c) Hybrid speciation.

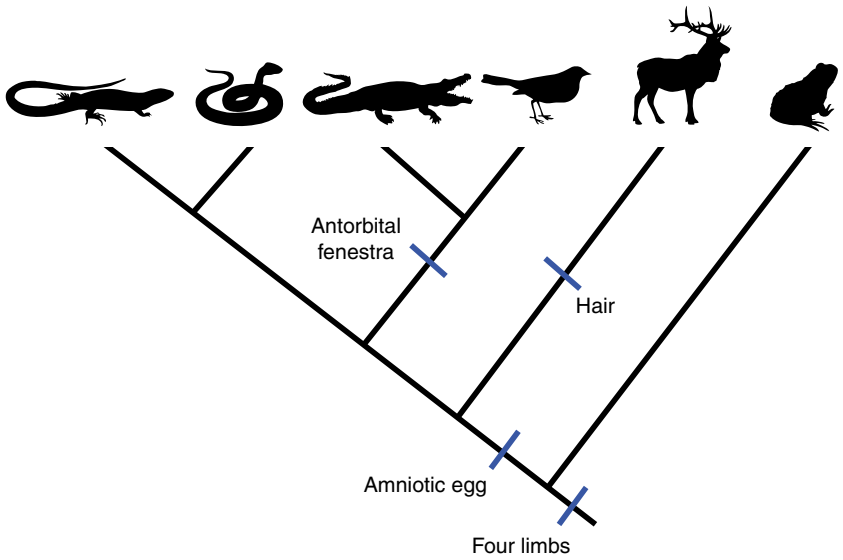

Figure 6 Cladogram of tetrapods showing trait evolution.

Lineage splitting is often mistakenly confused with trait evolution. A lineage splitting event is not necessarily accompanied by trait divergence (and even if it did, it might involve traits that we don't even know about). It is after lineage splitting that descendant lineages accumulate independent mutations, which over time may lead to novel traits. For this reason, as in Figure 6, traits are not usually depicted as evolving on nodes but on branches. If a certain clade has a unique trait, then we can infer that the last common ancestor of this clade, represented by the 'crown node,' had the trait. We can also assume that the last ancestor shared between this clade and its closest relative (its 'sib taxon') represented by the 'stem node' did not have the trait. This follows because, if stem node individuals had already evolved the trait, then the sister clade should have it too. Therefore, the correct mapping of the unique trait is on the 'stem lineage' of the clade, the branch linking its stem and crown nodes (Figure 7).

So far we have focused on trait evolution along a tree, which may imply that the products of evolution are always visible. It is important to note that evolutionary kinship is not always apparent just by looking at organisms. For example, the tree in Figure 6 shows that crocodiles are more closely related to birds than they are to lizards, even though superficially you might think lizards and crocodiles seem more similar. Relatedness does not equal similarity.

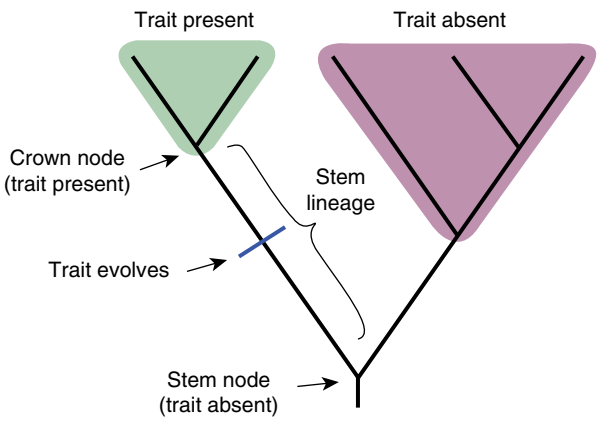

Figure 7 Trait evolution and crown/stem terminology.

There is a specialized group of terms used to describe traits, based on their distribution and origin. 'Synapomorphic' traits are ones that are unique to a particular clade. In Figure 6, snakes, lizards, crocodiles, birds, and mammals share the

synapomorphy of the amnion, which evolved after the divergence of amphibians; animals-with-amnions corresponds to a monophyletic group. 'Plesiomorphic' traits, in contrast, are those shared by a group of taxa that were inherited from an ancestor, where some other lineages lost this trait. For example, 'four limbs' is a plesiomorphic trait of tetrapods (Figure 6); vertebrates-with-four-limbs does not correspond to a monophyletic group because some tetrapods, such as snakes, legless lizards, caecilian, and whales, lack external limbs. If two independent lineages evolve a similar trait, they are said to share a homoplasious trait. Homoplasy arises when distinct lineages acquire similar traits.

Do Trees Show Advancement and Progress?

Despite the abandonment of overt ladder thinking there is still an unfortunate tendency to read a tree as though it supports a narrative in which some tips are more advanced or primitive than others. This is often apparent in the words biologists use to describe a tree. For example, vascular plants are sometimes referred to as 'higher plants' and vertebrates as 'higher animals.' But this is misleading since 'higher plants/animals' have had just as long to evolve from their common ancestors as 'lower plants/animals.' Likewise, some taxa with visible traits that make them resemble ancestors, such as lungfish, monotremes, liverworts, and bacteria, are called 'primitive' even though these lineages are all alive today and have many invisible differences from the ancestors they resemble. And species-poor lineages closely related to diverse clades are frequently labeled 'basal' or 'early-diverging' even though they diverged from their sib taxon at the same time that the sib taxon diverged from them. There isn't room here to discuss all of the potentially misleading labels and why they are wrong, but we advocate being mindful of these vestiges of ladder thinking. For more on this topic, see Krell and Cranston (2004), Crisp and Cook (2005), and Rigato and Minelli (2013).

Relatedness

When we say that taxa are closely related, what do we mean? 'Relatedness' refers to the amount of time since a group of organisms shared a common ancestor. For example, we say chimpanzees are the closest relatives of humans, because we share a common ancestor with chimps more recently than we do with any other organism (Figure 8). Using a tree diagram, it is simple to tease apart relatedness among taxa. Are chimps or humans more closely related to gorillas? Set aside your intuitions for a moment and look at Figure 8. Trace your way from one of the tips back through the tree to the node representing the common ancestor of chimps, humans, and gorillas (node *x*). Whether you start with the human tip or the chimpanzee tip, you reach the same ancestral node, which means that, given this tree, gorillas are equally closely related to humans and chimpanzees.

Two tips that are each other's closest relative are called sib taxa. In Figure 8, humans and chimpanzees are sib taxa. Gorillas are sibs to the human–chimpanzee clade.

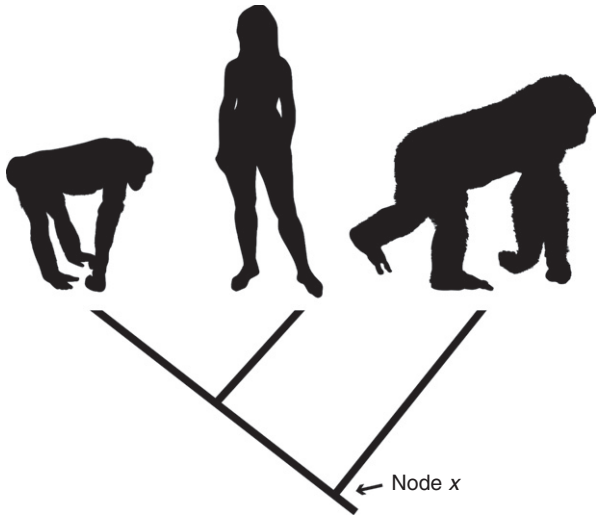

Figure 8 Cladogram showing relationships among primates.

Since tree topology refers to the list of clades that a tree includes and since all tips within a clade are more closely related to one another than to tips outside the clade, tree topology can be seen as a summary of evolutionary relatedness among all its tips. This is why tree topology is such an important evolutionary concept.

Trees can be depicted and modified in a number of different ways without changing their topology or the relatedness among organisms they represent. Regardless of whether a tree is orientated left to right, top to bottom, outward from the middle in a circle, or along a diagonal, the key information remains the same. For example, all the trees in Figure 9 show the same topology and imply the same set of evolutionary relationships. You can tell this because in all cases the list of clades is identical. Thus the orientation and style of a tree does not change the relationships it represents.

Along the same lines, the nodes of a tree can be rotated without modifying the topology (Figure 10). Looking at the two trees in Figure 10 you might think that they imply something different about evolutionary history. For example, you might think that the first tree implies that species E is the most advanced or evolutionarily derived, while the second tree implies something different. However, it turns out that these two trees have an identical topology, as you will see if you make a list of all the clades shown on each tree. This may serve to drum home the idea that trees do not show evolutionary progress even when drawn in a form that implies that some tips have changed less since the root.

Branch Length

Phylogenetic trees come in different flavors, depending on additional information they might contain. Trees may just show tree topology, in which case they may be called 'cladograms.' Sometimes, in trees called chronograms, branches are scaled to represent the opportunity for evolutionary change by drawing branch lengths proportional to time. If we assume that evolutionary changes occur at a fairly constant rate (sometimes called clocklike evolution), the length of a branch

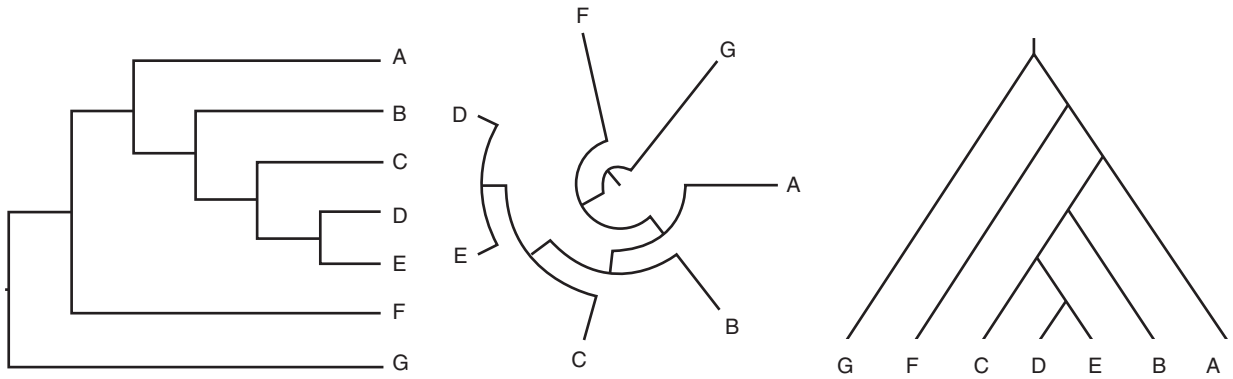

Figure 9 Trees can have different shapes and still retain the same topology.

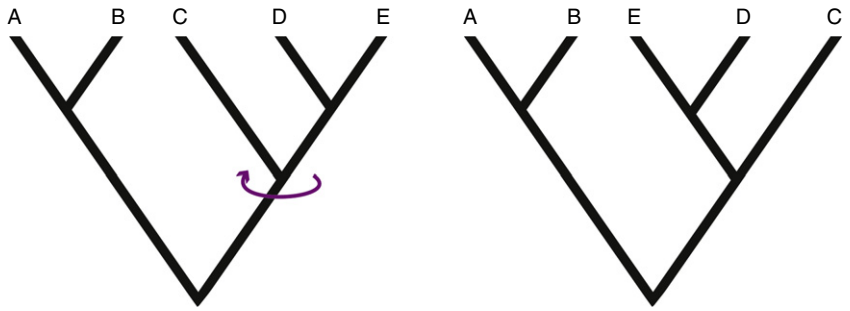

Figure 10 A tree can be rotated around any node without changing the relationships it depicts.

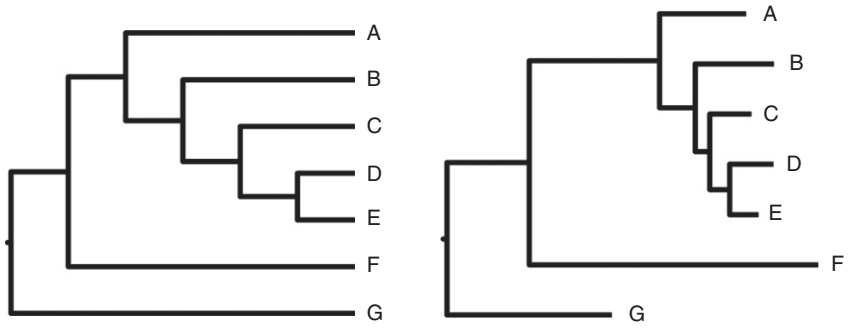

Figure 11 Cladogram (left) versus phylogram. Cladograms only communicate topology, while phylograms have branch lengths proportional to the expected amount of trait evolution.

on a chronogram tells us the relative probability that a particular trait evolved on this branch. However, this will not be true when the rate of evolution is not clocklike. In that case we can show instead a 'phylogram,' a tree whose branch lengths are drawn proportional to the expected amount of trait evolution. On a phylogram long branches indicate places where most of the changes occurred.

In a chronogram where all tips are living, all tips are equidistant from the root, since the branches represent the absolute time since divergence from a common ancestor. In a phylogram, in contrast, different tips may be different distances from the root, indicating the expectation that some tips may have accumulated more derived traits than others. Even when shown as a phylogram, however, it would be misleading to view tips or clades subtended by long branches (e.g., taxon F in Figure 11) as being more 'advanced' than ones with short branches. While they might have changed more from the common ancestor, they have had the same amount of time to evolve and may be just as well, or better, adapted to their specific ecological niche. Thinking clearly about evolution is best accomplished by completely dropping the concept of advanced and primitive taxa.

Unrooted Trees

So far, the tree diagrams we have shown are rooted trees. If a tree is rooted, all of the tips can be traced back through branches to a common ancestor. The 'root' of a tree therefore implies a chronological direction, starting in the past at the

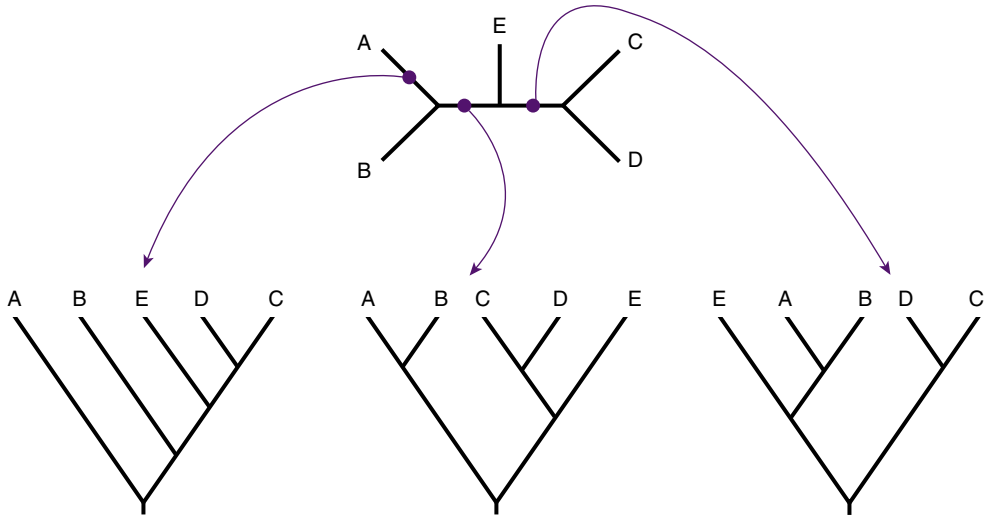

Figure 12 Different ways to root the same tree. Each of the three root positions changes the tree topology.

base of the tree and moving toward the present at the tips. However, as discussed in the following entries on tree estimation, methods for inferring phylogenetic trees often yield unrooted trees. An unrooted tree provides little information about the direction of trait evolution or the degree of relatedness of tips. This is because when a set of tips lie on one side of an internal branch we do not know if they will represent a clade on the correctly rooted tree. As a way to become more comfortable with phylogenetic trees, it is helpful to learn how an unrooted tree can be rooted (Figure 12).

The most common rooting method is outgroup rooting. To do this, we make sure that at least one tip included in a study (the outgroup) is distantly related to the other taxa in the tree (the ingroup). This requires some prior knowledge of phylogeny, since using an inappropriate outgroup can greatly change the rooted tree. As Figure 12 shows, where the root is placed can change the relationships, meaning it is important to root trees appropriately. Other rooting methods include molecular clock rooting, which assumes that all tips have had roughly the same opportunity for change since the root node, and duplicate gene rooting, which takes advantage of ancient gene duplication events.

Trees as Substrate

Understanding the relationships among organisms is just a small portion of what we can learn using trees. Once we build a phylogenetic tree, we can use it as a foundation for other analyses.

For a start, taxonomic decisions are informed by phylogenetic trees. For example, finding that one species in a genus is more closely related to members of a different genus might lead to it to be reassigned to that genus. Indeed, taxonomy now strives to only give formal names to monophyletic groups because all members of a clade are more closely related to each other than to any organisms outside the clade. A monophyletic group can thus be seen as occupying a single location on the tree of life, something that is not true for non-monophyletic groups.

Another major role for phylogenetic trees is in ancestral state reconstruction: with a tree and a trait that varies among tips, we can determine the likely characteristics of ancestral nodes in the tree. Analogous methods can also be applied to geographical distribution allowing us to infer an ancestor's historical range.

As discussed in other sections, trees have many other uses including making inferences about causes of character evolution and studying the movement of species in geographic space (see other articles within the Evolutionary Biogeography section). Indeed, scanning through the many sections of this encyclopedia you will see many phylogenetic trees – as clear an indication as any other that being able to think clearly about the process and pattern of evolution rests upon a solid foundation of phylogenetic trees and 'tree thinking.'

See also: Ancestral Reconstruction: Theory and Practice. Bayesian Phylogenetic Methods. Distance-Based Phylogenetic Inference. Maximum Likelihood Phylogenetic Inference. Parsimony Methods in Phylogenetics. Phylogenetic Invariants. Phylogenetic Networks. Rooting Trees, Methods for

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Phylogenetic Tree Comparison

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Glossary

Adjusted Bootstrap Proportion (aBP) A corrected bootstrap proportion of phylogenetic hypotheses which produces more accurate approximations of p-values when using the $p \approx 1-BP$. The aBP accounts for the complex boundaries between phylogenetic hypotheses.

Bootstrap It is a resampling procedure used to approximate the variance of a distribution. Many pseudoreplicate data sets are created by resampling the original data. An estimate is calculated for each pseudoreplicate. The standard error of the estimate on the actual data can be approximated by examining the variability across the collection of estimates from the pseudoreplicate data.

Bootstrap Proportion (BP) In the phylogenetic bootstrap, the BP is the proportion of bootstrap replicates that include a given clade. It is a measure of how well supported the clade is.

Kishino–Hasegawa test (KH test) A test of two competing topological hypotheses which is appropriate if both hypotheses are determined a priori.

Likelihood Ratio test statistic The test statistic is twice the difference in log-likelihoods between two hypotheses. Note that the ratio of likelihoods of different hypotheses becomes a difference when log-transformed.

Maximum likelihood (ML) A framework for statistical inference. The 'likelihood' of a hypothesis is the probability of generating a dataset identical to the observed data if that hypothesis were true. ML prefers the hypothesis that yields the highest likelihood.

Resampling of estimated log-likelihoods (RELL) A fast approximation of bootstrapping to assess variability in likelihood ratio test statistics. Instead of resampling characters and conducting a full phylogenetic analysis, in RELL we simply resample the site-likelihoods from the original analysis.

Shimodaira–Hasegawa test (SH test) SH test is an extension of the KH test which can be used to test one specific tree against another tree that was found by searching for the best tree among a large set of candidate trees.

Estimating the genealogical relationships between species is a computationally and statistically challenging endeavor. Whenever the tree that we estimate differs from the tree that we were expecting, a natural question is, 'Can we explain the discrepancy between our estimate and a previous hypothesis solely on the basis of sampling error?' If we have a small amount of character data or our data set has a large amount of internal conflict, we should be wary of our estimate. In such cases we may want to publish our best estimate, but not reject previous hypotheses about the phylogenetic relationships. We can treat our previous expectations for the topology as an null hypothesis for statistical testing.

The standard recipe for approaching this type of question in the frequentist approach to statistics is to:

1. choose a test statistic that describes how the data deviates from expectations under the null hypothesis;
2. describe the distribution of values that we would expect for this statistic if the null hypothesis were true – this is the null distribution of the test statistic;
3. compare the value of the test statistic to the null distribution to find out the probability of seeing a result that is at least this surprising if the null were true – this probability is the p-value; and
4. reject the null hypothesis if the p-value is small.

Likelihood Ratio Is (Almost Always) the Best Test Statistic

The choice of which test statistic to use in this form of testing is very open-ended. It may be tempting to use a test statistic that measures how different our estimated tree is from the trees that are a part of the null hypothesis. However, using only a tree-to-tree distance is suboptimal because it does not capture the strength of support for different groupings. Fortunately, the 'law of likelihood' assures us that the ratio of the likelihoods for competing hypotheses captures all of the evidence in the data for one hypothesis over another. So, we can focus our testing on a procedure that uses the popular 'delta' statistic:

$$\delta(T_1, T_0|X) = 2[\ln L(T_1|X) - \ln L(T_0|X)]$$

where T_1 and T_0 are the two trees that we are comparing, X represents the data, and L represents the likelihood of a tree for a data set. Note that δ is twice the difference in log-likelihoods for our two hypotheses. It is a measure of how much more support one hypothesis has than the other. We often call this the likelihood ratio test statistic. Note that the statistic is calculated on the log-scale. So, the logarithm of the ratio of two likelihoods becomes a difference between the log-likelihoods.

So what is the likelihood of a tree? The likelihood, $L(T_1|X)$, is simply the probability that we would generate a data set identical to our data (X) if tree 1 is an accurate depiction of the phylogenetic relationships. You can think of the likelihood as a measurement of how well our model (the tree) matches our data. Trees that would cause us to expect the patterns of data that we actually observe have higher likelihoods.

Because the likelihood is the probability of the entire data set, it uses all of the information in the data. Test statistics that are not functions of the likelihood are less powerful because they do not capture all of this information. While DNA sequence data is commonly used to reconstruct phylogenies,

these approaches can be applied to any data for which the likelihood of a tree can be calculated.

Null Hypotheses

Depending on the question at hand, our null hypothesis may be one of several types. The null may be that multiple topologies are equally good explanations, that one topology corresponds to the true set of relationships, or that one specific grouping is not a part of the true tree. Different tests described below are formulated with different null hypotheses. In addition, our hypotheses generally are statements about 'topologies' – the relationships among taxa. However, we calculate likelihoods on trees – topologies with branch lengths optimized according to the model of evolution to maximize the likelihood of the data. Thus, the testing procedures must include some method of handling the estimation of branch lengths, despite the fact that the hypotheses being tested rarely refer to branch lengths explicitly.

Calculating the δ Statistic in Phylogenetics

To conduct a test, we also need an alternative tree to contrast with the null hypothesis. As we will see later, we run into problems if the alternative tree that we want to test is not known when we start the study. This often occurs if we initiate a study to test a hypothesis. We may suspect that a particular grouping (the null) is not true, but we do not have a specific alternative. An appealing approach is to estimate the best tree for our data and then perform a test to see if we can reject the null. If we use the data to select the alternative tree to test, then we must alter the threshold that we use for deciding when we reject the null hypothesis (see Section 'Selection Bias Problems').

However, if we start our study interested in comparing two specific trees, then calculating δ is easy: we simply choose the most appropriate models of character evolution for our data and then calculate the log-likelihood of each tree. Calculating δ is easy in this case, but to conduct a test we also need to know the null distribution of the δ test statistic.

The Null Distribution of the Likelihood Ration Is Not Trivial to Obtain

The Null Hypothesis That Expected Likelihood Is the Same for Multiple Trees

To think about the null distribution, we have to think clearly about what hypothesis we are testing. Consider the null hypothesis that trees with or without a particular group are expected to be equally good explanations of the data. This may seem like an odd null hypothesis – after all when we are about to perform a test we do not actually expect all of the trees to be tied.

Imagine, for example, hypotheses for the relationships between humans, chimps, and gorillas, with orangutans as an outgroup (Figure 1). If our null was that in the true tree humans are the sister group to gorillas (Figure 1(a)), the most challenging form of this null would occur if the lineage that was the last common ancestor of only humans and gorillas persisted for an infinitesimal amount of time before speciating (Figure 1(d)). By 'most challenging' null, we mean the null hypothesis that will be hardest to reject. In that case, where the true tree has a zero-length internal branch, we would expect no character changes in the data set to support the grouping of humans and gorillas. Thus, we should expect each of alternative trees (e.g., trees with humans sister to the chimps (Figure 1(b)), or trees with chimps sister to gorillas (Figure 1(c)) to explain the data just as well as the true topology (Figure 1(a)). Frequentist hypothesis-testing tries to give the null hypothesis the benefit of the doubt at every step of hypothesis testing by focusing on these most challenging cases. Because of this focus on the most challenging case, the testing procedure will let us make very general statements such as 'either the null hypothesis is false or some rare form of sampling error occurred; we would only expect this extreme effect of sampling error in $P \times 100\%$ of tests.

Using Per-Character δ's to Generate a Null Distribution

Using the difference in log-likelihoods between the alternative and null hypothesis is appealing in terms of statistical power

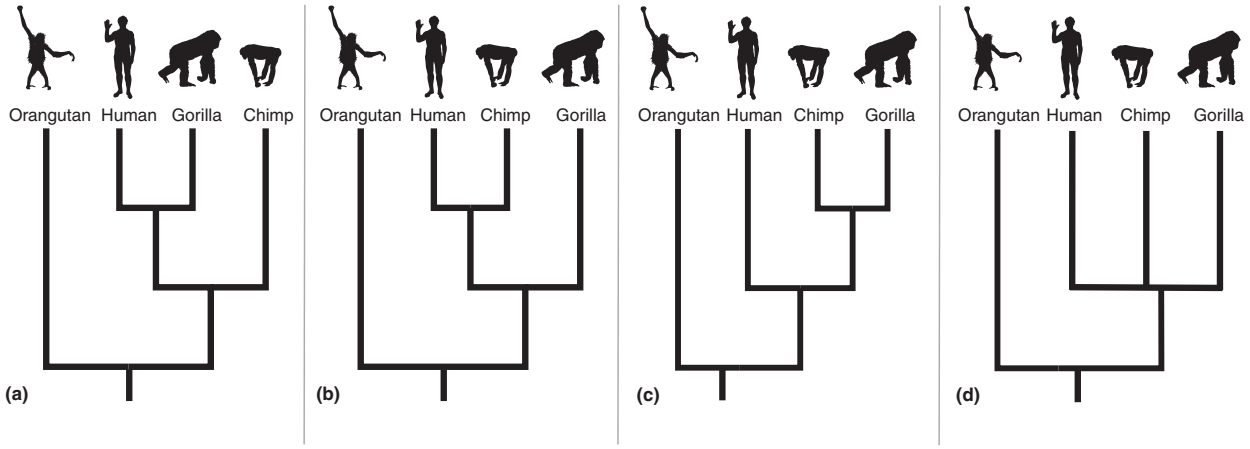

Figure 1 Three example trees demonstrating alternative potential topologies for the relationships between humans, chimps, and gorillas, with orangutans as an outgroup. (a), (b), and (c) are potential hypotheses, and (d) represents the border between these hypotheses. All branches which differ between (a), (b), and (c) are collapsed in (d). Silhouettes are reproduced from phylopic.org, credit Mike Keesey and Gareth Monger CC BY 3.0.

and making the best use of our data. Unfortunately, we do not have any clear, simple theoretical description of the null distribution of this statistic. In other words, if the null were true, we do not know how large the δ statistic might get solely as the result of sampling errors. Having a null distribution is crucial because it lets us convert an observed value of the test statistic to a p-value.

With no theoretical descriptions of the null distribution of the δ statistic, we seem to be incapable of conducting a hypothesis test. Fortunately, we can look at the phylogenetic signal within each character in our data set. This allows us to get a sense of whether the difference in log-likelihoods is coming from a few or many characters and whether or not there is much internal conflict. In almost all models used to describe character evolution in phylogenetics, different characters are assumed to evolve independently of one another. Thus the likelihood is calculated by taking a product over characters. On the log-scale the value of the δ statistic is simply the sum of the per-site versions of the δ statistic. So, looking at the distribution of the per-site differences in log-likelihoods gives us a rich view of the per-datum quantities that make up our test statistic.

Imagine that we have 50 characters in our data set and a total log-likelihood difference of 5.0 between our null hypothesis and an alternative tree. Is this degree of support statistically significant? The answer actually depends on the variance in the support from character to character.

Our null hypothesis states that trees with or without the clade of interest (e.g., Humans and Gorillas) are expected to explain the data equally well. If our tree inference method is not biased, we could imagine that every informative character has a 50% chance of favoring the null tree, and a 50% chance of supporting the alternative tree (as we will see below, this assumption is too simplistic because it ignores the number of trees that correspond to each hypothesis, but this simple case is a good starting point).

Consider the scenarios shown in Figure 2. In the case Figure 2(a), all 50 characters prefer the alternative tree by 0.1 log-likelihood units. In this case, the phylogenetic signal in the data is very repeatable and internally consistent. If the magnitude of character preference were only 0.1 and a tree with the clade was equally likely explain the data as a tree without the clade, what is the probability that we would see an overall difference in log-likelihoods of 5.0 from a data set of

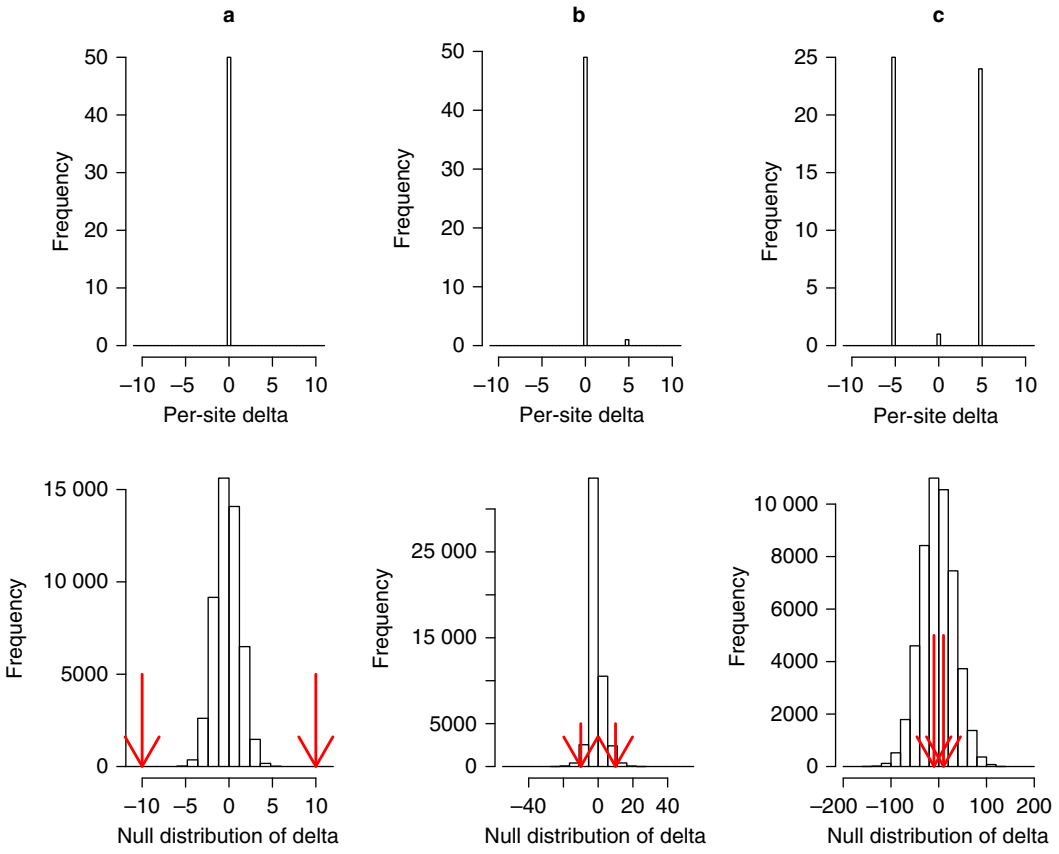

Figure 2 Null distributions under three different scenarios describing the distribution of per-site Likelihood Ratio statistics. Each column shows the per site delta distribution (top) and an approximation of the null distribution of the δ statistic (bottom). Note that the x-axis differs for each of the null distribution plots; red arrows on each plot indicate the test statistic value of 10.0 and –10.0 (the p-value is the probability of falling in the tails of the distribution more extreme than these arrows). In case (a), all 50 characters prefer the tree by 0.1 log-likelihood units (per site $\delta = 0.2$). In (b), 49 of the characters have no preference ($\delta_i = 0$) between the trees, and one character prefers the alternative tree by 5.0 log-likelihood units. In (c), we see a case of strong internal conflict in the data. One character has no preference between the hypotheses; 25 characters prefer the alternative tree; and 24 characters prefer the null tree. Among the 49 characters which have a preference for one tree over another, the magnitude of the preference is strong – centered around 5.0 log-likelihood units.

50 characters? To get a log-likelihood difference of 5, each of the 50 randomly selected sites would have to favor the same tree. The probability is tiny – it is the equivalent of flipping a fair coin 50 times and always seeing the same face of the coin (the probability is 0.5^{49} if you conduct a typical, two-sided hypothesis test). Seeing a total log-likelihood difference of 5.0 out of 50 characters each of which has a preference of around 0.1, is an extremely unlikely event if the null hypothesis is true. So we have reason to reject the null.

Figures 2(b) and **2(c)** depict two other possible outcomes of looking at the per-character log-likelihood differences. Note that the alternative tree is favored by 5.0 log-likelihood units in each of the **Figures 2(a)**, **2(b)**, and **2(c)**. In **Figure 2(b)**, 49 of the characters have no preference between the trees, and one character prefers the alternative tree by 5.0 log-likelihood units. In other words all of our signal is coming from one of our 50 data points. Unsurprisingly, this should not lead to a significant test result. If our character data is sampled from a universe with these sort of properties (probability of 0.02 of getting an informative site, and informative sites are strongly informative), then we would expect to see quite a bit of data set-to-data set variability in which tree is preferred. In fact there is a very strong chance ($\approx$0.63) that one of the trees will be preferred by at least 5.0 log-likelihood units even under the null hypothesis. Hence we do not have enough support in the real data set to make us reject the null hypothesis.

In **Figure 2(c)**, we see a case of strong internal conflict in the data. One character has no preference between the hypotheses; 25 characters prefer the alternative tree; and 24 characters prefer the null tree. Among the 49 characters which have a preference for one tree over another, the magnitude of the preference is strong – centered around 5.0 log-likelihood units. If this distribution described the signal in our data, then we should not be surprised at all to see a preference of about 5 log-likelihood units. Due to sampling error, we would almost always see this much of a difference.

Kishino–Hasegawa Test

The previous section tried to provide some intuition for why the distribution of differences in log-likelihoods per site has a strong effect on whether or not we consider a result such as $\delta = 10$ to be enough evidence for us to reject the null hypothesis (recall that the δ is twice the difference in log-likelihoods; so in the previous example a difference in log-likelihoods of 5 implies $\delta = 10$). To derive the null distribution requires more care than our intuitive exercise. Early efforts (Cavender, 1978; Templeton, 1983) had considered calculating a *p*-value from the total difference in scores using data that had integer scores only (or which scoring systems in which a character could just be defined as supporting or conflicting with a tree). Kishino and Hasegawa (1989) tackled this problem in a way that fully used the magnitude of the difference in log-likelihoods.

Kishino and Hasegawa (1989) reasoned that, because the difference in log-likelihoods between trees is simply the sum of many per-site differences, the statistic should (according to the Central Limit Theorem) follow a normal distribution if the data set is large. They used the empirically estimated per site log-likelihoods to estimate a variance of the normal distribution. They note that the sampled distribution of differences in log-likelihoods is not identical to the null distribution. However, the sampled distribution should have a similar variance to the null distribution. Under the null hypothesis, both trees should explain the data equally well. So, the null distribution should be centered at 0. Combining the mean of 0 with the sampled variance provides a normal distribution which approximates the null distribution.

Kishino and Hasegawa (1989) also noted that we can avoid the explicit assumption of normality by simply resampling the per-site differences in log-likelihoods many times to generate a distribution of δ statistics. This distribution will not be centered around 0, because whatever tree is favored by our real data will tend to be favored in the resampling procedure. We can correct for this bias very simply – by subtracting from each sampled δ value the mean over all of the simulation replicates. This enforces the null hypothesis expectation that the two trees would explain the data equally well. This procedure of resampling the per site-likelihoods is referred to as the 'resampling of estimated log-likelihoods' (RELL) bootstrap, and testing the significance of the difference between trees in this way is the 'Kishino–Hasegawa test (KH test),' after Kishino and Hasegawa (1989).

Susko (2014) questioned the assumption that the difference in log-likelihoods should follow a normal distribution. He has found the KH test using the normal as the null distribution and the RELL approximation to be too conservative. He introduced two new approaches for generating the null distribution for the KH test: a procedure that uses simulation from normal distributions to approximate the shape of the log-likelihood surface on phylogenetic trees, and a procedure using a null distribution created by mixing χ^2 (chi-squared) distributions. The mixture of χ^2 distributions is suitable for testing two trees that are very topologically similar to each other; Susko also introduced some conservative approximations that can be used in more general cases.

Selection Bias Problems

The largest impediment to the widespread use of the KH test is that it assumes that both the null hypothesis tree and the alternative are known a priori. In phylogenetics it is much more common for the null hypothesis to correspond to a set of trees and for the alternative hypothesis to be implicitly defined as 'all trees that do not fit the null.' This introduces a multiple testing problem: if you select the ML tree to use as your alternative, then you are implicitly wading through a huge number of possible alternative trees and selecting the one that is most likely to lead to rejection of the null. Selecting the most promising alternative tree makes sense in terms of constructing the most powerful test, but this selection of an alternative has a large effect on the null distribution of the test statistic. Failing to account for the multiple testing aspect of phylogenetic topology testing can lead to gross exaggerations of the strength of support against the null – this problem can be referred to as selection bias.

These considerations are even stronger in the case of another common pattern in phylogenetic data analysis: a researcher estimates a tree, and then would like to highlight the groupings that are significantly supported. In this case neither the null nor the alternative is specified a priori.

The Shimodaira–Hasegawa test (SH test) was the first phylogenetic testing procedure to deal with the problems related to selection bias.

Shimodaira–Hasegawa Test

Like the KH test, the SH test uses the likelihood ratio test statistic, and it uses a resampling procedure to generate the null distribution. In order to account for selection bias, the researcher must inform the testing machinery of the candidate set of trees that were considered plausible before the data set was examined. The SH test will test each of these trees in a way that acknowledges the fact that the researcher has searched through this set of trees to find the one with the highest score.

Recall that when performing the KH test we used the null hypothesized expectation of 0 for the difference in log-likelihoods. Because we are using the best of many trees as the alternative tree in the SH test, we can no longer assume that the δ statistic will be centered around 0. Clearly, the maximum likelihood (ML) tree will have a δ statistic that favors it when we compare it to any other tree – otherwise it would not be the ML estimate of the tree. In the SH test we allow each of the candidate trees to influence our null distribution for the delta statistic. Then we calculate a *p*-value for each tree individually to ascertain if its difference from the ML tree's score can be explained under the null hypothesis.

We can enforce the null hypothesis that a tree is no better than other members of the candidate set by:

1. resampling the sites to generate a estimate of the sampling distributions of log-likelihoods for each tree in the candidate set,
2. calculating the mean log-likelihood for each tree across all resampled replicates, and
3. subtracting that mean log-likelihood for a tree from each of its resampled log-likelihood values.

This centers the distribution of log-likelihoods for each tree across resampling replicates around 0.

To mimic the selection bias, for each replicate we find the highest *centered* log-likelihood over all of the trees. We use that value to stand in for the maximized likelihood. For each bootstrap replicate, each candidate tree's centered likelihood is compared to the highest centered log-likelihood for that replicate. This generates a sample of the null δ statistic for each tree.

For each topology in each bootstrap replicate we have generated a δ value under the expectation that on average that tree is no better or worse than the ML tree. For any candidate topology, we can then compare the observed δ to this distribution. The *p*-value for each tree is the fraction of the RELL bootstrap in which the resampled δ statistic for that tree is more extreme than that tree's δ statistic calculated on the real data set. In this way we are able to capture not only the effects of the number of candidate trees we are considering on the expectation of δ, but also the variance structure of the log-likelihoods for individual trees.

The SH test makes the pessimistic assumption that we should treat every member of the candidate set as equally likely a priori and that each contributes in an independent manner to the multiple testing problem. Because a few sites may strongly disfavor a large number of candidate trees, centering each tree's set of scores around 0 is probably too generous an assumption with respect to the worst trees in the candidate set. The result of making this cautious assumption is that the test tends to be too conservative.

A more pressing empirical problem with the test is that the researcher has to specify the candidate set of trees honestly. Omitting a tree from the candidate set will tend to exaggerate the significance of the trees tested. For even a moderate number of tips in a tree, there are a very large number of possible, and a priori plausible trees. Thus, the test can be infeasible to apply because it would requre storing and resampling the likelihood for too many trees.

Tests Based on Parametric Bootstrapping

The methods described above leverage the variation in the likelihoods across sites in observed data to generate distributions of expected δ values. This gives us the ability to estimate *p*-values. Alternatively, we can use our understanding of evolutionary process to generate expected distributions of data sets under a topology of interest. First, a substitution model is selected for the data. When we estimate a phylogeny using likelihood or Bayesian methods, we also estimate a model of evolution under which our sequences have evolved. For example, if we estimate a phylogeny using a general time reversible model for DNA sequences, we estimate the rate of change between each class of nucleotides. The choice of substitution model is often determined by a likelihood ratio tests or an information criterion. The parameter values for the chosen model, θ, are estimated for the null hypothesis topology.

Next a series of data sets are simulated on that topology under the parameters (θ) of that inferred model of evolution. A search for the ML tree is performed on each of these simulated data sets. In addition, the the likelihood of the optimized null topology is calculated for each simulated data set. The difference in likelihood score, δ, between the ML topology and the topology generating the data, is calculated for each simulated data set. This procedure produces a distribution of δ values that would be expected if the null tree were the correct topology. By comparing the observed δ to the distribution of simulated δ you can calculate the probability that the observed difference in likelihood between the ML tree and the null would have been observed if the null were the true tree. Testing phylogenies in this manner is often called the SOWH test, after Swofford, Olson, Waddell, and Hillis who first described using parametric bootstrapping to test topologies (Swoford *et al.*, 1996; Goldman *et al.*, 2000).

There are several advantages to using parametric bootstrapping for topology testing. Because this test uses the inferred model of evolution, the SOWH test has much higher power than the nonparametric approaches described above (Goldman *et al.*, 2000; Buckley, 2002). Also, conveniently, the SOWH test doesn't rely on topologies having been determined a priori. As the data sets are generated with respect to a specific topology, in comparison to the observed ML tree, these tests may be performed on the ML tree without the necessity of attempting to encompass all potentially plausibly trees, as is appropriate for the SH test. There may be millions of plausible trees, so this is a valuable advantage.

However, there are several important disadvantages to a parametric bootstrapping approach as well. Firstly, there is a large computational burden of estimating the ML phylogeny for hundreds of simulated data sets. If the individual ML searches do not find the best topologies, δ values in the null distribution will be underestimated; this can lead the test to reject too frequently. Although there are some shortcuts which can decrease the computational time, these may affect rejection rates in undetermined ways (Goldman *et al.*, 2000). Nonetheless, with increases in computational power, and the application of large computing clusters the technical issue of searching for ML trees can be overcome. More troubling is the issue that available models of sequence evolution under which we are able to simulate rarely capture the complexity of observed empirical data (Buckley, 2002). This oversimplification of simulated data likely results in phylogenetic inference on these data sets having an easier time inferring the correct tree. This will result in smaller values of δ statistics in the null distribution, and make it easer to reject the null topology as the generating tree. Researchers should be aware that if they reject a null hypothesis based on parametric bootstrapping, the appropriate conclusion is that the degree of support observed is too large to be easily explained by chance assuming that the simulated model of sequence evolution adequately mimics the level of conflicting signal in the true generating process. Indeed, in many comparisons of SH and KH tests to SOWH tests, parametric bootstrapping strongly rejects the null in cases where those nonparametric tests are unable to do so (Goldman *et al.*, 2000). This may be due to some differences in how the null and alternative hypotheses are generated under these different tests (rejecting one alternative tree vs. rejecting all other trees) and the general result that parametric tests have more statistical power. The more worrisome possibility is that SOWH may have excessive type I error when the model of evolution used is too simplistic (Buckley, 2002).

Susko (2014) has made some suggestions to improve the SOWH test and decrease the prevalence of type I error. If instead of using a fully resolved topology for the null distribution, a constrained topology in which branches differentiating the two hypotheses are collapsed (e.g., **Figure 1(d)**), the test would better capture the borderline between support for one topology or the other. By comparing this borderline topology, this modification gives a fairer chance to the null hypothesis and lowers the excessive rejection rates of the SOWH test (Susko, 2014).

Quick and Dirty Methods

Finding the likelihood ratio between the ML tree and the best tree that lacks a particular grouping can be computationally expensive. Often a nearest neighbor of the ML tree which has all of the groups with the exception of the clade of interest will be the 'next best' tree. A few testing methods have exploited this to produce quick statements of support for a branch in the ML tree by only considering the neighboring trees that lack that branch. This makes the δ test statistic quick to calculate. If a mixture of χ^2 distributions is used as a null distribution, this approach is referred to as an 'approximate Likelihood Ratio Test' (aLRT) (Anisimova and Gascuel, 2006). Anisimova *et al.* (2011) found that the aLRT rejects the null too frequently, and they recommend another fast approximate statement of support, the aBayes statistic. To calculate the aBayes score for a branch in the ML tree, one divides the likelihood of the tree containing the branch by the sum of the likelihood of that tree and the two trees that are nearest neighbors of the tree but lack the branch in question (see **Figure 3**). Simulations by Anisimova *et al.* (2011) indicate that using a 0.95 threshold for aBayes does not reject the null too often, but there are no theoretical results to indicate that this conclusion is general.

Pure Resampling Approaches

The methods discussed above all use the likelihood ratio test statistic – they only differ in how they generate the null distribution for that test statistic. It is also possible to take a fully resampling based approach in many cases. Felsenstein (1985) introduced the general statistical technique of bootstrapping to the field of phylogenetics as a method for determining confidence for different groupings in the tree. In the bootstrapping procedure, you create an artificial data set the same size as your data by resampling the characters (randomly and with replacement) from your original data set. You can estimate a tree on this bootstrap-pseudoreplicate data set. This process can be repeated many times to produces a collection of bootstrap trees. Clades that show up in all or almost all of the bootstrap trees must have substantial support. Even in the face of the sampling error of the bootstrapping procedure, these clades are almost always recovered. Thus, the amount of support for these clades in your data must be larger than the amount of noise that is typically produced by sampling error. If a clade appears across many different replicate subsamples of your data, you can have some confidence that the clade is not just an artifact of sampling error.

For some statistical problems, one can even approximate a p-value using 1.0 minus the bootstrap proportion (BP). So, if a result was found in 96% of the bootstrap replicates, its p-value would be approximately 0.04. Unfortunately, this simple approach does not apply to phylogenetic problems. The connection between the p-value and $1-BP$ is complicated (see Alfaro *et al.*, 2003; Newton, 1996, and references therein). Correction for the $1-BP$ approach have been developed based on theories about the geometry of tree space. This body of theory underlies the approximately unbiased test for phylogenies Shimodaira (2002) and the multi-level bootstrap method of Efron *et al.* (1996).

Adjusted Bootstrap Proportion

Susko (2010, 2014) developed improved phylogeny testing procedures that pay particular attention to the boundaries between hypotheses where the branches that differ between the trees all have branch lengths of 0. At that point, the different topologies make exactly the same prediction of what data we would expect to see – so it is impossible to distinguish data arising from one topology from data that arose from other trees. If we are treating one of the trees as the null, this point should be the hardest point of the null hypothesis to

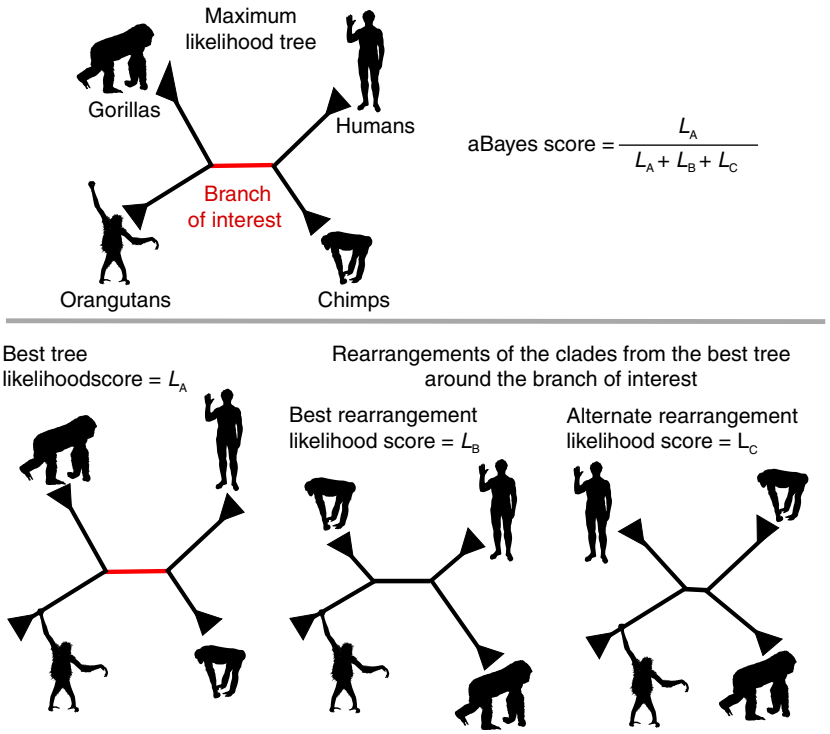

Figure 3 To calculate the aBayes score for a branch in the maximum likelihood (ML) tree, one divides its likelihood by the sum of the likelihood of that tree and the two trees that are nearest neighbors of the tree but lack the branch in question. Silhouettes are reproduced from phylopic.org, credit Mike Keesey and Gareth Monger CC BY 3.0.

reject. Not only are the boundaries between hypotheses curved, but more than two trees' boundaries come together at this point of zero branch lengths. Susko's approaches evaluate the curvature of the likelihood function for different trees at this point. After fitting normal distributions to mimic the shape of the likelihood surface, his 'adjusted bootstrap proportion' (a*BP*) can correct for the complexities of the hypothesis testing problem. Thus 1–a*BP* provides a better estimate of the *p*-value than the simple $1 - BP$ approach.

Further Considerations

This article has outlined methods for considering sampling error when phylogenetic hypotheses are tested. There are many other sources of error that a practitioner must be aware of that are completely ignored by the tests presented here. For instance:

- If the method of tree inference is not sophisticated enough to interpret the data, then the tree inference may be subject to systematic bias. For example, for some sets of branch lengths parsimony is guaranteed to estimate the wrong tree if given enough data. For you to have confidence in your estimated tree, you must be confident that it is not the result of either sampling error or systematic error.
- If you have data from one or a few loci, you may have confidence in your estimates of the gene trees. However, you should be aware that the species tree may differ from the gene tree due to hybridization, lateral gene transfer, problems of paralogy, or simply the failure of polymorphisms to fix during the duration of an ancestral species lineage. The topology tests described above do not accommodate these effects.
- Errors in the construction of the data set are ignored by the methods above. For example, the true alignment is not known and phylogenetic biases of alignment algorithms can affect the strength of support. See Karin *et al.* (2014) for recommendations about incorporating alignment uncertainty in parametric bootstrapping.

See also: Maximum Likelihood Phylogenetic Inference. Molecular Evolution, Models of. Support Measures, Phylogenetic Tree

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Relevant Website

http://phylo.bio.ku.edu/mephytis/lrt-null-nonparametric.html
Holder Lab.

Phylogenetic Tree Distances

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Introduction

The relatively recent development of cheap biological sequencing technologies has led to a sustained period of exponential growth in the amount of dat00a available to systematists. The analysis of this data presents some unique challenges that have attracted the attention of many researchers in the fields of mathematics, statistics, and computer science.

One object of particular interest is the phylogenetic tree. Phylogenetic trees summarize the most recent common ancestor relationships between a given set of taxa in the form of a mathematical graph. Figure 1 depicts a pair of unrooted trees on five taxa. These trees both specify branch lengths, which typically represent an expected number of substitution events per sequence locus, although other quantities may also appear (It is also common to encounter trees without branch lengths, as some methods only concern themselves with the relative relationships, and ignore the magnitudes of the differences.). These trees T_1 and T_2 will be used to demonstrate the calculations for all methods. If a distance measure does not require branch length information, then they may be ignored.

When observing real variables, there is little debate about how one ought to quantify the distance between two observed points, subtraction being the nearly universal choice. However, when the objects being observed are not real numbers, one must consider more carefully the question of how to define distance. There are numerous ways to define distances on the space of possible phylogenetic trees; some of these methods have convenient analytic or computational properties, while others have more natural biological interpretations.

In the subsequent descriptions, we use n to denote the number of terminal taxa (or leaves) in the tree. The space of all possible trees on n taxa is called $\mathcal{T}_n$. This space may or may not incorporate branch length information, and the trees may or may not be rooted, depending on the context. We use $\|x\|$ to represent the usual Euclidean length of a vector $x=(x_1,x_2,\ldots,x_d)\in\mathbb{R}^d$, that is defined as

$$\|x\| = \sqrt{x_1^2 + x_2^2 + \cdots + x_n^2} \qquad [1]$$

and $|\cdot|$ to indicate the cardinality of a set, that is, a measure of the number of elements in the set. A tree distance is a function, $d:\mathcal{T}_n\times\mathcal{T}_n\to\mathbb{R}^+$ that has, at a minimum, the properties $d(r,s)=d(s,r)$ and $d(t,t)=0$. Many of the methods also require a vectorization function, $v:\mathcal{T}_n\to\mathbb{R}^p$, for some p, which maps phylogenetic trees into Euclidean space. The symmetric difference between two sets is defined as $A\ominus B:=(A\backslash B)\cup(B\backslash A)$.

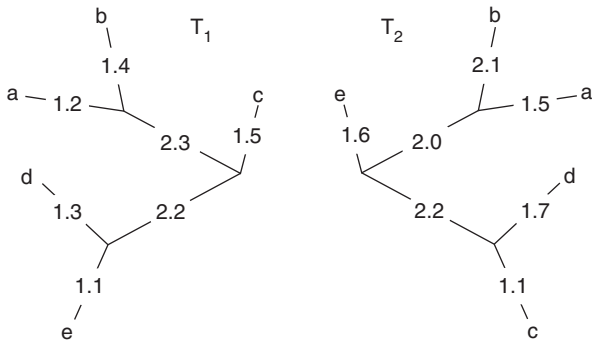

Figure 1 Example phylogenetic trees: T_1 and T_2. The trees represent proposed most recent common ancestor relationships between five taxa, labeled *a* through *e*. These trees have branch lengths specified, but not all trees need have such information.

Squared Euclidean Distances

A tree distance $d(\cdot,\cdot)$ is 'squared Euclidean' if there is a vectorization function v and a positive constant c, such that

$$d(r,s) = c\cdot\|v(r)-v(s)\|^2 \qquad [2]$$

Several popular tree distances are squared Euclidean distances as will be demonstrated below.

Robinson–Foulds Distance

A 'split' is a bipartition of the set of leaves of a tree. For example, $\{abc|de\}$ (sometimes shortened to simply *abc* when the context is clear) is one possible split of the taxa found in the example trees. This split is found in T_1, since by removing the branch with length 2.2 we can form two trees with the correct partitioning of leaves. However, this split is not found in $T2$; there is no way to remove a branch and obtain the desired partition.

Let $S(T)$ be the set of splits found in a tree T. The normalized Robinson–Foulds (RF) distance is defined as half of the size of the symmetric difference between the set of splits for each tree,

$$d_{RF}(T,T') := \tfrac{1}{2}|S(T)\ominus S(T')| \qquad [3]$$

The RF distance is a squared Euclidean distance, since we may define a vectorization function $v_{RF}:\mathcal{T}_n\to\mathbb{R}^{2n-1}-1$ where the components of v_{RF} form an enumeration of the indicator functions on all possible tree splits. In other words, for each possible split of the leaves, $A|A^c$, there is an element of $v_{RF}(T)$ which is 1 if $A|A^c\in S(T)$, and zero otherwise. We leave it as an exercise to the reader to demonstrate the connection between this vectorization scheme and the definition in terms of the symmetric difference, and to show that eqn [2] is satisfied, with $c=1/2$.

If the coordinates of v_{RF} are associated with splits in the following way,

$$(a,\ b,\ c,\ d,\ e,\ ab,\ bc,\ ac,\ cd,\ bd,\ ad,\ de,\ cd,\ bd,\ ae)$$

then the trees T_1 and T_2 from Figure 1 are vectorized as

$$\begin{aligned} v_{RF}(T_1) &= (1,1,1,1,1,1,0,0,0,0,0,0,0,0,1) \\ v_{RF}(T_2) &= (1,1,1,1,1,1,0,0,0,0,0,0,1,0,0) \end{aligned} \quad [4]$$

With these vectors, it is simple to calculate the normalized RF distance by applying eqn [2] and we find $d_{RF}(T_1,T_2)=1$.

Note that it is somewhat common for authors or programs (e.g., PHYLIP) to define the RF distance as the size of the symmetric difference without the normalizing constant $\frac{1}{2}$. When comparing RF values from different sources it is important to determine if the conventions used are compatible.

Quartet Distance

Given any tree with more than four leaves, it is possible to form a number of subtrees by pruning the tree down in various ways until it contains only four leaves. Such a subtree is called a 'quartet' of the original tree. Each quartet contains a single nontrivial split, obtained by removing the single interior branch. Since there are three possible unrooted topologies for each quartet, the splits which they define carry information about the topology of the complete tree.

We say that a tree T contains the quartet $ab|cd$, if the quartet comprising the listed nodes contains the given split. For example, T_1 contains the quartets $ab|de$ and $ab|cd$, but not the quartet $cd|be$. T_2, however, contains all three quartets.

Let $Q(T)$ be the set of quartets in a tree T. The quartet distance (Estabrook *et al.*, 1985) is defined as half of the size of the symmetric difference of quartets,

$$d_Q(T,T') := \tfrac{1}{2}|Q(T) \ominus Q(T')| \quad [5]$$

As in the case of the RF distance, d_Q can be written as a squared Euclidean distance such that using a vectorization function $v_Q : \mathcal{T}_n \to \mathbb{R}^{3\binom{n}{4}}$. This function maps tree T to the 0/1 vector $v_Q(T)$ whose entries are indicator functions of all possible quartet splits in T. For example, if the coordinates of v_Q are ordered in the following way,

$$\begin{aligned} &(ab|cd,\ ac|bd,\ ad|bc,\ bc|de,\ bd|ce, \\ &be|cd,\ ab|ce,\ ac|be,\ ae|bc,\ ac|de, \\ &ad|ce,\ ae|cd,\ ab|de,\ ad|be,\ ae|bd) \end{aligned} \quad [6]$$

then our example trees T_1 and T_2 from Figure 1 are vectorized as

$$\begin{aligned} v_Q(T_1) &= (1,0,0,1,0,0,1,0,0,1,0,0,1,0,0) \\ v_Q(T_2) &= (1,0,0,0,0,1,1,0,0,0,0,1,1,0,0) \end{aligned} \quad [7]$$

The distance between T_1 and T_2 is easily computed to be

$$d_Q(T_1,T_2) = \tfrac{1}{2}\|v_Q(T_1) - v_Q(T_2)\|^2 = 2 \quad [8]$$

Dissimilarity Map Distance

Given any tree T of n leaves with branch length information, one may produce a corresponding 'distance matrix,' $D(T)$. The distance matrix is a $n \times n$ symmetric matrix of nonnegative real numbers, with elements corresponding to the sum of the branch lengths between pairs of leaves in the tree. To calculate $D_{(ij)}(T)$, one simply determines which edges of the tree form the path from leaf i to leaf j, and then sums the lengths of these branches.

Since $D(T)$ is symmetric and has zeros on the diagonal, the upper-triangular portion of the matrix contains all of the unique information found in the matrix. We can vectorize T by enumerating this unique portion of the distance matrix,

$$v_D(T) := (D_{12}(T), D_{13}(T), \ldots, D_{23}(T), \ldots, D_{n-i,n}(T)) \quad [9]$$

The 'squared dissimilarity map distance' is defined to be

$$d_D(T',T) := \|v_D(T) - v_D(T')\|^2 \quad [10]$$

A discussion of the dissimilarity map distance can be found in Buneman (1971).

If we order the columns and rows of the distance matrix alphabetically, then the example trees are vectorized as

$$\begin{aligned} v_D(T_1) &= (2.6,\ 5.0,\ 7.0,\ 6.8,\ 5.2,\ 7.2,\ 7.0,\ 5.0,\ 4.8,\ 2.4) \\ v_D(T_2) &= (3.6,\ 5.1,\ 7.4,\ 6.8,\ 5.7,\ 8.0,\ 7.4,\ 5.5,\ 4.9,\ 2.8) \end{aligned} \quad [11]$$

From this point, computing the distance between the trees is simple,

$$d_D(T_1,T_2) = \|v_D(T_1) - v_D(T_2)\|^2 \approx 2.64 \quad [12]$$

Path Difference

The RF and Quartet distances are completely determined by the topologies of the trees, ignoring any edge lengths that may be present. Conversely, the dissimilarity map distance requires that the edge lengths be defined. The 'path difference' distance d_P is a distance analogous to the dissimilarity map, but which does not require edge length information.

The calculation of the path difference is identical to the dissimilarity map, except that elements in the distance matrix $D(T)$ are determined by counting the number of edges between the leaves, rather than summing the edge lengths (This is equivalent to the dissimilarity map distance with all edge lengths in the tree set equal to 1.). The path difference is studied and compared with the RF distances by Steel and Penny (1993).

Using the same vector ordering as in the dissimilarity map example, we find that the path difference vectorizations of our example trees are

$$\begin{aligned} v_p(T_1) &= (2,\ 3,\ 4,\ 4,\ 3,\ 4,\ 4,\ 3,\ 3,\ 2) \\ v_p(T_2) &= (2,\ 4,\ 4,\ 3,\ 4,\ 4,\ 3,\ 2,\ 3,\ 3) \end{aligned} \quad [13]$$

The path difference is therefore,

$$d_p(T_1,T_2) = ||v_p(T_1) - v_p(T_2)||^2 = 6 \quad [14]$$

Tree Rearrangement Distances

All of the tree distances discussed so far can be understood in terms of vector magnitudes in some Euclidean space. The

distances discussed in this section are defined in a different way: given a certain class of tree rearrangement operations, the distance between two trees is the minimum number of steps needed to transform one tree into another. These distances are all topological distances; they ignore any edge length information the trees may contain.

Nearest-Neighbor Interchange Distance

For each internal branch in a tree, there are three possible configurations for the connected subtrees, as shown in Figure 2. A nearest-neighbor interchange (NNI) operation (also known as a tree rotation) makes a small change to the topology of the tree by exchanging two adjacent subtrees, forming one of the alternative topologies (Robinson, 1971). The NNI distance between two trees is the minimum number of such moves required to transform one tree into the other. Although conceptually simple, computing the NNI distance is an NP-hard problem (Dasgupta *et al.*, 1997).

For instance, each of the example trees can be rotated about the length 2.2 branch, exchanging the leaves *c* and *e*, to form the other tree. Thus, the trees are separated by an NNI distance of 1.

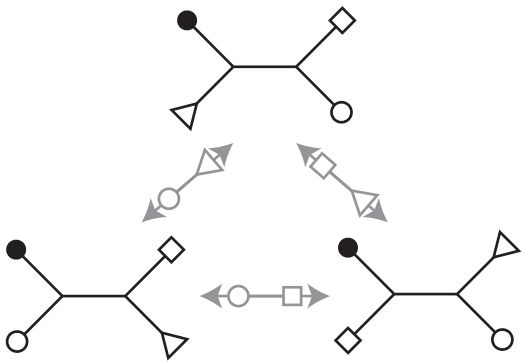

Figure 2 A nearest-neighbor interchange (NNI) move begins by selecting an internal branch from the tree. The selected internal branch defines four subtrees, which are represented in simplified form by different shapes. These four subtrees may be arranged in one of the three possible topologies. The NNI move is completed by exchanging two of the subtrees, forming one of the alternative topologies.

Subtree-Prune-and-Regraft Distance

Like the NNI distance, the 'Subtree-Prune-and-Regraft (SPR)' distance is defined by a minimum number of operations required to transform one tree into another. The steps of an SPR move are depicted in Figure 3. Succinctly, a subtree is pruned from the main tree, and then reattached to the middle of an edge elsewhere in the tree. An NNI move is a special case of an SPR move, where the detached subtree can only be moved to one of two possible locations.

Unfortunately, computing the SPR distance also is an NP-hard problem (Hickey *et al.*, 2008). However, since an NNI move is also an SPR move, we know that the example trees are also separated by SPR distance 1.

Tree-Bisection-and-Regrafting Distance

A further generalization of SPR, 'tree bisection and regrafting (TBR)' operations on trees can also be used to define tree distances (Semple and Steel, 2003). In a TBR operation, the pruned subtree can be reattached to the main tree in a more general fashion than in an SPR move. An example of a TBR move is depicted in Figure 4. Like the other distances in this section, the TBR distance is defined as the minimum number of such moves required to transform one tree into another.

Computation of the TBR distance is also NP-hard (Allen and Steel, 2001). However, in the case of our example trees, the distance is 1, since TBR is a generalization of both SPR and NNI.

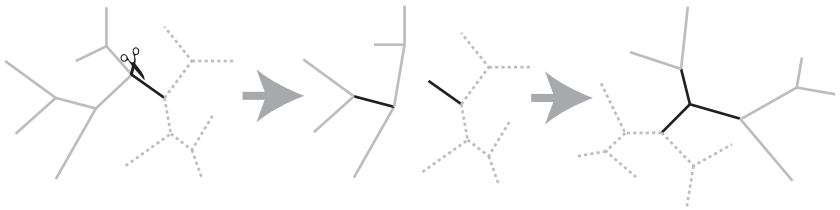

Figure 3 A subtree-prune-and-regraft (SPR) move: (Left) A subtree is selected and pruned from the main tree. (Middle) A branch is chosen from the main tree to receive the subtree. (Right) The subtree is regrafted onto the main tree.

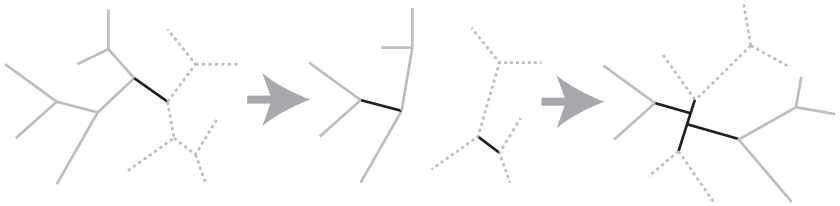

Figure 4 A tree-bisection-and-regraft (TBR) move: (Left) The tree is bisected by removing an internal branch. (Middle) Two branches are chosen from the resulting subtrees. (Right) The two branches are regrafted together in one of the three possible topologies.

Disagree Distance

Steel and Penny (1993) also described the 'disagree distance.' This distance is defined by the minimum number of taxa that must be removed from the phylogeny before the trees become congruent. For example, in our example, trees are separated by a disagree distance of 1, because removing any one of the taxa *c*, *d*, or *e* results in the trees being topologically congruent. Puigbó *et al.* (2007) discussed an algorithm for computing the disagree distance.

Billera–Holmes–Vogtmann Geodesic Distance

Billera *et al.* (2001) introduced a continuous space which models the set of rooted phylogenetic trees with edge lengths on a fixed set of leaves (Unrooted trees can be accommodated by using either the Ferras transform, or by designating an arbitrary leaf node as the root.). The Billera–Holmes–Vogtmann (BHV) tree space is not Euclidean, but it is non-positively curved, and thus has the property that any two points are connected by a unique shortest path through the space, called a 'geodesic.' The distance between two trees is defined as the length of the geodesic connecting them.

Consider a rooted tree with n leaves. Such a tree has at most $2n-2$ edges; there are n terminal edges, which are connected to leaves, and as many as $n-2$ internal edges. The maximum number of edges is achieved when the tree is binary, but the number of edges can be lower if the tree contains any polytomies. With each distinct tree topology, we associate a Euclidean 'orthant,' of dimension equal to the number of edges that the topology possesses (here, we may regard an orthant to be the subset of $\mathbb{R}^d$ with all coordinates nonnegative). For each topology, the orthant coordinates correspond to edge lengths in the tree.

Since all tree topologies have the same set of n terminal leaves, and each of these leaves is associated with a single terminal edge, the orthant coordinates associated with the terminal edges are of less interest than those of internal nodes. As a result, we will simplify our discussion by ignoring the terminal edge lengths, and concern ourselves primarily with the portion of each orthant which describes the internal edges. (Recall that this space has at most $n-2$ dimensions.)

Since each of the coordinates in our simplified orthant corresponds to an internal edge length, the orthant boundaries (where at least one coordinate is zero) represent trees with collapsed internal edges. These points can be thought of as trees with slightly different – but closely related – topologies. The BHV space is constructed by noting that the boundary trees from two different orthants may describe the same polytomic topology. With this insight, we may set about constructing the space by grafting orthant boundaries together when the trees they represent coincide.

Figure 5 depicts a portion of the BHV space on rooted trees with four leaves. The depicted portion of the space includes five orthants (topologies) and the structure of the connections between them. Since rooted binary trees on four leaves have two internal nodes, the space consists of two-dimensional orthants. Each point within an orthant corresponds to the tree with its associated topology and given internal edge lengths. The origin of each orthant corresponds to the tree with no internal edges (the 'star tree'), and the boundary rays correspond to trees where one edge is collapsed, forming a single internal node with three children.

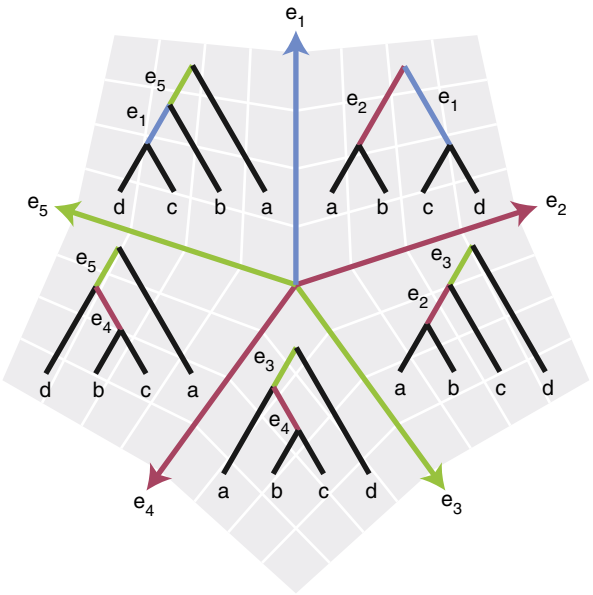

Figure 5 A portion of the space of rooted trees with four leaves. The space is formed by grafting together orthants, each corresponding to a particular topology. The full space contains several additional orthants, and is depicted schematically in Figure 6.

It is possible to represent the structure of the grafting, using the graph in Figure 6. In this graph, each edge represents a continuous path through a single orthant, from one boundary ray to the other. Conceptually, this path is formed by exchanging length between the internal edges of the tree. If two edges are joined by a node, then there are trees along the boundaries of the orthants which share a common (polytomic) topology. These boundaries are grafted together, making it possible to form a continuous path between the two orthants.

If two trees are within the same orthant, then we define the distance between them using the Euclidean distance between the corresponding points. However, if the trees have different topologies, then things become more difficult. In Figure 7 we have plotted the example trees (by arbitrarily designating node *e* as the root) onto the portion of the space from Figure 5. One possible continuous path between any two trees can be formed by shrinking the internal nodes of one tree down to zero (forming the star tree), and then expanding the tree again in the correct topology. This path is called the 'cone path' and is depicted by the dotted line.

However, there is a shorter path connecting the trees, in which only one internal edge is collapsed and the resulting polytomy can be resolved directly into the topology of the other tree. This path is depicted by the solid line. Considering only these two options, it is clear that cone path is longer. However, we have yet to establish that we have, in fact, found the shortest among all possible paths.

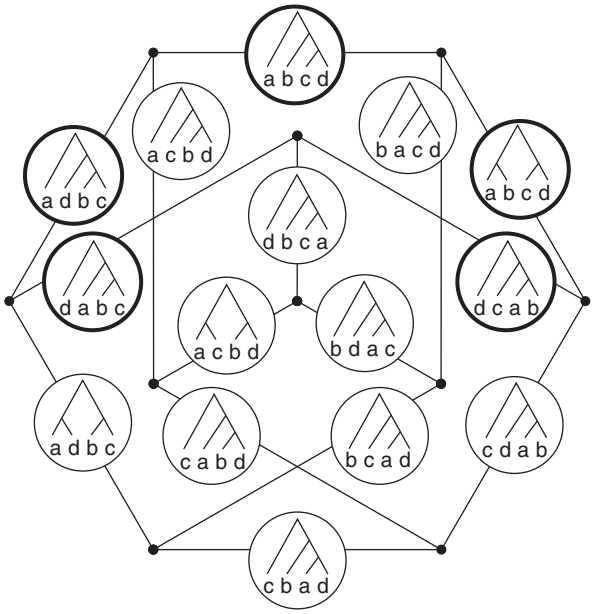

Figure 6 A schematic representation of the full space of trees on four leaves, forming a Petersen graph. Each edge represents a rooted binary tree topology, and each node represents the grafting together of orthant boundaries from the connected topologies. The portion of the graph depicted in **Figure 5** is bolded.

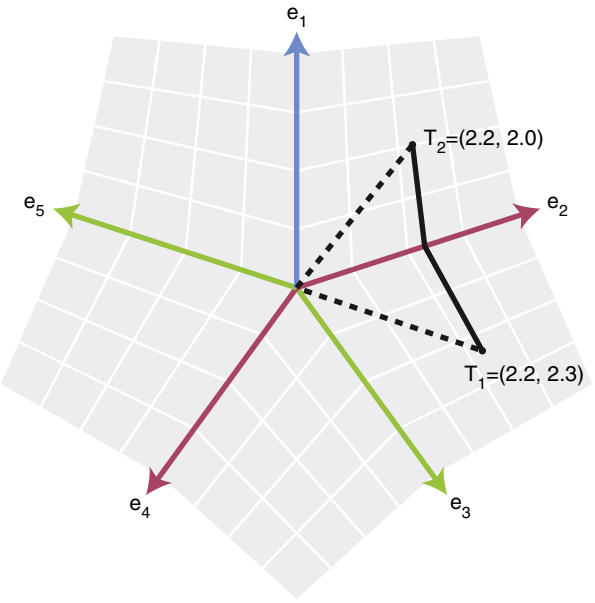

Figure 7 If we relabel leaf *e* as the root, then we can plot our example trees in the reduced tree space. (The orthants shown correspond to the same topologies as in **Figure 5**.) The cone path between the trees is shown as the dotted line. The shortest path connecting the trees is called a geodesic, and is plotted as the solid black path.

Since each orthant is locally a Euclidean space, the shortest path between two points within a single orthant is a straight line. The difficulty comes in establishing which sequence of orthants joining the two topologies will contain the geodesic. In the case of four leaves, we could do this through a brute-force search, but we cannot hope to do so with larger trees. Owen and Provan (2011) present a quartic-time algorithm (in the number of leaves) for finding the geodesic path between any two points in the space, but unfortunately the details are far beyond the scope of this article. However, once the geodesic is known, computing its length – and thus the distance between the trees – is a simple matter.

Other Tree Distances

This section contains a few other notable tree distances which do not fall into any of the previous categories.

Matching Splits Distance

A recently introduced distance is the 'matching splits distance,' developed by Bogdanowicz and Giaro (2011). Roughly speaking, the matching splits distance refines the RF distance by allowing splits to partially match each other when a portion of the split is shared by both trees.

Maximum Parsimony Distance

Fischer and Kelk (2015) introduced a notion of the 'Maximum Parsimony (MP) distance' between phylogenetic trees. The MP distance between trees is the difference between the MP scores of the given trees. The MP is a nonparametric phylogenetic tree reconstruction method and a score of taxa is computed how similar to each other among them based on a score matrix. Then the MP tree score is computed by picking the best score of all possible scores. One can find an example of the MP distance on the 'ParametricBootstrappingLab' wiki web page of Special Topics Courses: Workshop on Molecular Evolution (Hillis *et al.*, 2013).

See also: Consensus Methods, Phylogenetic. Phylogenetic Tree Comparison

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Phylogeography

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Glossary

Alpha-diversity Species richness, or the number of species present at a location; a measure of biological diversity (biodiversity).

Beta-diversity Species turnover between locations, or the number of species present at one location which are not present at the second; a measure of biological diversity (biodiversity).

Coalescent The population genetic theory by which all alleles present in an extant population trace their genealogy back to a shared ancestor at some point in time.

Genetic drift The random change in frequency of alleles within a population.

Glacial refugia A contraction in a species' geographic range during glacial conditions, often resulting in a genetic bottleneck; that part of the range in which suitable environmental conditions allow a species to persist until conditions improve.

Macroecology The discipline which investigates ecological patterns and processes across multiple study systems or at large spatial scales, to identify general rules governing the distribution of biological diversity.

Macroevolution Evolutionary patterns and processes detectable at the species level or above, or the study thereof.

Microevolution Evolutionary patterns and processes detectable within species, or the study thereof.

Phylogeography The discipline which investigates how genetic diversity within species is related to geography and geological events.

Integrating Across Disciplines

Avise *et al.* (1987) originally envisioned phylogeography – the study of the geographical context for genetic variation within species – as a bridge between the processes acting on populations (e.g., genetic drift and selection) and supra-specific evolutionary patterns (see **Figure 1**). Since its inception, phylogeography has rapidly grown in popularity, resulting in a vast quantity of data from a myriad of taxa and environments, a blossoming of methods to analyze those data, and conceptual links with disciplines across evolution, ecology, and earth and climate sciences, making phylogeography "one of the most integrative disciplines in all of biology" (Hickerson *et al.*, 2010). Phylogeographic studies have facilitated a deeper understanding of the prevalence of gene flow among spatially structured populations (Knowles and Carstens, 2007); the prevalence of cryptic diversification within species (Martin and McKay, 2004); the complex roles of refugia, persistence, and dispersal in shaping species' geographic ranges after the last glacial period (Moritz *et al.*, 2009; Dasmahapatra *et al.*, 2010); and invaluable perspectives on genetic evolution (Singhal and Moritz, 2012). Multi-taxon comparative phylogeography studies have indicated the pervasiveness of idiosyncratic responses to past events among even co-distributed species with apparently similar ecologies, providing important insights into dispersal, adaptation, and other factors which mediate these differences (Taberlet *et al.*, 1998; Bell *et al.*, 2012; Marske *et al.*, 2012). Like all popular disciplines, phylogeography has also been the subject of pointed critique, particularly directed at the initially descriptive way in which phylogenies were related to historical events (Soltis *et al.*, 2006). This criticism – while largely warranted – sparked an explosion of analytical development and synthesis with nongenetic methods which have left phylogeography well placed to remain highly relevant to our understanding of questions as varied as speciation and evolution to ecosystem stability and species responses to climate change.

History and Development

Avise *et al.* (1987) coined the term 'intraspecific phylogeography' after observing that genetic discontinuities for multiple North American fishes corresponded with long-term geographic barriers, and described intraspecific phylogeographic structure as a function of geographic isolation and species' dispersal ability. As well as launching the nascent discipline of phylogeography, their foundational paper elucidated two guiding principles which formed the basis for subsequent study: First, Avise *et al.* (1987) recognized that phylogeography represented the theoretical evolutionary link necessary to infer species' histories across timescales, from population genetic patterns to phylogenetic systematics, and proposed that these microevolutionary processes reflected – and could predict – macroevolutionary phenomena. Second, phylogeography was presented from the very beginning as a comparative endeavor, namely, that "strong geographic barriers should mold the genetic structure of independently evolving species in concordant fashion" (Avise *et al.*, 1987). A decade after its inception, Bermingham and Moritz (1998) implicitly underscored these two principles when they predicted that continued progress within comparative phylogeography would allow 'investigation of the fundamental links between population processes and regional patterns of diversity and biogeography.'

A third feature which has characterized many phylogeographic studies from the beginning is a focus on Pleistocene processes and glacial refugia (reviewed by Hewitt, 1996; Taberlet *et al.*, 1998). Avise *et al.* (1987) never characterized their phylogeographic patterns in terms of glaciation – they described "long-term, extrinsic (i.e., zoographic) boundaries to

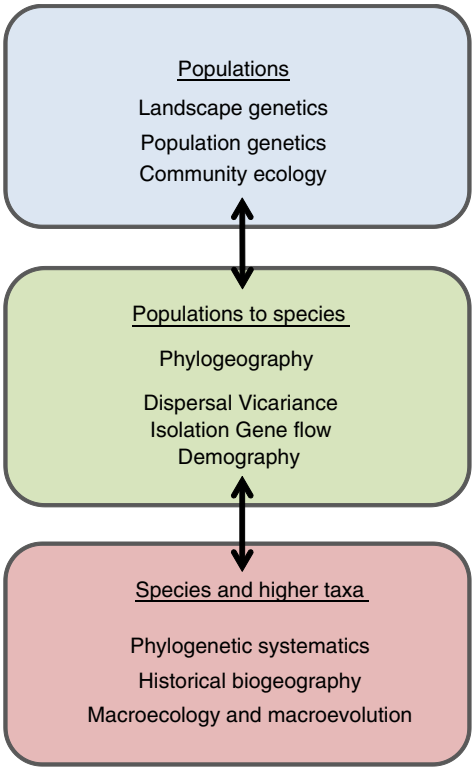

Figure 1 Phylogeography was originally envisioned as the conceptual bridge connecting processes acting on populations with evolutionary patterns detectable among species or higher taxa (Avise *et al.*, 1987). Implicit within this conceptualization is a temporal and spatial component, with population-level processes acting over shorter periods and smaller spaces but scaling up to historical biogeographic and macroecological patterns, which reflect deeper evolutionary history acting at a broader spatial scale. Thus, the population-level patterns detected using phylogeography are at the center of a continuum of ecological and evolutionary processes (Marske *et al.*, 2013). Adapted from Avise, J.C., 2009. Phylogeography: Retrospect and prospect. Journal of Biogeography 36, 3–15.

gene flow"; rather, this focus evolved as a natural result of the temporal span encompassed by intraspecific variation in mitochondrial DNA (mtDNA), upon which phylogeography was initially based (Avise *et al.*, 1987; Bermingham and Moritz, 1998). In their comparative syntheses of the effects of Quaternary glaciation on genetic diversity within Europe, Hewitt (1996, 2000) and Taberlet *et al.* (1998) emphasized both the idiosyncratic histories of individual species, and similar patterns of range expansion out of the peninsular refugia (the "southern richness, northern purity" pattern; Hewitt, 2000) with predictable suture zones where expanding lineages met. Based on these patterns, Taberlet *et al.* (1998) hypothesized that while phylogeographic congruence might occasionally occur within restricted areas, it was highly unlikely across large spatial scales, such as continents. Subsequent studies confirmed these patterns: Sullivan *et al.* (2000), in one of the first studies which explicitly tested a priori phylogeographic hypotheses, found that phylogeographic histories of two rodent taxa in a geologically complex region were neither entirely unique nor identical, but somewhere in between. In a later synthetic analysis of multiple North American taxa, Soltis *et al.* (2006) warned of the risk of pseudocongruence, where similar spatial patterns are driven by causal factors across different timescales. They also suggested that pseudocongruence might stem, in part, from the tendency among researchers to visually interpret and categorize phylogeographic patterns across species (Soltis *et al.*, 2006; but see Pyron and Burbrink, 2010), echoing a growing number of critics demanding a higher level of rigor in the testing of phylogeographic scenarios (Edwards and Beerli, 2000; Knowles and Maddison, 2002; Carstens *et al.*, 2005; Kidd and Ritchie, 2006).

One response to this criticism was the adoption of statistical phylogeographic methods, such as ancestral state reconstruction, which directly estimates the geographic locations of ancestral populations (Lemmon and Lemmon, 2008; Lemey *et al.*, 2009; Nylinder *et al.*, 2014), or model selection methods, which provide statistical support for explicit phylogeographic hypotheses (e.g., Carstens *et al.*, 2005; Hickerson *et al.*, 2006; Chan *et al.*, 2014; Pelletier and Carstens, 2014), mostly using an approximate Bayesian computational framework (Beaumont *et al.*, 2002). A key motivating factor driving the development of these methods and their implementation within a coalescent framework (e.g., Rosenberg and Nordborg, 2002) was the realization that stochastic, as well as deterministic, processes had left their mark on species' genealogies and hence, phylogeographic patterns (Edwards and Beerli, 2000; Arbogast *et al.*, 2002; Knowles and Maddison, 2002; Carstens *et al.*, 2005). These stochastic processes can result in discordant diversification among genetic loci, resulting in gene trees which do not reflect the species tree (Arbogast *et al.*, 2002). Statistical phylogeography has thus promoted both the widespread adoption and further development of coalescent tools and the increasing prevalence of multi-locus datasets within phylogeography (Knowles, 2009).

A second response to criticism was to explicitly or implicitly associate genetic data with other information sources, such as species distribution models (SDMs), to validate phylogeographic patterns (Kidd and Ritchie, 2006; Richards *et al.*, 2007; Kozak *et al.*, 2008). SDMs, which predict species' geographic distributions by relating collection localities to environmental data (Hugall *et al.*, 2002; Richards *et al.*, 2007), were first combined with phylogeography to test whether glacial refugia identified by genetic data coincided with areas of long-term climatic stability (Hugall *et al.*, 2002). SDMs are now hugely popular within phylogeography as a means of providing independent support for phylogeographic scenarios (Waltari *et al.*, 2007; Alvarado-Serrano and Knowles, 2014). Further, the two avenues of statistical phylogeography and independent verification with SDMs very quickly converged, with SDMs generating explicit spatial hypotheses (Richards *et al.*, 2007; Carnaval *et al.*, 2009), testing for potential environmental divergence underlying genetic differentiation (McCormack *et al.*, 2010), or setting the baseline conditions for direct simulations of species' histories under different dispersal scenarios (Brown and Knowles, 2012).

Current Status

Phylogeography provides a hypothetical framework with which to test the processes underlying diversification,

providing invaluable insights into how biodiversity is generated and maintained. As such, the questions currently addressed by phylogeography cover the full spectrum of ecology and evolution. A tremendous body of data across a variety of taxa and systems, from relatively easily sampled regions such as North America (Soltis *et al.*, 2006) to the farthest reaches of the globe, including Antarctica and the sub-Antarctic islands (Fraser *et al.*, 2012), suggest a myriad of responses to past climate change and other geological events. Notably, the late Quaternary history of many species was characterized by persistence in relatively small, isolated refugia (e.g., Carnaval *et al.*, 2009; Moritz *et al.*, 2009), many of which preserved evidence of much deeper divergence than just the last glacial period (Marske *et al.*, 2011; Bell *et al.*, 2012). Many studies have also yielded a much deeper understanding of dispersal, ranging from persistence in dynamic landscapes which function as temporally shifting habitat mosaics (Brown and Knowles, 2012), to movement through intermittent or leaky dispersal barriers (Burridge *et al.*, 2008; Marske *et al.*, 2009) to how dispersal potential relates to life history traits (Dawson, 2014). In spite of these dramatic changes in environment and species' geographic ranges, phylogeographic studies have largely discounted Late Pleistocene events as significant for initiating speciation (Bermingham and Moritz, 1998), but have illuminated other, older drivers of the speciation process, in conjunction with a variety of phylogenetic methods (Kozak *et al.*, 2005, 2006).

Phylogeography has also yielded insights into the origin and maintenance of regional to global biodiversity patterns, such as the distribution and drivers of alpha- and beta-diversity and the extent to which genetic diversity reflects these species-level patterns. For example, Martin and McKay (2004) proposed that if the global latitudinal diversity gradient results from higher rates of species origination at lower latitudes, genetic divergence among populations within species should be greater at the lower latitude portion of species' ranges. They synthesized data for 60 vertebrates from regions across the globe, and found that lower latitude populations do experience greater evolutionary independence, even when controlling for geographic distance and recent glacial activity (Martin and McKay, 2004). Dexter *et al.* (2012) sampled community composition and population genetic data for a genus of tropical trees along a transect in Amazonian Peru, and found that an ecological pattern of distance decay in compositional similarity masked a zone of heightened turnover where species expanding from separate refugia came into secondary contact, signifying the necessity of accounting for both contemporary and historical processes when examining the mechanisms behind biodiversity patterns. Finally, Emerson *et al.* (2011) have proposed using bulk genetic extraction techniques to simultaneously infer comparative phylogeography, historical biogeography, and phylogenetic beta-diversity among communities of soil invertebrates, using DNA barcodes to directly sample community composition for each soil sample rather than trying to demarcate community boundaries a priori.

Numerous phylogeographic studies have also sought to elucidate the processes shaping species' contemporary geographical ranges. In particular, the central-marginal or abundant center hypothesis states that species should achieve highest abundance at the range core where conditions are most optimal, with populations growing smaller and more isolated toward the range margins (Hengeveld and Haeck, 1982; Brown, 1984; Brussard, 1984). Therefore, genetic diversity and gene flow should be highest at the center of a species' range, with peripheral populations exhibiting less diversity but higher differentiation from each other and from the core (Eckert *et al.*, 2008). In a synthetic study incorporating population genetic data spanning the ranges of 115 taxa (animals and plants), Eckert *et al.* (2008) detected this pattern, but found that the difference in genetic diversity was relatively small. Notably, relatively few of the studies they included incorporated a phylogeographic perspective or invoked historical mechanisms, which Eckert *et al.* (2008) suggested might underlie the relatively weak support for the central-marginal hypothesis. Johansson *et al.* (2013) revisited the central-marginal hypothesis in a phylogeographic study of five European damselfly species, and found no general adherence to the patterns described above; rather, they found that genetic patterns were better explained by historical and contemporary ecological factors. Moritz *et al.* (2012) went a step further and explored whether variation in environmental conditions across the ranges of three skink species had resulted in different selection regimes between central and marginal populations. They found that the marginal populations were both physiologically differentiated from the core populations, and possessed thermal adaptations which might make them more likely than the core populations to adapt to and persist under contemporary climate change (Moritz *et al.*, 2012).

Other studies have centered on the formation of range margins, with a particular focus on where phylogeographic breaks, or points of spatial turnover between phylogeographic lineages, are shared across species. For example, Rissler and Smith (2010) tested whether phylogeographic breaks clustered together at contact zones between species as predicted by Taberlet *et al.* (1998) and Hewitt (2000), using available data for North American amphibians. They found significant clustering of phylogeographic breaks and contact zones in regions of known amphibian species richness, and highlighted these areas as important natural laboratories for further investigating the speciation process (Rissler and Smith, 2010). Moritz *et al.* (2009) focused on the Australia Wet Tropics, a narrow habitat corridor which forms a natural transect connecting regions of long-term climatic stability, to test the roles of historical versus contemporary climate conditions in driving diversification among lineages. They found that the majority of phylogeographic breaks occurred within a suture zone between two glacial refugia, but within this zone, individual phylogeographic breaks occurred in areas of relatively low contemporary environmental suitability (Moritz *et al.*, 2009). In contrast, Dasmahapatra *et al.* (2010) detected suture zones which, while shared across multiple Amazonian butterflies, failed to correspond to expectations based on several biogeographic hypotheses. They suggested that these zones might instead reflect contemporary ecological conditions, with multiple phylogeographic breaks shifting independently and becoming stuck on a common 'ecological hiatus' (Dasmahapatra *et al.*, 2010).

Phylogeographic studies have also generated a wealth of insight on modes of diversification and speciation. Allopatric divergence is regarded as one of the most common modes of

speciation (Coyne and Orr, 2004), and Pyron and Burbrink (2010) explored how allopatry around hard physical barriers versus environmental gradients has produced distinctly different phylogeographic patterns. They proposed that lineages should show strong concordance in the location of the phylogeographic break in cases of 'hard allopatry' around hard physical barriers, while break points might be more scattered where 'soft allopatry' reflects historical climatic disjunctions or environmental gradients. A synthetic study of genetic discontinuities around the Mississippi River Embayment and Cochise Filter Barrier in North America confirmed these predictions, as well as clarifying the temporal distribution of lineage breaks associated with each type of allopatry: under soft allopatry, divergence coincided with the timing of climate change, whereas under hard allopatry, divergence was dependent upon rare dispersal events across the barrier, and was therefore taxon specific rather than temporally clustered (Pyron and Burbrink, 2010). Jordal *et al.* (2006) utilized phylogeography to explore a putative case of sympatric speciation between two Canary Islands beetles. Rather than a sympatric origin, however, they found that the taxa likely diverged on separate islands before coming into secondary contact on La Palma, highlighting that contemporary sympatry does not rule out previous opportunities for allopatric phases of diversification (Jordal *et al.*, 2006). The mechanisms driving ecological speciation are more controversial than those for allopatric speciation, with disagreement over whether divergent natural selection leads to (Schluter, 2001) or follows (Wiens, 2004) speciation. McCormack *et al.* (2010) investigated whether allopatric lineages of *Aphelocoma* jays in the process of speciation were more different than expected based on a null model, and found little evidence for ecological divergence except in lineages which exist in partial sympatry. In contrast, Cooke *et al.* (2012) found that neutral and selected genetic divergence in Amazonian fish was more strongly driven by water color than either river system or biogeographic history. Further, they found that both selection and neutral population structure were heightened at the environmental interface between water types, providing strong evidence for environmental, rather than allopatric, divergence (Cooke *et al.*, 2012). These studies, and those detailed above, represent but a small sample of the diverse ecological and evolutionary insights stemming from phylogeographic studies, and these data still retain immense potential for broad-scale ecological and evolutionary synthesis (Bermingham and Moritz, 1998; Marske *et al.*, 2013).

Challenges and Future Directions

Numerous analytical challenges remain within phylogeography, such as the impact of parameter selection (e.g., Carstens *et al.*, 2013), model specification (e.g., Pelletier and Carstens, 2014), or underlying theoretical assumptions (e.g., Chikhi *et al.*, 2010; Heller *et al.*, 2013) on phylogeographic inference. One persistent analytical challenge is a dearth of methods which can directly deal with community-level data, and with the notable exception of hierarchical approximate Bayesian computation (Hickerson and Meyer, 2008), comparative phylogeography typically consists of inferring individual species histories and comparing them post-hoc (Andrew *et al.*, 2013). One reason is the computational challenge of dealing with large comparative datasets (Andrew *et al.*, 2013), but the more critical aspect is conceptual: an increasing number of studies indicate that co-distributed species often exhibit consistent – but not concordant – phylogeographic histories (Taberlet *et al.*, 1998; Sullivan *et al.*, 2000; Soltis *et al.*, 2006; Moritz *et al.*, 2009; Bell *et al.*, 2012; Marske *et al.*, 2012). This may be related to the methods with which species' histories are estimated: for example, detailed spatially explicit simulations of range expansion (e.g., Brown and Knowles, 2012) or estimated dispersal routes leading out from multiple refugia (e.g., Marske *et al.*, 2012); repeated across multiple taxa, may be more likely to highlight differences between species' histories, even where similarities exist. In contrast, methods which rely upon summary statistics to test specific hypotheses of population size changes (e.g., Chan *et al.*, 2014) can classify histories as similar or different, but lack the spatial component which might tie together divergence events on different timescales. These alternate views have implications for interpretation of community assembly and stability over time (i.e., Taberlet *et al.*, 1998); for example, most species likely have idiosyncratic histories, but the regional species pool may have behaved in a more predictable way (Zink, 2002; Marske *et al.*, 2013). A more nuanced analytical approach is needed to integrate these individual histories into a model of community evolution.

The data challenge – what types of data are most appropriate and how best to procure them – remains one of the longest-running conversations within the phylogeographic community. Avise *et al.* (1987) first envisioned phylogeography as the 'mtDNA bridge' between evolutionary disciplines, but relatively early on, Bermingham and Moritz (1998) highlighted the rich potential for large-scale, multi-locus comparative studies to tease apart evolution across timescales. Adoption of coalescent methods quickly identified a weakness inherent to many phylogeographic datasets, that mtDNA often lacked sufficient resolution for robust parameter estimation (Edwards and Beerli, 2000; Carstens and Knowles, 2007), but gave rise to a variety of methods which integrate information across multiple loci to estimate divergence history while accounting for stochastic genetic processes (Knowles, 2009). In that same vein, one of the greatest contemporary challenges in phylogeography – and one of the areas with greatest promise – is how to best make use of high-throughput sequencing technologies. To date, the major hurdles have included designing data capture in a way relevant for phylogeography, developing the informatics pipeline to process the data, and finding the right balance between spatial sampling and sequencing depth of individuals and populations (Carstens *et al.*, 2012; Lemmon and Lemmon, 2012; McCormack *et al.*, 2012; Andrew *et al.*, 2013; O'Neill *et al.*, 2013). Once these challenges are addressed, the pitfalls and promises are the same – how best to glean maximum evolutionary and ecological insight from the data available.

This new era in phylogeography, in which data acquisition is no longer a significant limiting factor on experimental design, offers unprecedented opportunity for creating new syntheses across disciplines and exploring creative new questions at the interface of ecology and evolution. Data-rich,

geographically comprehensive comparative studies within and between regions and taxonomic groups, currently the purview of macroecology and macroevolution, can tackle issues like diversification and community assembly and significantly contribute to our understanding of the organization of biodiversity. As new genomic sequencing methods erode the distinction between model and non-model organisms, the tantalizing possibility of associating neutral genetic diversification with selection at the functionally important genes underlying divergence draws closer to reality. Phylogeography's most important contribution to ecology and evolution may be that it is integrative and flexible, and unraveling the next set of interesting questions will likely require pairing phylogeography with novel types of external data and methods from other disciplines. However, moving toward a more complete and process-based understanding of evolution, adaptation, community assembly, and the generation of biodiversity patterns such as richness gradients requires a multifaceted approach, of which phylogeography is an integral part.

See also: Biogeography, Evolutionary Theories in. Dispersal Biogeography. Molecular Evolution, Models of. Population Structure and Gene Flow. Quaternary Biogeography and Climate Change. Ring Species

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Plant–Pollinator Interactions and Flower Diversification

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Glossary

Bilaterally symmetrical flower (zygomorphic) Flowers with one axis of symmetry, usually a vertical one, such that the right and left sides are mirror images.
Clade A group of organisms with a common ancestor.
Character displacement Divergence of phenotypic traits in response to selection to reduce competition or reproductive interference between sympatric species.
Generalized pollination Ecological state of being pollinated by many species or functional groups of animals.
Nectar spurs Tubular structures containing nectar or other pollinator rewards.
Radially symmetrical flower (actinomorphic) Flowers with several to many axes of symmetry (as in a starfish, for example).
Reproductive isolation The tendency for members of two different species (or other groupings) not to mate or not to be interfertile; reproductively isolated populations do not exchange genes.
Reinforcement Reinforcement of reproductive isolation as a response to selection against mating or exchanging genes with members of other species (or other groupings) in secondary sympatry.
Specialized pollination Ecological state of being pollinated by few species or functional groups of animals.
Species richness The number of species (e.g., in a clade) is referred as species richness.
Sympatry/sympatric When organisms occur in the same region, near enough to one another potentially to mate or interact ecologically (opposite: allopatry).

Darwin (1859, 1877) was probably the first to recognize that specialized plant–pollinator interactions could influence both the evolution and the diversification of plants and interacting animals. Grant (1949, 1994) and most subsequent researchers (see review in Kay and Sargent, 2009) have focused primarily on the influence of floral specialization on speciation rates. However, a group's standing diversity (better termed 'clade species richness,' where a clade is a group of organisms with a common ancestor, and species richness is the number of species) is the difference between speciation and extinction rates over time since origin. It is thus important to consider the effects of pollination mechanisms on both differential extinction and speciation. Indeed, Stebbins (1950, 1951, 1974) focused on the possible influences of floral traits and trait combinations on 'adaptive success' (i.e., lower extinction rates), while suggesting a more limited influence of floral specialization on speciation rates.

The strongest clues that plant–pollinator interactions influence plant diversification rates come from analyses of the species richness of clades versus. aspects of their floral morphology or pollination ecology. For example, Dodd *et al.* (1999) found that clades of plants that are biotically pollinated are more diverse than their abiotically pollinated relatives (e.g., those with wind or water pollination). Hodges (1997) and Hodges and Arnold (1995) found greater diversification rates in plants bearing specialized flowers with nectar spurs (tubular structures containing nectar or other pollinator rewards) than in relatives with flowers not having nectar spurs. Schiestl and Schlueter (2009) found similar trends in orchids. Sargent (2004) found that clades containing plants with bilaterally symmetrical flowers were more diverse than sister clades with radially symmetrical flowers, where bilateral symmetry is assumed to increase specialization in, and/or precision of, pollination (see also Kay and Sargent, 2009). All these authors have generally interpreted the association between floral specialization and clade species richness as the result of floral specialization promoting the effectiveness of reproductive isolation between related sympatric species, and hence potentially increasing speciation rates. However, as noted above, one also needs to consider other possible causes of such correlations, such as the influence of floral specialization on extinction rates, as Stebbins (1951, 1974) and others have pointed out (see reviews in Armbruster and Muchhala 2009; Armbruster, 2014). Below I consider, in turn, speciation, extinction, and other factors that could individually, or together, contribute an association between specialized pollination and increased plant diversification (see **Figure 1**).

Effects of Floral Traits on Speciation Rates

Floral traits that limit the number of pollinator species visiting and pollinating flowers could enhance the likelihood of reproductive isolation developing between related sympatric species. The expected mechanism is that specialization in pollination ecology allows related species to use different pollinators (leading to 'ethological isolation;' Grant, 1949, 1994) or place pollen in, and pick up pollen from, different locations on the pollinators' bodies (leading to 'mechanical isolation;' Grant 1949, 1994). Both isolation mechanisms are assumed to enhance the potential for speciation either with initial divergence in sympatry (sympatric speciation) or on secondary contact (reinforcement of reproductive isolation).

Ethological Isolation

Some plants have flowers that attract only very specific pollinators (e.g., one species), and this specificity may generate ethological isolation from related species. Such pollinator

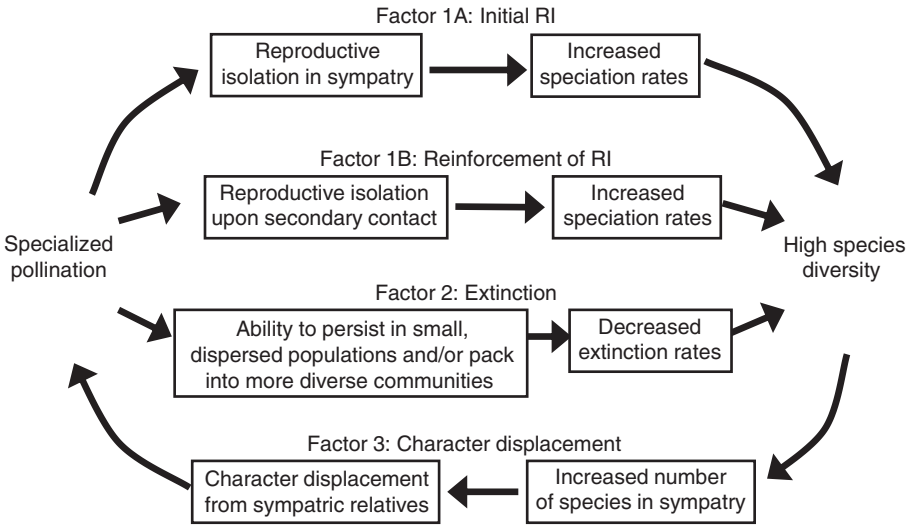

Figure 1 A graphical model of the mechanisms interlinking pollination ecology and species richness (diversity) of clades.

specificity is seen among nursery-reward species (where larval food is the motivating reward for the pollinator), such as figs and yuccas, as well as among some orchids and other plants with extreme flower morphologies, such as the Madagascar star orchid (*Angraecum sesquipedale*), with approximately 30 cm nectar spurs that restrict access to a species of sphinx moth with a similarly long proboscis (Wasserthal, 1997). Flowers pollinated by male euglossine bees (Apidae: Euglossini) may also be sufficiently specialized for ethological isolation to operate. For example, some orchids pollinated by male euglossine bees attract one or a few species of pollinators by having unusual compounds or complex mixtures of compounds in their fragrance attractants (Dodson, 1962; Dodson *et al.*, 1969; Dressler 1982; Ramirez *et al.*, 2011). Extremely similar, closely related orchid species may be virtually identical in all features but fragrance chemistry and yet remain reproductively isolated. Their speciation may have involved only small shifts in the chemistry of their floral fragrances (which are, importantly, rewards as well advertisements). That speciation can occur with little phenotypic or genetic change suggests isolation can evolve rapidity, and there should hence be increased rates of diversification in such lineages.

Outside of orchids and nursery-reward plants, however, extreme specialization and specificity of plant–pollinator relationships is generally thought to be rare, at least in most geographic regions (e.g., Waser *et al.*, 1996; cf. Johnson and Steiner, 2000; Armbruster, 2006; Schleuning *et al.*, 2012; Trojelsgaard and Olesen, 2013; Pauw and Stanway, 2015). This means that floral ethological isolation through pollinator specialization probably contributes only infrequently to diversification of plant lineages (see Armbruster (2014) for further discussion). Ethological isolation could also, in theory, be manifested through floral constancy, the tendency of individual bees to restrict foraging to one species of flower for extended periods of time (Grant, 1950; Waser, 1986). Despite being adaptive in reducing floral handling time, however, floral constancy is never absolute and is generally too incomplete to generate reproductive isolation between sympatric plant species (Chittka *et al.*, 1999; Armbruster, 2014). For example, bumble bees, which are often highly constant, still make many interspecific-flower transitions when they make periodic assessments of resource availability (Heinrich, 1976). Euglossine bees (Apidae: Euglossini) can be completely inconstant, at least when the mechanics of handling flowers is similar across plant species (Armbruster and Herzig, 1984).

Mechanical Isolation

Floral 'mechanical isolation' operates through differential pollen placement on pollinators (Grant 1949, 1994). As Stebbins (1950) pointed out, some of the strongest cases for this mechanism are seen in orchids that have pollen united into packets ('pollinia'). For example, sympatric orchids pollinated by male euglossine bees in the tropical Americas usually differ in the location of pollinarium attachment on pollinators (Dressler, 1968). This may be critical in the maintenance of reproductive isolation and play a role in speciation and diversification. Stebbins (1950) suggested that milkweeds (Apocynaceae), which also have their pollen united into pollinia, also have the potential for mechanical isolation and enhanced speciation rates, although fewer data support this contention. The critical element in these two examples is the organization of pollen into packets that can be placed precisely on pollinator and stay put until removed by other conspecific flowers. This is in contrast to most plants, which have granular pollen; granular pollen rarely lands and stays in a small enough space on the pollinator to preclude interspecific pollination (Armbruster and Muchhala 2009; Armbruster *et al.*, 2014). Indeed, recent research by Huang and Shi (2013) and Armbruster *et al.* (2014) on specialized *Pedicularis* (Orobanchaceae) flowers indicated that the granular pollen was distributed too widely on the pollinator to effect reproductive isolation between sympatric species, even when the modal sites of pollen deposition and stigma contact differed among species.

Thus, the original paradigm established by Grant (1949) and partially reinforced by Stebbins (1950), wherein

specialized pollination increases plant speciation rates by increasing the likelihood of ethological and mechanical isolation among closely related species, may be more the exception than the rule among flowering plants (but cf. Kay and Sargent, 2009). Stebbins (1974, pp. 11–13) himself suggested that ethological and mechanical isolation were unlikely to be sufficiently effective to affect rates of speciation in most plants.

Effects of Floral Traits on Extinction Rates

Another route to clade species richness is reduced extinction rates (**Figure 1**). Lowered extinction rates may occur in plant lineages that have particularly effective combinations of floral traits (Stebbins, 1951, 1974), which lead to effective attraction of, and mechanical fit with, pollinators (Armbruster, 2014). These may sometimes be associated with ecological specialization of flowers (i.e., flowers being pollinated by only a few animal species), although some models (e.g., Waser *et al.*, 1996) predict the opposite: greater reproductive success of plants with generalized pollination systems. The role that adaptive character combinations and adaptive specialization play in diversification has received very little empirical investigation. It is, unfortunately, very difficult to estimate extinction rates from phylogenetic data, so direct study of the relationship between specialization and extinction is difficult in the absence of a good fossil record. Needless to say, fossil evidence of plant–pollinator interactions is extremely limited.

One mechanism through which specialized pollination may reduce extinction rates is that more species can coexist in a plant community if they partition limiting pollinator resources. This is an axis of ecological differentiation, where limiting similarity in pollination may reduce the number of pollination generalists that can coexist in a community, relative to pollination specialists (Pauw, 2013; Benadi, 2015). Thus, as species become more specialized in their pollination, more can coexist in communities without reproductive exclusion occurring. This should lower the extinction rate in lineages with specialized pollination (Armbruster and Muchhala, 2009; Armbruster, 2014).

Effects of Diversity on the Evolution of Specialized Flowers

A third route to the association between specialized flowers and clade species richness has been pointed out only recently: members of species-rich clades may regularly experience sympatry with one or more relatives, and this selects for floral specialization (**Figure 1**; Armbruster and Muchhala, 2009; Armbruster, 2014). The logic is as follows. Closely related species are likely to share pollinators, at least when they initially come into sympatry. Members of large clades (e.g., genera) are more likely to occur sympatrically with close relatives than are members of small clades. When related species share pollinators, there will be strong selection for them to diverge in pollinator use (character displacement), such that one or both of them may become more specialized in their pollination. This is a largely untested idea, but it is consistent with the fairly common observation of floral character displacement occurring in flowering plants (e.g., Hopkins *et al.*, 2012; Beans, 2014).

Conclusions

Although there is little doubt that plant–pollinator interactions and plant-diversification rates are interrelated, the actual mechanisms involved, and their relative importance, remain to be fully elucidated. In some groups, such as orchids, specialized relationships with pollinators may increase the likelihood of reproductive isolation between similar plant taxa and thus increase speciation rates. More commonly, however, effective flower–pollinator relationships may contribute to plant diversification rates by enhancing the effectiveness of pollination and seed production, increasing population viability, and hence reducing rates of extinction. Finally, in some plant lineages, the association between specialized pollination and species diversity may reflect specialization in response to selection generated by pollinator sharing with closely related, sympatric species.

See also: Angiosperm Phylogeny and Diversification. Mutualism, the Evolutionary Ecology of. Reinforcement. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic

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Plasmid Driven Evolution of Bacteria

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Glossary

Coevolution Evolutionary change that is reciprocated between two interacting groups (usually species). For example, evolutionary change in group A leads to evolutionary change in group B which in turn causes evolutionary change in group A and so forth.

Conjugation Transfer of genetic material directly from one bacterium to another via cell–cell contact, mediated by plasmids or integrative conjugative elements (ICEs).

Geographic mosaic theory (of coevolution) A framework set down by J. N. Thompson for examining the coevolutionary process and its outcomes. In particular the GMTC emphasizes that coevolutionary interactions are spatially and temporally variable, which has led to novel theoretical and empirical findings in coevolutionary systems.

Horizontal gene pool or mobilome The collection of mobile genetic elements that are available to a bacterium for uptake and contained in the local environment.

Mobile genetic element (MGE) DNA fragments that can be transferred from one organism to another via methods other than inheritance. In bacteria these methods are conjugation, transformation, and transduction. There are several types of MGEs including plasmids, insertion sequences, transposons, and ICEs (integrative and conjugative elements).

Plasmid host range The diversity of bacterial species in which a plasmid can replicate. Host ranges are often classified as being 'broad' for those plasmids that can replicate in many hosts across distantly related taxa (such as IncP-1 plasmids in multiple classes within the phylum Proteobacteria), or 'narrow' for those plasmids that are constrained to closely related taxa (such as IncF plasmids).

Plasmid incompatibility Two plasmids are considered incompatible if they cannot stably coexist in the same lineage over multiple generations. Incompatibility typically arises due to high similarity in replication or partitioning machinery.

Plasmid stability or persistence Highly stable plasmids are those that remain within a bacterial lineage for long periods of time, mostly due to efficient partitioning mechanisms and/or post-segregational killing systems (those plasmids that are always present in a species are considered 'native'). Conversely, plasmids that remain in a lineage for only a few generations are called unstable.

Transduction Transfer of DNA from one bacterium into another by a virus (bacterial viruses are called bacteriophages).

Transformation Transfer of genetic material directly from the abiotic environment (i.e., free-floating DNA) in a bacterium. Bacterial cells that are naturally able to take up free DNA are called competent. Transformation of cells using an electrical field (also known as electroporation) is commonly used in the laboratory to cause uptake of extracellular DNA.

The emergence and rapid spread of antibiotic resistance in bacterial species is one of the major challenges facing public health. According to a recent study in the United States (Magill *et al.*, 2014), it is estimated that there were more than 700 000 hospital-acquired (nosocomial) infections resulting in 75 000 deaths in 2011; however, others (Klevens *et al.*, 2007) have previously estimated the number to be as high as 1.7 million infections that accounted for nearly 100 000 deaths based on data available from 2002. A large percentage of these infections are caused by bacteria that have acquired multidrug resistance (MDR), such as the Gram-positive bacteria *Clostridium difficile* and *Staphylococcus aureus* and the Gram-negative bacteria *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and are associated with severe health outcomes. For example, hospital patients infected with MDR *Acinetobacter* are more than two times as likely to have prolonged stays in both intensive care units (13.3 d vs. 6.7 d) and general care wards (27.5 d vs. 19.8 d); furthermore, mortality rates were 26% for MDR strains versus 17.6% for susceptible strains (Sunenshine *et al.*, 2007).

There are multiple methods by which bacteria become MDR but horizontal gene transfer (HGT) is thought to be one of the more common and problematic mechanisms. Numerous studies have indicated that HGT leads to rapid adaptation and evolution of bacteria (Gogarten and Townsend, 2005; Jain *et al.*, 2003; Ochman *et al.*, 2000). The rapid evolution is evidenced by the divergence of genome sequences among bacteria that are supposedly of the same species, with some reports of conspecifics sharing less than 50% of their genome (Abby and Daubin, 2007; Welch *et al.*, 2002). Furthermore, HGT between distantly related prokaryotic organisms is not a rare event (Bellanger *et al.*, 2014; Polz *et al.*, 2013; Frigaard *et al.*, 2006) and may even commonly occur between prokaryotes and eukaryotes (Bruto *et al.*, 2014). HGT therefore provides a method by which genes favored by natural selection – such as drug resistance – can rapidly be acquired from other members of the bacterial community. This has led to the terms 'horizontal gene pool' (Zechner and Bailey, 2004) and 'mobilome' (Frost *et al.*, 2005) which refer to the reservoir of mobile genetic elements (e.g., plasmids, insertion sequences,

transposons) available to bacteria for the production of a wide array phenotypes.

Plasmids and Horizontal Gene Transfer

One of the primary mechanisms for HGT is gene acquisition via the transfer of plasmids during bacterial conjugation. The term plasmid was first introduced by Lederberg (1952) and defined "as a generic term for any extrachromosomal hereditary determinant." However, more modern definitions of plasmids include the caveat that these extrachromosomal DNA determinants are self-replicating genetic elements. Several plasmids are self-transmissible in that they encode the necessary machinery for transferring a copy of their DNA to adjacent cells by means of conjugation. The genes carried by plasmids are typically not essential for normal cellular function but, as in the case of antibiotic resistance, could be vital to survival in certain environments. Most plasmids are circular dsDNA but linear plasmids also exist (Stewart *et al.*, 2005; Hinnebusch and Tilly, 1993); they typically consist of (1) a 'backbone,' which contains the genes necessary for self-replication, maintenance, control, and conjugative transfer and (2) various 'accessory' genes that provide other functions to the host bacterium. It is these plasmid-borne accessory genes that contribute, at least in part, to the rapid spread and emergence of traits across Archaea and Bacteria.

Numerous types/classes of traits are encoded for by the accessory genes of plasmids. As previously mentioned, antibiotic resistance is one of the most concerning of the traits. The transfer of antibiotic resistance by means of plasmids was first documented between members of the Enterobacteriaceae in the late 1950s and early 1960s (Leclercq, 2002; Watanabe and Fukasawa, 1960). However, as shown in **Figure 1**, increased resistance to many drugs, and in particular 'last line' drugs like vancomycin (Chang *et al.*, 2003; Weigel *et al.*, 2003), has been spreading rapidly in the last several decades and is also tied to plasmid encoded genes (Friães *et al.*, 2014). Resistance to toxic heavy metals (such as cadmium, cobalt, silver, lead, and mercury) is also often encoded by plasmids. For example, the plasmid pWR501 has been demonstrated to transfer genes for resistance to mercury in *Shigella* (Venkatesan *et al.*, 2001). Heavy metal resistance genes allow bacteria to exist and thrive in harsh, contaminated environments. Moreover, due to co-localization of metal and antibiotic resistance genes on the same plasmids, the presence of heavy metals may co-select antibiotic resistance, as suggested by recent alarming examples such as in the MRSA strain ST398 (Gómez-Sanz *et al.*, 2013). Plasmids also often transfer virulence factors between bacteria. A classic example of virulence encoded by plasmids occurs in

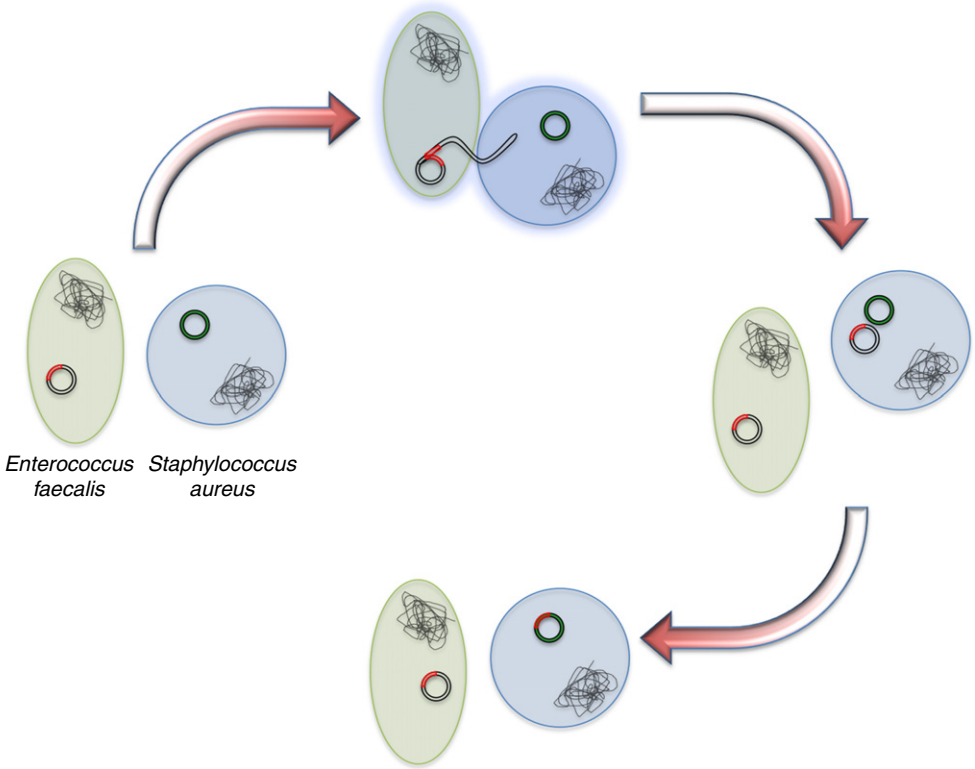

Figure 1 Hypothesized transfer of vancomycin resistance from *Enterococcus faecalis* (green) to a methicillin resistant *Staphylococcus aureus* (MRSA) (blue), resulting in a vancomycin resistant MRSA strain (VRSA), based on the findings of Weigel *et al.* (2003). Each bacterium carries its chromosomal DNA (black lines) and a plasmid (circles). The vancomycin resistance gene is carried on a transposon (red segment of *E. faecalis* plasmid; left). Although rare, conjugation can occur between *E. faecalis* and *S. aureus* when they come in contact. The *E. faecalis* plasmid is then transferred to *S. aureus*, resulting in *S. aureus* carrying both its normal plasmid and the plasmid carrying vancomycin resistance. Finally, the transposon encoding vancomycin resistance moves to the *S. aureus* MRSA plasmid via transposition. The *E. faecalis* plasmid is eventually lost from *S. aureus*, but now the *S. aureus* lineage carries the vancomycin resistance on its own plasmid, and is thus a VRSA (bottom).

the *Bacillus cereus* group of which the species *B. cereus*, *Bacillus thuringiensis*, and *Bacillus anthracis* are all highly genetically related members. However, *B. anthracis* is the etiological agent of the dreaded anthrax disease. The plasmids pXO1 and pXO2 carry the genes that encode for the exotoxin and acid capsule (both virulence factors) of *B. anthracis*, respectively (Kolstø *et al.*, 2009). Degradation of organic compounds is another function plasmids may provide bacteria. These catabolic plasmids are usually large (>50 kb) because degradation of organic compounds often requires numerous genes that are part of catabolic cascading pathways. Classical examples of this are naphthalene and toluene/xylene degradation by the plasmids NAH7 and pWWO in *Pseudomonas putida*, and the many catabolic plasmids of the broad-host-range plasmid group IncP-1 (Fernández *et al.*, 2012; Top and Springael, 2003). It should be noted that this is not an exhaustive list of the many phenotypes plasmids can encode in bacteria (Top *et al.*, 2000), just those that are often considered highly important.

Plasmids are typically classified in incompatibility groups. Incompatibility is defined as the inability of two plasmids to be vertically co-transmitted within a cell lineage for multiple generations (Couturier *et al.*, 1988). Traditionally, this was determined using selection for plasmids and then releasing the selection pressure and observing which plasmids remained in a lineage. More recently incompatibility types are being determined by DNA sequence information on the replication region of the plasmid (Sota and Top, 2008; Carattoli *et al.*, 2005). Plasmids of Gram-negative bacteria are typically classified into an alphabetical grouping (i.e., IncA through IncZ). Classification of plasmids from Gram-positive bacteria follows various methods, for example, the plasmids of *S. aureus* are broken into 15 families (Inc1 through Inc15). Needless to say, the classification of plasmids is confusing and overlap between classification systems exists (Couturier *et al.*, 1988), but the concept of plasmid coexistence within a bacterium is important to how groups of plasmids can shape evolution in bacteria.

In broader biological terms, the relationship between plasmids and bacteria can be thought of as an interaction between species where bacteria fulfill the role of the host. Thus a great deal of research has focused on the host range of plasmids, with the delineation of having either broad or narrow host range (BHR vs NHR). The host range of a plasmid is normally defined by the group of hosts in which a plasmid can successfully replicate. This does not necessarily indicate which bacteria a plasmid may be found in. Without selective pressure, replicating plasmids may be rapidly lost from some lineages but can also be efficiently retained in other strains, even within the same species (De Gelder *et al.*, 2007). Conjugative transfer of some plasmids can occur to bacteria or even eukaryotes in which the plasmid cannot replicate, resulting in a transfer range that is much wider than the replication range (Thomas and Smith, 1987). In terms of the evolution of unwanted phenotypes, like antibiotic resistance, BHR plasmids are the most problematic because of their ability to pass genes for such traits to numerous species of bacteria and may be the most important means of HGT between distantly related bacterial hosts (Mazodier and Davies, 1991). Thus, one of the central questions related to plasmid-mediated bacterial evolution is how host ranges and the stability of a plasmid within a host evolve.

Coevolution of Plasmids and Bacteria

The evolution of plasmid persistence and host range is dependent on the coevolution of plasmids and their bacterial hosts. Coevolution, using the broadly accepted definition of Janzen (1980), occurs when reciprocal evolutionary change occurs between interacting groups (typically species). Interactions between species can broadly be classified in several categories: competitions, mutualisms, antagonisms, commensalisms, and amensalisms. These classifications come from the fitness consequences of two species interacting together. If we let (+) indicate a fitness benefit, (−) indicate a fitness loss, and (0) indicate neutral fitness consequences, then a (−,−) species pair is a competition, a (+,+) species pair is a mutualism, a (+,−) species pair is an antagonism, a (+,0) species pair a commensalism, and a (0,−) species pair is amensalism ((0,0) is sometimes referred to as a neutralism, for completeness, but is typically considered a lack of interaction). Understanding these types of interactions and their consequences is central to understanding coevolutionary outcomes (Thompson, 1982). Thus, studying the fitness costs and benefits of plasmids interacting with their host is an important topic; Harrison and Brockhurst (2012) have gone so far as to claim that, "HGT via conjugation can only be fully understood in a coevolutionary framework."

On the surface, it often appears that the gain of a plasmid by a bacterium would be beneficial to its host. The host is often gaining a phenotype needed for its survival, such as antibiotic resistance. For the plasmid, the benefit would be the access to the hosts replication machinery which allows the plasmid to propagate. Therefore, at first pass, it might seem that plasmid–bacterium interactions fall into the category of mutualisms. A classic example of a mutualistic interaction between plasmids and bacteria is that of the pSym plasmids carried by *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*. All three genera are Gram-negative soil bacteria. Rhizobia form root nodules where they provide nitrogen fixation services to their host plant in exchange for photosynthates. However, this plant–bacteria symbiosis requires the presence of pSym plasmids which encode genes necessary for rhizobia to actually perform nitrogen fixation (Mercado-Blanco, 1996). The pSym plasmids are nearly always present in rhizobia. Interestingly, non-symbiotic plasmids of rhizobia (non-pSym) also exists and are not required to form the symbiosis host plant. These non-pSym plasmids seem to be acquired opportunistically by rhizobia, particularly during periods of root colonization in which traits encoded on these plasmids may confer competitive advantages such as increased nodulation in beans (Martinez-Romero and Rosenblueth, 1990).

The mutualistic relationship between plasmids and their hosts can easily change. For example, once selective pressure for a trait encoded by a plasmid is removed, a bacterium is no longer benefiting from the plasmid. Worse yet, the plasmid is now acting as a parasite because it is utilizing the replication machinery and energy stores of its host in order to propagate itself. The fitness consequences of interacting with a bacterium can also change for a plasmid. For example, if the host is in an environment where conjugation often results in transfer to bacteria outside the plasmid's host range, or if transfer results in direct competition between the plasmid and other members

of its incompatibility group, then persisting within that particular host could have negative fitness consequences for the plasmid. Or worse, if that host's population size becomes severely reduced due to competition for resources or antagonistic interactions with other members of the microbial community, that host is no longer favorable and the plasmid is doomed if it cannot escape by horizontal transfer to fitter neighbors in time (Bergstrom *et al.*, 2000). Therefore, depending on context, the relationship between a plasmid and its host could shift to other forms of interactions.

One particularly fascinating question is why plasmids exist (Fox *et al.*, 2008; Lundquist and Levin, 1986; Levin and Stewart, 1980; Stewart and Levin, 1977; Bergstrom *et al.*, 2000). It has been demonstrated numerous times that, in the absence of selection for some accessory gene, plasmids impose a fitness cost on their host, due to use of DNA replication and maintenance machinery, the energetic cost of protein production, possible negative interference of expressed functions with host processes, etc. (Corchero and Villaverde, 1998). Evidence from *Escherichia coli* suggests that plasmids that code for either numerically more or more complex gene products impose the highest fitness costs on their hosts (note that this not necessarily the same as the base pair length of the plasmid). Given the fitness cost of maintaining a plasmid, it would be more sensible for bacteria to integrate advantageous genes from a plasmid and then discard the plasmid. Thus a conflict exists regarding what is best for the plasmid (to replicate within or across bacterial lineages) and what is best for its host (chromosomal integration of selectively advantageous genes). Coevolution has served as primary hypothesis behind why plasmids still exist (Harrison and Brockhurst, 2012). Several studies have investigated this hypothesis. For example, when comparing models of two potential bacterial mitigation strategies (either suppression of plasmid gene expression or simply resisting invasion by a plasmid), it was determined that host regulation of gene expression should be the favored strategy (Mc Ginty and Rankin, 2012). However, other models have suggested that simply modeling such strategies without including competition among plasmids can lead to incorrect conclusions, and that rescue by nonresident/immigrant plasmids could disrupt the coevolutionary process (Tazzyman and Bonhoeffer, 2014).

In some ways, it seems obvious that the relationship between plasmids and bacteria must be coevolutionary. Clearly, uptake of genes via HGT leads to the evolution of bacteria, but this is just one side of a 'reciprocal' coevolutionary relationship. The issue of plasmid persistence being increased due to the amelioration of costs via coevolution has long been considered a possibility (Duncan *et al.*, 1995), and several recent studies have provided evidence for this (De Gelder *et al.*, 2008; San Millan *et al.*, 2014; Sota *et al.*, 2010).

More concrete evidence has been accumulating however that bacteria also affect plasmid evolution. Recently, it has become evident that bacteria may use clustered, regularly interspaced short palindromic repeats (CRISPRs) as a form of 'adaptive immunity' to the invading genetic elements of viruses and plasmids (Marraffini and Sontheimer, 2010; Bhaya *et al.*, 2011). Along with CRISPRs come CRISPR-associated proteins (Cas) which together then constitute a CRISPR–Cas system. By incorporating DNA from plasmids into a CRISPR array, bacteria can recognize specific plasmid sequences as nonself. The Cas protein can then target these invading genetic elements, interfere with plasmid gene expression, and degrade foreign nucleic acids. Reciprocally, plasmids must evolve to elude the host's CRISPR recognition system in order to produce proteins necessary for their propagation. Models have shown that complex coevolutionary dynamics arise (which often lead to the proliferation of novel genotypes akin to the dynamics in many host–pathogen models) for plasmids and CRISPR–Cas systems (Berezovskaya *et al.*, 2014; Childs *et al.*, 2012; Levin, 2010). It has been suggested that the toxicity of anthrax is actually due to coevolutionary relationship where *B. anthracis* has lost its ability to regulate protein production in pXO1 and pXO2 due to a mutation in the P1cR regulatory gene (Kolstø *et al.*, 2009).

Another example of how incoming plasmid encoded genetic information can be affected by the host machinery is the transcriptional silencing of horizontally acquired plasmid genes by host H-NS, one of the nucleoid-associated proteins of bacteria (Dorman, 2014; Will *et al.*, 2004). A good example is the repression of conjugative transfer of the F plasmid by H-NS during entry into stationary growth phase in *E. coli* batch cultures (Will *et al.*, 2004). The finding that H-NS-like proteins are also encoded on some plasmids makes it even more intriguing (Suzuki *et al.*, 2014), and suggests that plasmids may be coevolving with each other as well.

Further evidence of the coevolution of plasmids and bacteria has been discovered by looking for shared genomic signatures (e.g., looking at frequency of particular dinucleotide repeats with the genome and comparing those between plasmids and hosts). Notably, it has been shown using genomic signatures that the backbone of the BHR plasmid IncP-1 adapts/evolves in accordance with the hosts to which it is exposed (Norberg *et al.*, 2011). Similarly, Suzuki *et al.* (2010) found that genomics signatures can actually be used to predict the host range of plasmid. For example, the signatures of IncP plasmids do indeed suggest they are BHR plasmids, while the IncF plasmid signatures are very similar to those of Enterobacteriaceae, their known hosts. Such evidence reinforces the idea that not only are plasmids shaping bacterial evolution, but that bacteria are reciprocally shaping plasmid genomes.

The support for ongoing coevolutionary interactions between plasmids and bacteria points to the need to study such interactions in the broader context of coevolution. The geographic mosaic theory of coevolution (GMTC) proposed by Thompson (1994, 2005) acts as a general framework for much of current coevolutionary thinking. This framework emphasizes sources of heterogeneity within the coevolutionary process. First, coevolutionary outcomes are expected to vary among populations due to differences in selection pressure and environments. This type of dynamic is exemplified by the differences observed in plasmid–bacteria interactions in spatially structured biofilms versus liquid cultures (Madsen *et al.*, 2012). For example, two plasmids (pMCBF1 and pMCBF6) isolated from the same marine biofilm have identical backbones, but have evolved to carry to different versions of their accessory genes (namely mutations in their mercury resistance transposons) (Norberg *et al.*, 2014); this indicates that diverse evolutionary outcomes are possible within spatially structured biofilms. Second, looking at a population (or a

metapopulation) at one point in time is an incomplete representation of a coevolutionary system because these interactions often display complex (e.g., cyclic) dynamics that are highly variable through time. Thus to truly understand coevolution, one must look at both spatial and temporal variability. The previously mentioned CRISPR–Cas systems predicted such highly complex, cyclical (perhaps even chaotic), temporally heterogeneous outcomes (Childs *et al.*, 2012; Berezovskaya *et al.*, 2014), but this remains to be tested empirically. The ongoing and future integration of plasmid studies with coevolutionary theory will greatly enhance our understanding of plasmid-bacteria interactions.

Conclusions

The role of plasmids in bacterial evolution can be thought of in two different lights: that of short-term, rapid evolution via HGT between bacterial lineages and that of the long-term coevolutionary relationships that dictate the host range boundaries for HGT. Gogarten and Townsend (2005) wrote: "Throughout the decades, mutualism and reticulation have often been considered the most important processes in species evolution." In bacteria, the reticulate evolution via HGT by plasmids and coevolutionary mutualisms with plasmids definitely fulfill this paradigm. The majority of research to date has focused on HGT and its implications for bacterial evolution. This focus is largely due to the observed rapid spread of phenotypes that are dangerous to human health – such as antibiotic resistance and virulence – via HGT. Research in this area has led to shocking discoveries about the (high) degree to which distantly related bacteria share genetic information, and plasmids have been implicated as a primary mechanism by which such distant transfers occur. From this, it is clear that plasmids are a driving factor in shaping the genomes of bacteria. This remains an area of intense research and new methods of analysis (such as network analysis) are being brought to bear on reticulate evolution within microbes (Dagan *et al.*, 2014; Conlan *et al.*, 2014).

Newer research is focusing on the ecological and coevolutionary relationships between plasmids and bacteria that dictate who-transmits-what-to-whom. Mounting evidence suggests that these relationships are indeed reciprocal and not simply plasmids driving the evolution of bacteria (as one might assume with HGT). The fitness benefits and consequences of conjugative transfer of plasmids, to both the plasmid and the host bacterium, must be studied in order to understand what makes one plasmid more stable within a lineage than another and why some plasmids have NHRs while others have BHRs. When considering the fitness outcomes of an interaction between a plasmid and its host, it becomes apparent that there is a large role for context dependence, as promoted by the GMTC. In particular, spatial and temporal heterogeneity in both the biotic and abiotic environment will lead to different coevolutionary outcomes. For example, such heterogeneity has been demonstrated to have an effect when plasmids and bacteria interact in spatially structured biofilms rather than homogeneous liquid cultures (Król *et al.*, 2011; Madsen *et al.*, 2012). However, there is currently a lack of studies that have focused on issues outside of HGT by plasmids, and thus the coevolution of bacteria and plasmids is far from well understood. For a truly synthetic understanding of the role of plasmids in bacterial evolution, much more research on the ecological and (co)evolutionary underpinnings is still needed. Such research will enable predicting and controlling the emergence and spread of unwanted phenotypes such as antimicrobial resistance.

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See also: Genome Plasticity, Bacterial. Recombination in Bacterial Populations

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Polyandry and Female Postcopulatory Choice

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Glossary

Adaptation(s) A feature/trait that has become prevalent in a population because of the selective advantage of that feature in the improvement in function typically increasing the probability of offspring survival and viability.

Aedeagus The male copulatory, intromittent, or sperm-depositing organ of insects.

Anisogamy Sexual reproduction involving the fusion of gametes (haploid sex cells) that differ in size with males being defined as the sex with the smaller gametes and females with larger gametes. Contrast with isogamy, in which the gametes are the same size.

Antiaphrodisiacs A substance that reduces sexual receptivity or attractivity, often delivered by a male to female during mating to delay or reduce the likelihood that she will remate.

Artificially inseminate (AI) The deliberate introduction of sperm, by an investigator or doctor, into a female's reproductive tract for the purpose of fertilizing eggs by means other than sexual intercourse.

CM-PCR (competitive polymerase chain reactions) A method that amplifies small repeated segments of DNA (i.e., microsatellites) in a pool of cell samples from different individuals to assign a relative contribution of cells to the pool. This method was developed to assess the relative number of mixed sperm cells from different males within a female's reproductive tract.

Comparative method A procedure for inferring the adaptive function of a character by correlating its states in various taxa with one or more variables, such as ecological factors hypothesized to affect its evolution, while controlling for phylogeny (i.e., shared evolutionary history).

Conspecific Members of the same species. Contrast with heterospecific which means members of a different species.

Copulatory plugs (also known as mating plug, sperm plug, vaginal plug) This is gelatinous secretion or sometimes a piece of the male's intromittent organ that is deposited by a male into the female genital tract, such as the vagina, and that often later hardens into a plug or glues the tract closed. Commonly proposed functions of copulatory plugs are to reduce female remating rates by preventing further copulations, prevent sperm leakage or ejection, enhance sperm performance.

Counter-adaptation A coevolutionary response that generates an adaptive response to another adaptation. For example, in bedbugs the male's intromittent organ pierces the female's abdomen. This is an adaptation that increases the male's chances at reproductive success but it inflicts harm on the female. Females of this species have evolved a counter-adaptation to reduce the harm by thickening the cuticle in the area where males most commonly pierce them.

Cryptic female choice (CFC) A form of intersexual sexual selection whereby 'nonrandom paternity biases resulting from female morphology, physiology, or behavior that occur after coupling.'

Ejaculate (noun) Is a fluid issued by males that is composed of seminal fluid and sperm. The seminal fluid is a complex matrix containing many different molecules and compounds that are largely produced by sex accessory glands that nourishes sperm, can affect female physiology and form copulatory plugs.

Fisherian process (or runaway sexual selection) A model of sexual selection in which a male displays character and female preference for the character become correlated and reinforce one another so that both evolve to be more extreme.

Genetic incompatibility Reduction in the fitness of an offspring because of interaction between certain alleles in one parent with specific alleles at other loci in the other parent. This is most commonly seen in hybrid offspring produced in between population, subspecies or species crosses.

Genotype The set of genes possessed by an individual organism; often, its genetic composition at a specific locus or set of loci singled out for discussion.

Heritable variation The proportion of the variance in a trait among individuals that is attributable to differences in genotype and therefore can be passed on to a parent's offspring. In quantitative genetics it is expressed as the ratio of additive genetic variance to phenotypic variance.

Heterospecific A different species. Contrast with conspecific which means the same species.

Honest signal A signaling trait that accurately conveys the 'quality' of the sender to the receiver.

Immune response The process by which an organism recognizes and defends itself against substances that appear foreign and harmful, for example, bacteria and viruses.

In vitro A process performed or taking place in a test tube, culture dish, or elsewhere outside a living organism: contrast with *in vivo*, which are processes performed or taking place in a living organism.

Intersexual selection Selection that occurs due to interactions between the sexes, such as mate choice.

Intrasexual competition Selection that occurs due to the interactions between members of the same sex, usually in direct competition for mates or fertilization success.

Intromittent organ A general term for an external organ of a male that is specialized to deliver sperm during copulation such as the mammalian penis. But any organ that functions to delivery sperm can be an intromittent organ. For example, the tail of the tailed frog (*Ascaphus truei*) functions to deliver sperm into the female reproductive tract.

Knockout lines or RNA interference experiments to knock down gene expression These molecular methods allow researchers to experimentally manipulate trait expression to test the function of the target trait. Knockout lines are lineages of laboratory animals (typically mice or fruit flies) that have been bred to lack certain genes that determine the expression of the trait of interest. RNA interference introduces RNA molecules to inhibit gene expression (e.g., by damage of specific mRNA molecules), and thereby affecting the trait of interest.
Lag load dynamic The idea that if the selective environment changes such that the optimum genotype is always shifting, then there will be a 'lag' between average trait value in a population and the optimum trait value. This can lead to rapid evolution as there is always selection to move the trait value to its optimum.
Major histocompatibility complex (MHC) A set of cell surface molecules encoded by a large, highly diverse gene family which aids in the recognition pathogens by the immune system in all vertebrates.
Mate order effects The effect that the order of mating males (e.g., first male to mate with a female vs. second male to mate with the same female) has on the probability of fathering the offspring of female mates with more than one male.
Mate-guarding When a male remains close by a female, or prolongs copulation to prevent or reduce the likelihood that the female will mate again with a different male.
Non-transitive In terms of a competitive mating experiment (a given set of males are competing against each other to fertilize the eggs of a set of separate females), it means that the probability of a particular male being more or less successful at gaining fertilizations depends on the identity of the female and less on his competitors.
Ovarian fluid Fluid released from an ovarian follicle at ovulation. In externally fertilizing fish, females usually release large volumes of ovarian fluid along with their eggs. In some species, sperm of particular males exhibit greater motility and swimming speed in the ovarian fluid of particular females but less so in the ovarian fluid of other females.
Paternity Being the father of offspring (to father or sire offspring).
Paternity patterns The proportion of paternity that can regularly be ascribed to males under defined conditions. For example, in some species the last male to mate with a female usually fathers most of the offspring regardless of the identity of the female or competitors.
Phagocytosis The ingestion of a smaller cell, cell fragment, microorganism, or foreign particles by engulfing the object by the cell's membrane.
Phenotype The characteristics of an individual resulting from the interaction of its genotype with the environment.
Polyandry When females mate with multiple males.
Postcopulatory sexual selection When the processes of intrasexual competition and intersexual selection continues during and after copulation in the form of sperm competition and cryptic female choice.
Precopulatory sexual selection When the processes of intrasexual competition and intersexual selection occur in securing mates prior to copulation.
Promiscuous Demonstrating or implying an unselective approach to mate choice typically resulting in multiple matings.
Reproduction (sexual) Production of offspring whose genetic constitution is a mixture of those of two potentially genetically different gametes that unite at fertilization.
Sexual cascade The idea put forward by Geoff Parker that with the evolution of syngamy (sexual reproduction by the union of gametes) there is an inevitable evolution of copulation (internal fertilization) and sex roles (leading from anisogamy). Sexual conflict as an expected consequence of anisogamy and precopulatory sexual selection has a relatively late appearance in the evolution of the sexual cascade.
Sexual conflict When the evolutionary interests of males and females do not coincide.
Sexual selection Selection resulting from differential success in mate acquisition and fertilization.
Sexually antagonistic coevolution A dynamic and iterative process in which selection favors traits in males and females that increase their fitness at the expense of the opposite sex.
Sperm competition When the sperm of more than one male compete for the fertilization of a set of ova.
Sperm competition defense Traits that aid a male in preventing sperm competition from occurring and thus preserving his share of paternity, for example, mate guarding, copulatory plugs, prolonged or frequent, repeated copulations. Typically the first male to mate is in a defensive role.
Sperm competition offense Traits that aid a male in gaining paternity when mating with a previously mated female, for example, removing or displacing previous male's ejaculate.
Sperm storage Extended maintenance of sperm. This can be within the male prior to mating, and/or can be within the female after mating.

Polyandry is common and widespread across animal and plant taxa (Jennions and Petrie, 2000; Pizzari and Wedell, 2013; Taylor *et al.*, 2014). Therefore, the battle for reproductive success does not always end after intrasexual competition for mates (usually males) or intersexual selection of mates (usually by females) (Darwin, 1871; Andersson, 1994). Instead, sexual selection continues within the ovarian fluid plumes of external fertilizers and, in internal fertilizers, inside the reproductive tracts of promiscuous females, where the ejaculates of competing males vie for fertilization (Parker, 1970; Parker and Pizzari, 2010). There the ejaculates compete and female traits bias the contest for fertilization success (Thornhill, 1983;

Eberhard, 1996; Arnqvist, 2014). Many studies confirm the ubiquity and strength of both these forms of postcopulatory sexual selection (Smith, 1984; Birkhead and Møller, 1998; Birkhead and Pizzari, 2002; Andersson and Simmons, 2006; Pitnick and Hosken, 2009; Kvarnemo and Simmons, 2013; Arnqvist, 2014).

Although there are many ways that females benefit from polyandry (Arnqvist and Nilsson, 2000; Arnqvist and Kirkpatrick, 2005; Simmons, 2005; Snook, 2014), here we will focus on the fact that polyandry provides females with the opportunity to bias paternity toward male phenotypes that are superior and/or genetically compatible and away from incompatible or inferior male phenotypes and thus increase the fitness of her offspring (e.g., Tregenza and Wedell, 2000; Tregenza and Wedell, 2002; Pilastro *et al.*, 2004; Bretman *et al.*, 2009; reviewed in: Slatyer *et al*,. 2012). Given the limited space, our review of CFC cannot be exhaustive but instead we present a historical overview of research in this field, which we hope has heuristic value and then suggest where we think the field is headed.

Sexual selection can be conveniently divided into selection generated through intrasexual competition and intersexual mate choice (Andersson, 1994). Intrasexual competition occurs when males must compete directly with one another for access to females or control of resources females require (Emlen and Oring, 1977; Shuster and Wade, 2003). Traits that aid males in directly monopolizing these resources or the females will be under selection. Examples of these traits are sexual dimorphism in body size and defensive ornaments (e.g., antlers) (Andersson, 1994; Emlen, 2008). Intersexual mate choice occurs when a female exhibits a preference for a male with particular phenotypes. Classic examples of these traits are colorful plumage, displays, and elaborate tail feathers of male birds. Those traits that females find more attractive will be under selection and become exaggerated through evolutionary time through a Fisherian process or because these traits honestly signal male quality and are augmented by selection (Lande, 1981; Arnold and Wade, 1984; Andersson, 1994; Jones and Ratterman, 2009; Prum, 2010). In each of the examples listed above, selection occurs before copulation thus is termed precopulatory sexual selection. However, sexual selection does not end at copulation.

When females are promiscuous, that is, mating with multiple males during a single breeding season, the sperm from two or more males may overlap within the female's reproductive tract (or plume of ovarian fluid). The sperm from multiple males may then compete to reach ova and/or preferred fertilization sites closest to the ova. Parker (1970) coined the term 'sperm competition' to describe this situation. To the extent that females are promiscuous, sperm competition is predicted to generate selection for more, faster and/or longer-lived sperm (Parker and Pizzari, 2010). By analogy it follows that, if sperm competition is the postcopulatory equivalent to precopulatory male–male competition, then there is likely a postcopulatory equivalent to female mate choice. In 1983, Thornhill suggested that "in principle, female animals may choose males before, *during or after mating*." Although others had hinted at this idea (e.g., Lloyd, 1979; Halliday, 1983), Thornhill was the first to make an explicit claim that female choice could occur during or even after copulation (Eberhard, 1996); he called it cryptic female choice (CFC). Because of the inherent difficulty of segregating male and female effects within the female's reproductive tract, much has been written on methods and experimental designs that could definitively distinguish between sperm competition and CFC.

In 2000, four papers were published concurrently in *Evolution*, each of which aimed to more clearly define CFC, establish criteria and develop experimental methods for demonstrating CFC to the exclusion of sperm competition (Birkhead, 2000; Eberhard, 2000; Kempenaers *et al.*, 2000; Pitnick and Brown, 2000). As Eberhard (1996) suggested, and others have acknowledged since, to separately focus on male traits for sperm competition that have evolved to excel within the female reproductive tract from those very female traits that evolved to bias paternity toward 'superior' sperm competitors in some way misses the point of CFC (Eberhard, 1998; Pitnick and Hosken, 2009; Pitnick et al., 2009; Arnqvist, 2014). No doubt, the focus on delineating male and female processes was fueled by disbelief of the existence of cryptic female sperm choice and was analogous to the skepticism about female precopulatory mate choice in previous decades (Milam, 2010). However, we now recognize the inescapable fact that female traits influence male fertilization perhaps especially in internally fertilizing species (Olsson *et al.*, 1996; Birkhead *et al.*, 2009; Pitnick *et al.*, 2009; Arnqvist, 2014).

Defining Cryptic Female Choice

In an encyclopedic book treatment, Eberhard (1996) described 20 potential mechanisms of CFC (**Table 1**) and defined CFC as "a female-controlled process or structure that selectively favors paternity by conspecific males with a particular trait when the female has copulated with both types." Eberhard's book has been of great utility to evolutionary biologists for its numerous examples of potential cases of CFC and because it brought much needed attention to postcopulatory female processes (Holland and Rice, 1997). However, several authors criticized Eberhard's emphasis on supposed "female control" (e.g., Holland and Rice, 1997). Female control implies that females have direct and purposeful influence over sperm use (Birkhead, 2000; Pitnick and Brown, 2000). To illustrate the potential confusion introduced by Eberhard's use of the term "female control," consider *Calopteryx* damselflies (Pitnick and Brown, 2000). A male *Calopteryx* damselfly stimulates the female with horns at the tip of his aedeagus (intromittent organ) during copulation and the female responds by dumping sperm from her previous sexual partner. The amount of sperm she dumps from her previous partner is significantly correlated with the size of the male's aedeagus (Córdoba-Aguilar, 1999). Male copulatory stimulation can be interpreted as an example of CFC or as male manipulation of the female (i.e., sexual conflict, Arnqvist and Rowe, 2005; Córdoba-Aguilar, 2006; see sexual conflict below). Under Eberhard's definition, the evolutionary history of the trait must be known in order to answer this question (i.e., why the trait evolved) before we can claim it is CFC (Pitnick and Brown, 2000). It is possible to infer a trait's evolutionary history using modern comparative methods, although reconstructing the evolutionary history of a trait before assigning its function is a nearly

Table 1 List of the potential mechanisms by which a female may bias the relative fitness of her mates

Transport sperm of different males to different storage sites and then preferentially use sperm from particular storage sites based on male phenotype
Preferential ejection of sperm for one mate or another during or after copulation
Control of intromission, male genitalia, or copulation duration to increase or decrease ejaculate transfer
Affect deposition of copulatory plugs or allow (or resist) mate guarding
Produce or contribute to copulatory plug material or affect quick dissolution of the copulatory plug to allow subsequent matings
Behavioral, physiological, or morphological modifications that prevent or increase the likelihood of subsequent matings or ejaculate transfer of subsequent matings
Recruit more primary oocytes and/or ovulate more ova
Differential provisioning of eggs fertilized by particular males
Increase or decrease the rate of oviposition relative to male phenotype
Control transport of sperm such that they are closer or farther from prime fertilization sites relative to male phenotype
Transport sperm of different males to different storage sites and then preferentially use sperm from particular storage sites (i.e., from particular males)
Upregulate immune responses that may damage or remove sperm from the fertilization set (e.g., phagocytosis, absorption into epithelia cells, etc.) Gold medal research question: how are these sperm identified?
Differential nourishment of sperm in transport or of those in storage
Differential abortion of zygotes or embryos
Prepare uterus(i) for implantation or not
Differentially invest in offspring sired by preferred males or the converse (at any point in their ontogeny)

impossible and unnecessary burden (Arnqvist, 2014). We should only need to demonstrate that a female trait is maintained by CFC (Arnqvist, 2014). For example, researchers are not required to answer the analogous question regarding the origin of female precopulatory choice, only whether variation in male mating success is associated with the male trait (Andersson, 1994). Eberhard's definition also misplaced the focus solely on the female's active response to different males, while it neglected potential passive mechanisms that may bias paternity (Birkhead, 2000; Pitnick and Brown, 2000). In response to Eberhard's (1996) problematic definition, Pitnick and Brown (2000) proposed a more general definition: "nonrandom paternity biases resulting from female morphology, physiology, or behavior that occur after coupling." This definition includes both active and passive proximate mechanisms, and avoids confusion over the need to establish female intent, which would be nearly impossible in any experimental setting (Birkhead, 2000). Furthermore, this definition places the emphasis on traits which are much more tangible than patterns or processes of paternity (Arnqvist, 2014).

A female may actively respond differently to one male versus another male, either behaviorally or physiologically. After copulating with a subdominant male, a female jungle fowl is likely to eject his sperm (Pizzari and Birkhead, 2000) and female sperm dumping has been found in several other taxa as well (e.g., Dunnocks (*Prunella modularis*), Davies, 1983; Kittiwakes (*Rissa tridactyla*) to avoid old sperm, Wagner *et al.*, 2004; fruit flies (*Drosophila melanogaster*), Snook and Hosken, 2004; Spiders (*Silhouettella loricatula*), Burger, 2007; *Physocyclus globosus*, Peretti and Eberhard, 2010); female scorpionflies (*Harpobittacus nigriceps*) and field crickets (*Gryllus bimaculatus*) may remate with a preferred male using mate order effects (i.e., systematic biases in paternity due to the order in which a pair of males mate) to control paternity (e.g., *in sensu* Thornhill, 1983; Simmons, 1987); and female water frogs (*Rana lessonae–R. esculenta* complex) reduce clutch size when they are amplexed by undesired hybrid males (Reyer *et al.*, 1999). Importantly the timing of sperm ejection is heritable, and thus can evolve, in female fruit flies (*Drosophila melanogaster*) and this timing affects sperm usage of first versus second males (Lüpold *et al.*, 2013). These are active behavioral responses that conform to Eberhard's definition of CFC. It is less clear whether a female's physiological responses to different males should be considered 'active.' For example, a differential increase in immune response to different male's sperm may be considered either an active or passive action (Ziegler *et al.*, 2005; Poiani, 2006; Birkhead and Brillard, 2007; Pitnick *et al.*, 2009), as could differential nourishment, transport, and storage or displacement of sperm (e.g., Villavaso, 1975). The female immune response is activated by insemination in a diverse range of taxa from humans (Robertson, 2007) to fruit flies (Kapelnikov *et al.*, 2008; Pitnick *et al.*, 2009). Some predicted that females may exhibit different immunological responses to different males' sperm (Olsson et al, 1997), and although it still remains controversial, mechanisms for major histocompatibility complex (MHC) expression on sperm and female recognition of MHC within the reproductive tract have been proposed and supported (Olsson *et al.*, 1996, 1997; Ziegler *et al.*, 2002; Ziegler *et al.*, 2005; Alcaide *et al.*, 2012; Løvlie *et al.*, 2013).

In addition to active mechanisms, a female may passively affect sperm within her reproductive tract via differences in the size and shape of her sperm storage organs or their lumen pH (Pitnick and Brown, 2000; Poiani, 2006, respectively). In *Drosophila*, male fertilization success is determined by a correlation between the length of his sperm with the length of the individual female's sperm storage organ (Miller and Pitnick, 2002). Both active and passive selective mechanisms within the female's reproductive tract are important drivers of male fertilization success, and the mechanisms need not invoke a female's purposeful intent as Eberhard's definition does.

The Problem of Paternity Patterns in Investigating CFC

One of the central problems in demonstrating CFC that was comprehensively discussed in *Evolution* (2000) is that paternity patterns do not always identify and differentiate among

competing mechanisms (e.g., a male that has mated without siring offspring may have been both unsuccessful in sperm competition and/or been selected against by the female using CFC mechanisms; Birkhead, 2000; Eberhard, 2000; Kempenaers *et al.*, 2000; Pitnick and Brown, 2000; Olsson and Uller, 2009). The problem with paternity patterns is what misled Ward (1993) to credit CFC for the patterns of precedence he observed (large-male precedence: Simmons *et al.*, 1996; Simmons *et al.*, 1999). Thus, the problem with pattern of paternity is not exclusive to CFC but also complicates the interpretation of results in studies of sperm competition (Simmons, 2001). In sperm competition studies, mate order patterns are often due to either displacement of, or a decline in the number of the first male's sperm, i.e., sperm competition is won by a fair raffle; the male with the most sperm inside the female wins. However, in some extreme cases mate order effects may not be due to sperm competition. For a classic example, a male damselfly (*Calopteryx maculate*) removes the sperm from a female's previous mate with hooks on the end of his aedeagus (Waage, 1979). The second male receives close to 100% of the paternity, not because he is superior in sperm competition, nor because of some peculiarity of the female's reproductive tract; the second male gains paternity by avoiding sperm competition altogether (Waage, 1979). This example illustrates the problem inherent in assigning mechanistic cause via patterns alone. Studies of CFC have a similar problem in that observed biases in paternity toward or away from certain males are difficult to attribute solely to the female (Birkhead, 2000; Pitnick and Brown, 2000). One way forward to resolve this is the statistical partitioning of variance in paternity patterns.

Variance in the proportion of offspring sired by the second male to mate – P_2 (Simmons, 2001), can be attributed to different components, including: mate order, interval between matings, copulation duration (sperm numbers or volume of important ejaculate components), and time to oviposition or ovulation (Smith, 1984; Birkhead and Møller, 1998; Simmons, 2001). After controlling for these factors, different males (i.e., genotypes) may have different levels of success in sperm competition (Lewis and Austad, 1990; Simmons and Parker, 1992; e.g., Clark *et al.*, 1995; Fiumera *et al.*, 2005). In addition, female genotypes vary in their effect on P_2 (Wilson *et al.*, 1997; e.g., Clark and Begun, 1998; Mack *et al.*, 2002; Fedina and Lewis, 2008; Lüpold *et al.*, 2013). Thus, variance in patterns of paternity can be attributed to male effects, female effects, and if those effects are not independent of one another, the male × female interactions (e.g., Wilson *et al*, 1997; Clark *et al.*, 1999; Mack *et al.*, 2002). For example, male A may outperform male B when mated with female C, but their relative performance may reverse if they are mated with female D (Clark *et al.*, 2000; Birkhead *et al.*, 2004; Bjork *et al.*, 2007). Because paternity is dependent on the complex interactions of the individuals involved, these are said to be non-transitive outcomes. In short, if sperm choice is occurring at all, we may not expect all females within a population to prefer the same male's sperm; although this may be the case in some populations or species (Arnqvist, 2014; Kempenaers *et al.*, 2000; Pitnick and Brown, 2000). In fact, variation in precedence within a male pair that has inseminated a series of females would be evidence that female mechanisms are affecting the outcome. This is because the male pairing should produce similar patterns of paternity across all females if those patterns are due to sperm competition alone (Birkhead, 2000; Pitnick and Brown, 2000). Pitnick and Brown (2000) suggested that an experiment could be designed such that male and female variance components could be isolated, and that a significant male × female interaction term would be indicative of CFC. However, this experiment would not reveal CFC if all females biased paternity toward the same males (Pitnick and Brown, 2000; Arnqvist, 2014).

Pitnick and Brown's (2000) experimental design was based on the work of Clark *et al.* (1999). In *Drosophila*, there is significant variation in male fertilization success among experimental, inbred lines (Clark *et al.*, 1995). When pairs of males from these lines were crossed with females from these same lines ($6 \times 6 = 36$ crosses), there was a significant male × female interaction in paternity outcomes (Clark *et al.*, 1999). This result cannot be explained by genetic incompatibility between the lines (e.g., Zeh and Zeh, 1997), because in a separate experiment they found no significant differences in egg viability among the 36 crosses (Clark *et al.*, 1999). Therefore, the male × female interaction is strong evidence for female-mediated effects in this case. However, a weakness with this design is that copulation occurred naturally and the male × female interaction could be due to interactions during copulation; sperm choice was not unequivocally demonstrated. Domestic fowl (*Gallus gallus domesticus*) have also been shown to exhibit non-transitivity of paternity. Birkhead *et al.* (2004) used sperm from nine pairs of males to artificially inseminate (AI) 14 hens per male pair up to seven times at monthly intervals. Thus they had seven replicates within each individual female and across 14 females per male pair. With this design they were able to partition the variance as Pitnick and Brown (2000) described, and demonstrated a significant male × female interaction. One strength of this study was that they used AI. Using AI they eliminated all male effects due to interactions during copulation as well as controlled for sperm numbers. However, we note that there are pitfalls to AI, because stimulation during copulation or during postcopulatory courtship may be an important cue for females using CFC (Eberhard, 2009, 2010, 2011), as Løvlie *et al.* (2013) subsequently found in red jungle fowl (*Gallus gallus*). Unlike Clark *et al.* (1999), Birkhead *et al.* (2004) could not fully dismiss the potential effect of differential viability (genetic incompatibility) because of the limits of their study organism. However, they were dubious that there were, in fact, viability differences given their knowledge of domestic fowl reproduction (Birkhead *et al.*, 2004). In well-studied model organisms, such as *Drosophila*, when strong male × female interactions are characterized with crosses of established genetic lines, the reproductive genes of males and females can be screened for associations within the interacting lines (e.g., Reinhart *et al.*, 2015). Male × female interactions may also be prevalent in external fertilizing species. Sperm velocity is a primary determinant of fertilization success in externally fertilizing salmonid fish (*Oncorhynchus tshawytscha*, Evans *et al.*, 2013; *Salmo salar*, Gage *et al.*, 2004). A female releases her eggs along with ovarian fluid as male(s) deposit sperm in the plume of ovarian fluid. Studies conducted using pooled ovarian fluids from several females have shown that ovarian fluid increases motility and longevity of salmonid

sperm (e.g., Lahnsteiner, 2002; Turner and Montgomerie, 2002; Rosengrave *et al.*, 2009; Butts *et al.*, 2012; Evans *et al.*, 2013). In competitive *in vitro* fertilization trials between trout and salmon sperm; ovarian fluid mediated fertilization success of conspecific sperm over heterospecific sperm (Yeates *et al.*, 2013). In conspecific trials there was a significant male × female interaction on sperm velocity of different males in the ovarian fluid from single females (Chinnok salmon (*Oncorhynchus tshawytscha*), Rosengrave *et al.*, 2008; Arctic char (*Salvelinus alpinus*), Urbach *et al.*, 2005). Similar results were found in guppies (*Poecilia reticulata*), with internal fertilization, probably explaining similar biases in paternity (Gasparini and Pilastro, 2011), which are likely to be influenced by components of ovarian fluid (Gasparini *et al.*, 2012).

These examples clearly demonstrate that females have an effect on the fertilization success of particular males. In the first two examples, only paternity patterns were established. Clark *et al.* (1999) were able to rule out genetic incompatibility as a cause for the male × female interaction, while Birkhead *et al.* (2004) controlled for all male effects except those interactions between a male's sperm and a female's reproductive tract and ova. Apart from the practical advantages of working with an external fertilizer for sperm competition/CFC experiments, a strength of the last example using fish is that it provides us with a probable mechanism, ovarian fluid, whereby females can enhance the velocity of particular male's sperm, a trait that has been shown to increase fertilization success. There is no doubt that some species have inherent limitations for the study of CFC, but in general, the strengths of each of these experiments should be emulated. A conclusive demonstration of CFC by sperm choice would need to account for genetic incompatibilities, control for all male effects except for the effects of sperm × female reproductive tract interactions, and establish the mechanism that correlates with patterns of paternity (Birkhead, 2000; Arnqvist, 2014).

Differential Sperm Storage as Mechanism

The most important mechanism for CFC by sperm choice is likely to be differential sperm storage (Birkhead, 2000). In species with sperm storage where females mate with several partners, females may influence the fate of individual spermatozoa. Nearly nothing is known, for any taxon, about the mechanisms by which a female might bias paternity after copulation, for example, sperm choice (Pizzari and Birkhead, 2000; Andersson and Simmons, 2006; Bussiére *et al.*, 2006).

However, several recent studies have shown that different male's sperm are stored in different amounts in the female reproductive tract. Paul Ward's lab group utilized CM-PCR (competitive polymerase chain reactions) to quantify the amount of sperm of different males in the female reproductive tract in yellow dung flies (*Scathophaga stercoraria*) and field crickets (*Teleogryllus commodus*) (Bussiére *et al.*, 2010; Hall *et al.*, 2010). Bussiére *et al.* (2010) confirmed previous work by Simmons *et al.* (1999), which suggested that sperm displacement explains second male precedence in yellow dung flies (Bussiére *et al.*, 2010). Bretman *et al.* (2009) showed that when female field crickets (*Gryllus bimaculatus*) mated with their brothers and an unrelated male, they stored fewer sperm from their brother, and that this difference was reflected in paternity. This result indicates that the costs associated with genetic incompatibility can be avoided by CFC mechanisms that result in differential storage of sperm from a preferred male. Populations of sand lizards (*Lacerta agilis*) with low genetic diversity may facilitate the evolution of detection mechanisms that identify the sperm for elimination (Olsson and Madsen, 2001), which is CFC by differential sperm storage or 'sperm choice' (Olsson *et al.*, 1996). As suggested above, differential sperm storage may result from differential immune responses and selective targeting of sperm from related or outbred individuals for phagocytosis, based on sperm surface proteins and/or components of the ejaculate (Pitnick *et al.*, 2009). This area needs much more attention, if we are to fully work out the mechanisms and thus the traits under selection by CFC.

Female morphology can also potentially exert a strong influence on male fertilization success. Male traits, such as sperm and genital morphology, covary with female sperm storage organs that drive their evolution (Miller and Pitnick, 2002; Simmons and Kotiaho, 2007; Simmons and Garcia-Gonzalez, 2011; e.g., Higginson *et al.*, 2012), this provides strong support for sexual selection of sperm traits by reproductive tract traits (i.e., male postcopulatory phenotypes selected by female postcopulatory phenotypes – or CFC). Expanding on the groundbreaking work of Clark *et al.* (1998, 1999, 2000), the perhaps most sophisticated study of female-mediated differential sperm storage are those that actually visualize different fluorescently labeled sperm within the female reproductive tract in real time to model the factors that determine paternity in a competitive context (Manier *et al.*, 2010; Lüpold *et al.*, 2013; Manier *et al.*, 2013; Marie-Orleach *et al.*, 2014). For example, the experiments from Scott Pitnick's lab using transgenic lines of *Drosophila* show that heritable variation in the timing of female sperm ejection influences the relative proportions of first and second males' sperm, which in turn influences which male sires a female's offspring (Lüpold *et al.*, 2013). The female tract morphology and anatomy controlling sperm ejection is a potential CFC trait, as is the size differences of the sperm and spermatheca (Miller and Pitnick, 2003). Male morphological traits involved in copulatory and postcopulatory courtship are known to influence female sperm usage and thus may have evolved via CFC (Eberhard, 1996, 2009, 2010b, 2011). Female genitalia can also function to influence paternity. Although couched in terms of sexual conflict – where the evolutionary interests of the sexes do not coincide (Parker, 1979; Arnqvist and Rowe, 2005) – the convoluted reproductive tract in some species of waterfowl (family Anatidae), with blind tubes into which the phallus, and therefore, the sperm of coercive males are directed. Thus the complex female anatomy is a morphological mechanism by which females can bias paternity (Brennan *et al.*, 2010). The female reproductive tract and phalluses also show signature of male–female coevolution indicative of both sexual conflict and sexual selection via CFC (Brennan *et al.*, 2007; Brennan and Prum, 2014). A more subtle example of potential CFC and sexual conflict occurs in garter snakes (*Thamnophis sirtalis*) where copulation duration affects paternity and the female's muscularized vaginal pouch and body-rolling behavior shortens the duration of copulation and reduces the size of the male's copulatory plug (Friesen *et al.*, 2014a,b,c). Female

resistance may be evidence of CFC in response to male harassment (Shine *et al.*, 2005; King *et al.*, 2009; Friesen *et al.*, 2014c). Indeed, distinguishing between the effect of postcopulatory sexual selection and sexual conflict on the evolution of reproductive traits has recently become a central focus of evolutionary and behavioral ecology (Arnqvist, 2014; Brennan and Prum, 2014; Simmons, 2014).

Sexual Conflict and CFC

The example of female–male genital interactions highlight the difficulty of delineating between postcopulatory processes and sexual conflict (reviewed in Arnqvist, 2014; Brennan and Prum, 2014; Simmons, 2014). Increasingly, researchers recognize that sexual conflict is a nearly inescapable consequence of anisogamy and the resulting 'sexual cascade' (Parker, 2014) culminating in the advent of sexual selection (see the following reviews for in-depth treatment of the relationship between sexual selection and sexual conflict: Parker, 1979; Arnqvist, 2014; Brennan and Prum, 2014; Kokko and Jennions, 2014; Perry and Rowe, 2014). Polyandry not only leads to the evolution of male adaptations that aid males in both sperm competition offense (e.g., better performing, more sperm and seminal fluids (Parker and Pizzari, 2010; Fitzpatrick and Lüpold, 2014)) it also leads to evolution of defense mechanisms (e.g., mate-guarding, antiaphrodisiacs, and copulatory plugs (Knowlton and Greenwell, 1984; Stockley, 1997; Parker and Pizzari, 2010)). These defense mechanisms can inflict direct harm on females, preventing them from mating at their optimal rate and/or preclude female choice (Knowlton and Greenwell, 1984; Arnqvist and Rowe, 2005). Likewise, although generally overlooked (Arnqvist, 2014), CFC traits such as sperm ejection, spermicides, and bet hedging via extreme promiscuity may reduce male fitness. Under these circumstances there is the opportunity for sexually antagonistic coevolution where each sex evolves adaptations and counter-adaptations to control the outcome of copulation in a lag load dynamic as each sex alternately gains the upper hand at the expense of the other sex through evolutionary time (Rice *et al.*, 2006; Kokko and Jennions, 2014).

Conclusion

CFC has a history of controversy. Like any nascent field of scientific study, controversy yields discussion, clearer definitions, and explicit recommendations for research programs. Taking a reductionist approach of dissecting functional interactions between male and female traits via experimental manipulation or utilizing natural phenotypic variation associated with paternity bias should identify traits involved in CFC (e.g., Eberhard, 2010; Eberhard, 2011). With recent technological advances such as CM-PCR or fluorescently labeled sperm, patterns of sperm storage can be deduced or directly visualized (e.g., Manier *et al.*, 2010; Marie-Orleach *et al.*, 2014). By correlating patterns with processes (e.g., sperm storage and survival), investigators disentangle which male traits may have evolved because of the 'environmental conditions' imposed by the multivariate traits of the female reproductive tract (e.g., Lüpold *et al.*, 2013). Controlled crosses after a number of generations of experimental evolution at different densities can be used to identify traits and genes that respond to selection on male phenotypes relative to female phenotypes, and determining whether increased female fitness associated with these traits can reveal CFC or sexual conflict (e.g., Simmons and Garcia-Gonzalez, 2011). In addition, when reproductive genes associated with strong male × female interaction effects on paternity are identified (e.g., Reinhart *et al.*, 2015), the functional significance of these candidate genes can be assessed in knockout lines or RNA interference experiments to knock down gene expression (e.g., Bretman *et al.*, 2010; Findlay *et al.*, 2014). However, the tractability of these methods in non-model species in natural populations is a serious limitation. Greater focus on the naturally standing heritable variation in fitness associated with both male and female traits suspected to be involved in CFC 'in the wild' is crucial to understanding which modes of selection (natural selection, sperm competition, sexual conflict, and CFC) predominate their evolution (Arnqvist, 2014; Snook, 2014). These studies could be augmented by experiments concentrated on the functional significance and fitness consequences of female traits that interact with particular male phenotypes, but understanding their consequences in the 'real world' should be a focus if we want to understand the costs and benefits of adaptive traits.

See also: Sexual Selection, Theory of. Sperm Competition

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Polyploid Speciation

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Glossary

Allopolyploid A polyploid formed by the combination of genomes from two different species.
Autopolyploid A polyploid formed by the combination of genomes from within a single species (from the same or different parental individuals).
Diploid Having two sets of each chromosome. For example, humans are diploid (the majority of our cells have two sets of chromosome, one from each of our parents).
Diversification rate The net rate at which a group of species grows in number. The diversification rate equals the speciation rate minus the extinction rate.
Haploid Having one set of each chromosome (as found in the eggs and sperm of mammals). For example, the leafy green parts of most mosses are haploid.
Homolog/homeolog Chromosome pairs inherited from one's mother and father are known as 'homologs' (e.g., the two X chromosomes in a daughter are homologs). Chromosomes that are similar to each other because they both descend from a polyploidization event are known as 'homeologs.'
Minority cytotype exclusion The idea that when there is a mixture of ploidy levels within a population, the rarer type would tend to disappear because it more often mates with another ploidy level, producing offspring of intermediate ploidy and lower fitness (i.e., it suffers from a triploid block).
Neopolyploidy A polyploidization event that occurred in the recent past.
Paleoploidy A polyploidization event that occurred in the distant past. It is typically reserved for events that happened long enough ago that they have to be inferred from data other than chromosome counts alone.
Ploidy The number of complete chromosome sets a cell contains. For example, a human egg cell contains one set whereas the cells of an adult human have two sets.
Polyploid Having more than two sets of each chromosome in the majority of cells of an organism (3 sets = triploid, 4 sets = tetraploid, 5 sets = pentaploid, 6 sets = hexaploid, etc.).
Polyploidization The process by which an organism (or cell) has more genome copies than did its progenitors.
Triploid block The idea that triploids prevent the establishment of polyploids because of their low viability and fertility. In particular, if newly formed tetraploids are rare they might predominantly mate with diploid relatives and produce only low-fitness (or sterile) triploids.
Triploid bridge The idea that triploids may provide an important stepping stone to the establishment of tetraploids because they can produce some haploid, diploid, or triploid gametes that can combine with the gametes from other individuals in a population to generate additional polyploid individuals. The triploid bridge also increases gene flow between ploidy levels and introduces genetic variation to the polyploids.
Unreduced gametes The production of gametes that have not undergone the normal process of reductive division, such that the gamete has the same number of chromosomes as the parent instead of half the number.

Introduction

> Particularly remarkable is it that tetraploids while crossing with each other, yield a sufficient quantity of seeds, but in crosses with [the progenitor diploids] almost no formation of seeds occurs, i.e. the tetraploid hybrids prove already singled out from the parental species.
>
> (Karpechenko, 1928, p. 62)

The structure and size of genomes are fluid, changing over evolutionary time via a variety of mechanisms including gene duplications, translocations, inversions, and, most strikingly, polyploidization. The 'ploidy' level of an organism refers to the number of copies of each chromosome it contains: haploid for one (think of a human egg or sperm cell), diploid for two (e.g., a human adult), and polyploid for any larger number (triploid: three, tetraploid: four, pentaploid: five, etc.). Differences in ploidy are frequently observed among species, particularly in plants, with some of the most famous polyploids illustrated in Figure 1. Furthermore, individuals of different ploidy levels are often reproductively isolated from one another, leading biologists to consider 'polyploid speciation' to be one of the most direct routes to the formation of new species.

Karpechenko (1928) was one of the first to describe the experimental formation of a new polyploid species, obtained by crossing cabbage (*Brassica oleracea*) and radish (*Raphanus sativus*). Both parent species are diploids with $n = 9$ ('n' refers to the gametic number of chromosomes – the number after meiosis and before fertilization). The vast majority of the hybrid seeds failed to produce fertile plants, but a few were fertile and produced remarkably vigorous offspring. Counting their chromosomes, Karpechenko discovered that they had double the number of chromosomes ($n = 18$) and featured a mix of traits of both parents. Furthermore, these new hybrid polyploid plants were able to mate with one another but were infertile when crossed to either parent. Karpechenko had created a new species!

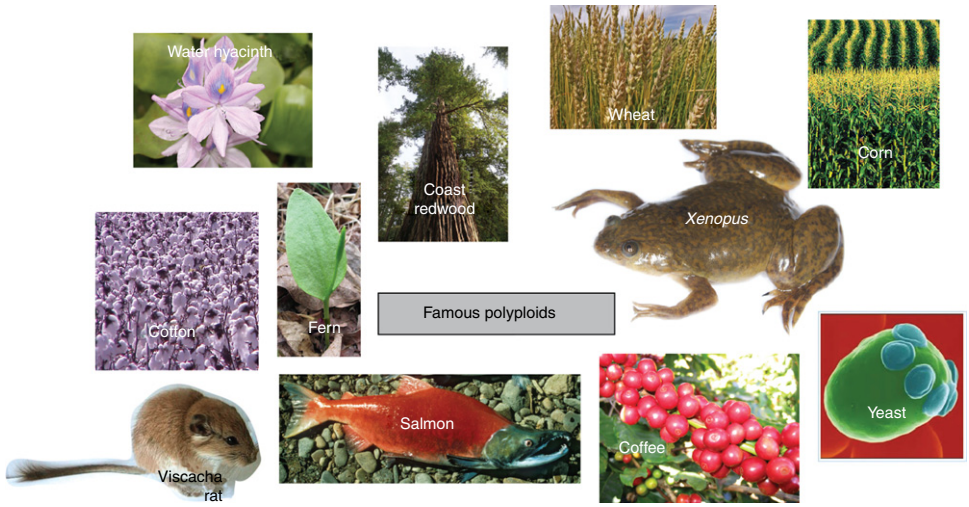

Figure 1 Illustrated are some of the most famous polyploid species, from the beautiful but highly invasive water hyacinth to the red viscacha rat, one of two known polyploid mammals. Also shown is the fern *Ophioglossum reticulatum*, the record holder for most chromosomes ($n = 720$) with about 100 copies per homologue.

This newly formed species, now called 'radicole' and used as a crop for animal fodder, represents an 'allopolyploid,' as coined by Kihara and Ono (1926) – it is a polyploid formed by the union of genomes from different species. Not all polyploids form in this way. An alternative possibility, 'autopolyploidy,' refers to the increase in ploidy level within a species. These categories are not absolutes, however, because polyploids formed from crosses between subspecies or distant populations may have characteristics intermediate between the two.

Polyploids are common in nature, especially in plants, and many of our most economically important plants – including both crop species and destructive weeds – are polyploids (**Figure 1**). For example, recent estimates suggest that 35% of vascular plants are recent polyploids ('neopolyploids'), having doubled in genome size since their genus arose (Wood *et al.*, 2009). Moreover, if one goes back far enough, all seed plants (Jiao *et al.*, 2011) and tetrapods (i.e., four-limbed vertebrates; Postlethwait *et al.*, 1998) have descended from polyploid ancestors.

Polyploidy is thought to play a major role in speciation for two reasons. The first is that chromosome doubling causes polyploids to be incompatible with their diploid parents, with crosses between them leading to low-fitness offspring (e.g., triploids). Consequently, polyploidy could be a rapid route to reproductive isolation, reducing gene flow between newly formed polyploids and their parental populations, and hence taking a key step toward speciation. The second reason is that polyploids often differ phenotypically from their diploid parents. These differences can be the immediate consequence of a doubled genome size (see next section) or be a consequence of the polyploids combining adaptations from different parents, allowing the polyploid to outperform both parents, at least in some environments. Polyploid hybrids are particularly interesting because they can maintain both parental genomes for long periods of time (illustrated as red and blue chromosomes in **Figure 2(d)**), perpetuating the advantages displayed by some hybrids ('hybrid vigor'). Furthermore, polyploids often avoid the sterility problems that can plague diploid hybrids by balancing the contributions of each genome and providing each chromosome with a closely related partner (a homolog) for pairing.

Given the prevalence and apparent success of numerous polyploid species and the ease with which changes in ploidy can contribute to reproductive isolation, it is natural to assume that polyploidy has played an important role in speciation. In this article, we discuss the current evidence for polyploid speciation and its consequences. We address two distinct but related questions. What role do ploidy *changes* play in speciation (i.e., in the instantaneous formation of new species)? And what influence does polyploidy have on subsequent speciation events (i.e., do polyploid species, *once formed*, have a greater or lesser tendency to speciate themselves)?

Polyploid Speciation I: The Formation of New Species by Polyploidization

Mechanisms of Polyploidization

Before discussing the impact of polyploidy on speciation, we briefly review the mechanisms by which polyploids form. An increase in ploidy level ('polyploidization') occurs via three primary mechanisms: somatic doubling, polyspermy, and unreduced gamete formation.

Somatic doubling occurs when DNA replication is not followed by a cell division. If this doubling occurs early in development, the entire (otherwise diploid) animal or plant can become tetraploid. If later in development, only part of the organism will be tetraploid. Although such tetraploid cells are often associated with cancer, they also arise normally during development in several tissues, including the heart, bone marrow, and liver in humans and other mammals (Zimmet and Ravid, 2000; Ganem *et al.*, 2007). However, for the doubled genome to be inherited – for there to be a chance of a new species forming – the doubling must occur in the germline. There is evidence that some polyploids do form in this way, including one of the first described allopolyploids,

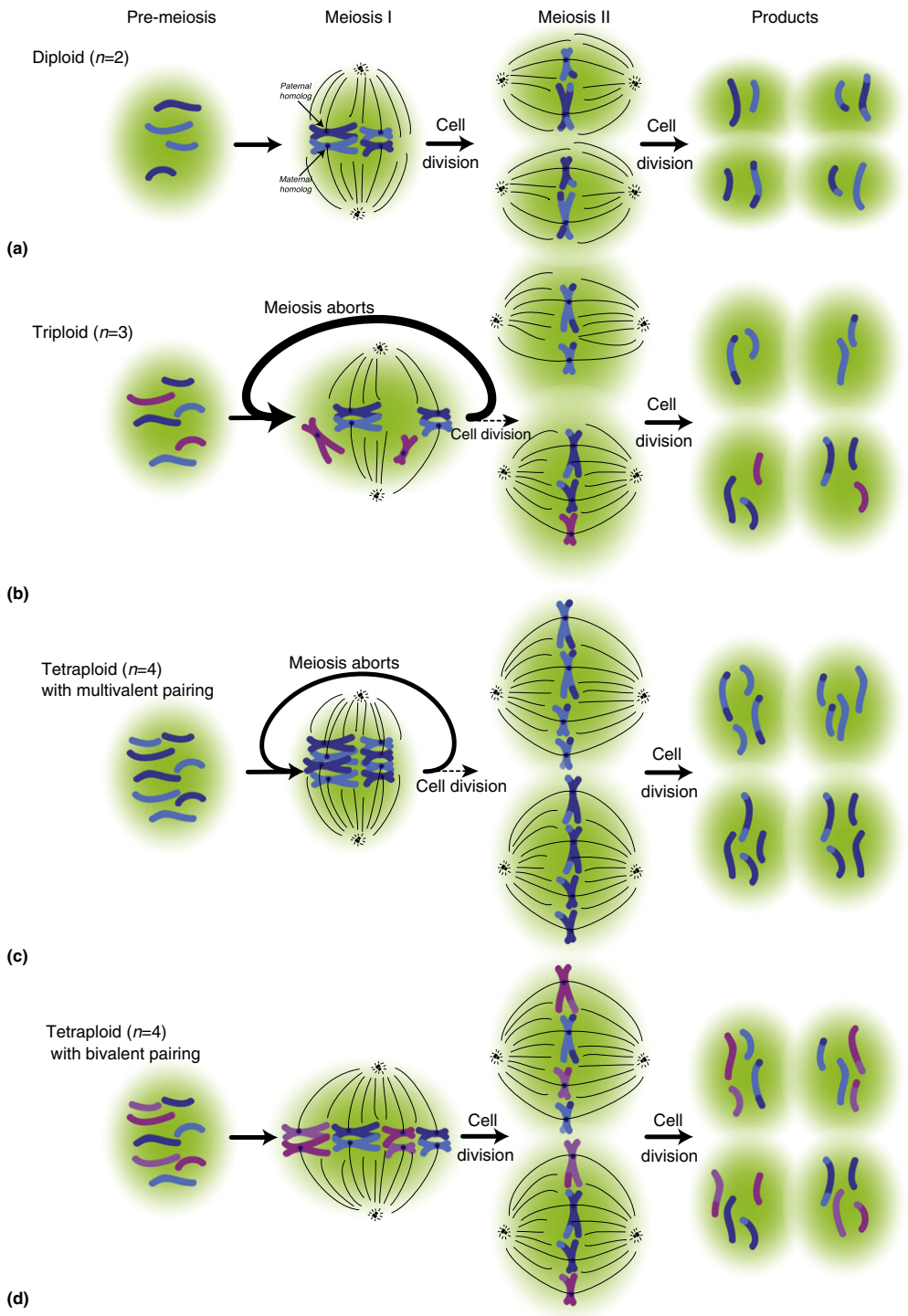

Figure 2 The segregation of chromosomes in diploids (a), triploids (b), and tetraploids (c, d). In triploids, unpaired chromosomes typically float in the cytoplasm during meiosis rather than being drawn to the metaphase plate, resulting in the abortion of meiosis or the production of highly unbalanced gametes, although occasionally balanced gametes are produced. Tetraploid segregation patterns are shown both for the case where all four sets of chromosomes come together at metaphase ('multivalent formation' (c)) and where only two sets come together ('bivalent formation' (d)). While meiosis is more likely to proceed normally via bivalent formation in allopolyploids, autopolyploids also frequently exhibit bivalent meiosis (Ramsey and Schemske, 2002).

Primula kewensis (Newton and Pellew, 1929). Somatic doubling is, however, thought to be a relatively uncommon route to polyploidy (Ramsey and Schemske, 1998).

Another route to polyploidization is *polyspermy*, the fertilization of an egg by more than one sperm. This mechanism is also thought to be rare in plants (Ramsey and Schemske, 1998),

but it may be more common in animals. For example, in humans, polyspermy is a frequent cause of polyploid conceptions (60%); these polyploid conceptions generally do not come to term and account for a relatively large fraction (10%) of spontaneous abortions (Zaragoza *et al.*, 2000).

Finally, the production of *unreduced gametes* through a failure in meiosis is the predominant route to polyploidy in plants (Ramsey and Schemske, 1998; De Storme and Mason, 2014) and the second most common route to polyploidy in humans (Zaragoza *et al.*, 2000). Unreduced gametes can arise by a failure to divide during meiosis I or meiosis II (referred to as 'first division restitution' and 'second division restitution,' respectively; Hermsen, 1984); these two forms can be distinguished based on the pattern of segregation of markers near and far from the centromere (**Figure 3**). Unreduced gametes can also arise when there is an endomitosis – an extra doubling of a cell's genome – prior to meiosis I ('Döpp–Manton sporogenesis'; Döpp, 1932; Manton, 1950).

Through any of these mechanisms, a triploid offspring would be expected in the next generation (assuming that unreduced gametes are rare and most likely to combine with reduced gametes). There are, however, circumstances under which the production of unreduced gametes is sufficiently common that two unreduced gametes might fuse, leading directly to tetraploidy. One such circumstance is cold shock (Fankhauser, 1945; Bogart *et al.*, 1989; Ramsey and Schemske, 1998), which might account for the association between polyploidy and high altitude and high latitude populations. Interestingly, unreduced gametes are also more common among hybrids (Harlan and deWet, 1975; Kobel, 1996; Ramsey and Schemske, 1998), occurring at 50-fold higher rates in hybrid plants than in non-hybrids

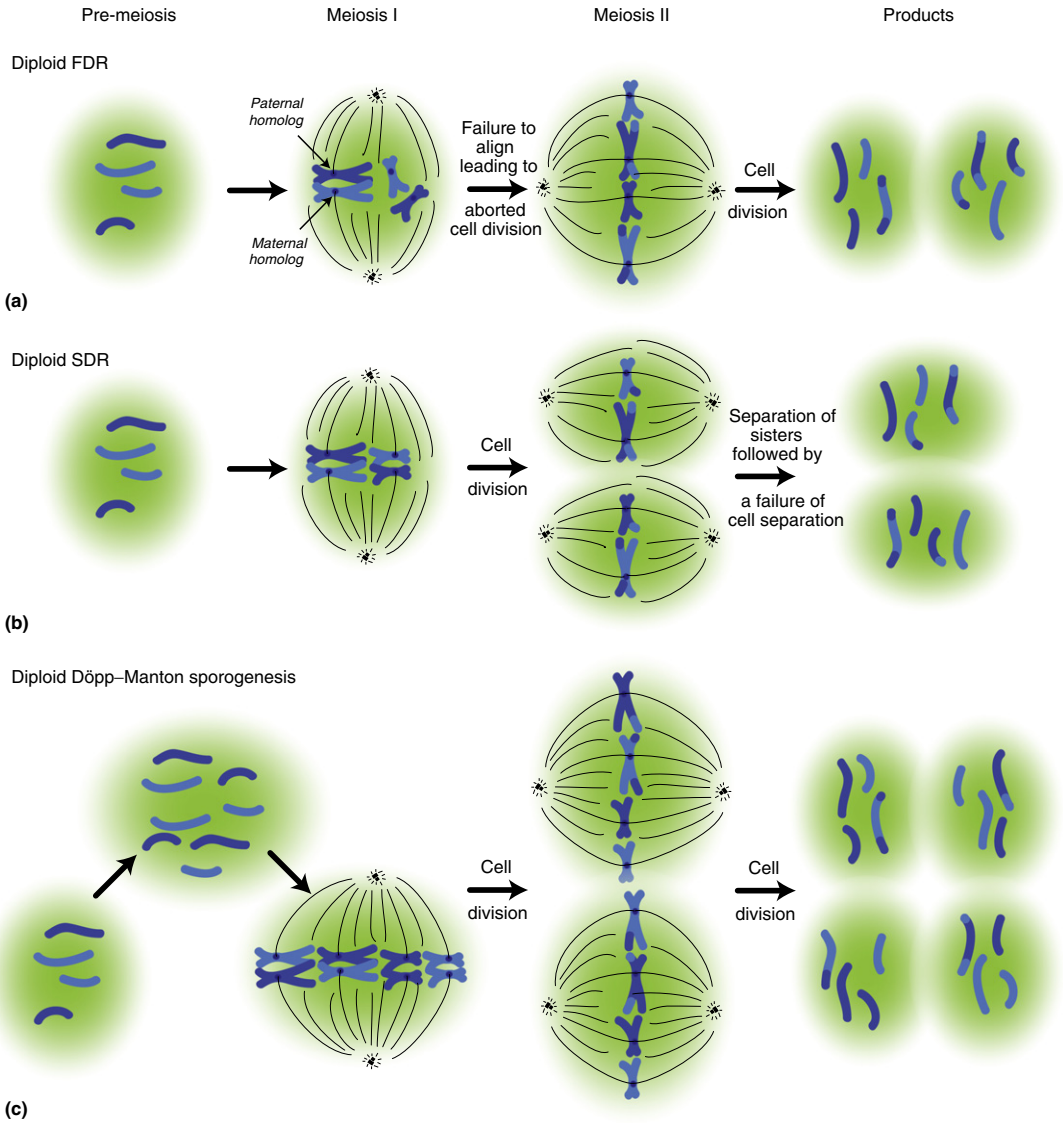

Figure 3 Abnormal segregation patterns leading to unreduced gametes. The three panels illustrate the three main routes to unreduced gametes (Köhler *et al.*, 2010): failure to divide the cell during meiosis I ('first division restitution' (a)), failure to divide the cell during meiosis II ('second division restitution' (b)), or an extra doubling of the genome prior to meiosis ('Döpp-Manton sporogenesis' (c)).

(Ramsey and Schemske, 1998). Unreduced gamete formation among hybrids is thought to be particularly important for allopolyploid formation, whereby, as in the radicole example, a mostly sterile diploid hybrid is able to produce some unreduced gametes, which when crossed with each other, restore fertility.

The Nature of New Polyploids

The pathway by which polyploidization occurs can have a major impact on the genetic variation observed among the newly formed polyploids. For example, tetraploids formed from two unreduced gametes from the same parent (selfing) bear a maximum of two alleles across their four gene copies, whereas four alleles can be captured if the unreduced gametes come from different parents. In addition, more genetic variation is captured when several polyploids are formed independently within a population ('multiple origins'). Although observing multiple polyploidization events may seem highly unlikely, circumstances that make it more likely for one polyploid to form (e.g., cold shock, hybridization, or genotypes predisposed to produce unreduced gametes) also make it more likely for multiple polyploids to form, as has been observed in several recent studies (Soltis and Soltis, 1999; Kaur *et al.*, 2014; Sigel *et al.*, 2014). Genetic diversity can be further augmented by matings between polyploids and diploid relatives. While such crosses often lead to partially sterile triploids, the few unreduced gametes produced by these triploids may contribute substantially to the number of tetraploids as well as their genetic diversity (the 'triploid bridge' mechanism, Ramsey and Schemske, 1998). For all of these reasons, young polyploid populations may not be as genetically depauperate as one might initially expect.

Phenotypically, newly formed polyploids often differ from their diploid progenitors. The most reliable phenotypic difference is increased cell size (Cavalier-Smith, 1978). Larger cell size can lead to larger body size in multicellular organisms, an association common in invertebrates, sometimes observed in plants, but rarely found in vertebrates (Otto and Whitton, 2000). In addition, polyploidization can affect development time, with polyploids often taking longer to develop (Ramsey and Schemske, 1998; Otto and Whitton, 2000). In plants, newly formed polyploids often differ from their diploid progenitors in morphology (e.g., thicker leaves), reproductive characters (e.g., larger flowers and later flowering times), and physiology (e.g., altered water transpiration and photosynthesis; Ramsey and Schemske, 1998). Ecologically, diploids and polyploids often differ in resistance to pests, sensitivity to nutrient stress, susceptibility to drought, and tolerance of extreme abiotic conditions (heat, cold, etc.; Levin, 1983). Many of these differences are idiosyncratic (e.g., with some tetraploids being more cold tolerant and some less so), making it impossible to predict the exact phenotypic shift likely to emerge in a new polyploid.

What is important is that polyploids are, immediately upon their formation, phenotypically different in ways that may make them better suited to some environments and less suited to others, shifting the ecological niche or the 'adaptive gestalt' of a population (Levin, 1983).

Moreover, some phenotypic shifts may additionally contribute immediately to reproductive isolation between polyploids and their diploid progenitors. For example, changes in flowering time associated with polyploidy can immediately isolate (at least partially) the new polyploids from their diploid progenitors (Husband and Schemske, 2000). Similarly, frogs in the genus *Hyla* both sing at lower frequencies (the males) and prefer lower frequency songs (the females) following increases in ploidy level, likely caused by increased cell sizes (Tucker and Gerhardt, 2012). By contributing both to ecological divergence and to reduced gene flow, polyploidization in such cases may represent a particularly easy route to speciation (a so-called magic trait; Coyne and Orr, 2004).

The Rate at Which New Polyploids Establish

Shifts between ploidy levels are inferred by two main signatures. The first is genomic: evidence that whole tracts of genes have been duplicated at the same point in time. This signature can last for hundreds of millions of years and is typically the information used to infer ancient polyploidization events (e.g., Bowers *et al.*, 2003; Jiao *et al.*, 2011). The second signature is a wholesale shift in the number of chromosomes within a lineage. For example, if most species in a genus have seven chromosomes after meiosis, but one recently derived species has 14, the latter is likely polyploid. In addition, because genome doubling always yields an even number of chromosomes, excesses of even over odd gametic chromosome numbers can be used to infer rates of polyploidization. This pattern is common in plants (63.2% of ferns have even numbers, 59.4% of monocots, and 54.9% of dicots, Otto and Whitton, 2000), which, assuming a conservative estimate of how often chromosome numbers change, yields an estimate of the rate of polyploidization relative to the rate of speciation of 2–4% in angiosperms and 7% in ferns (Otto and Whitton, 2000). A more refined approach maps chromosome number shifts along the phylogeny of a group of species. Using this approach, Wood *et al.* (2009) estimated that the rate of polyploidization was, on average, 15% that of the rate of speciation across a set of 123 angiosperm genera and 31% across 20 fern genera.

The above estimates do not account for differences in the rate of speciation and extinction between diploids and polyploids. Studies that have done so far have yielded much higher estimated rates of ploidy change. In one recent study, Scarpino *et al.* (2014) fitted a model to data from 60 genera of angiosperms. Their model estimated how much speciation and polyploidization is needed for each genus to have evolved from one species to the known number of species of each ploidy level that exist today, allowing diploids and polyploids to speciate at different rates (but ignoring extinction). This study inferred that, on average, diploids undergo polyploidization at a rate that is 39.9% the rate of speciation. Performing a phylogenetic analysis that allowed for differences in speciation and extinction; Mayrose *et al.* (2011) also obtained high estimates for the rate of polyploidization. Angiosperms polyploidized at a rate that was 29.6% that of the speciation rate, a number that rose to 41.0% for non-seed

plants, averaged across 50 angiosperm and 13 non-seed plant phylogenies, mostly at the genus level (Mayrose *et al.*, 2011). These numbers were inferred using a model that assumed polyploidization occurs over time, during the evolution of a lineage. An alternative model that allowed polyploidization to occur only at speciation events yielded similar estimates (29.7% for angiosperms, and 38.7% for non-seed plants). Thus, our best inferences at the moment suggest that plant species become polyploid at roughly one-third the overall speciation rate.

Importantly, these analyses only provide estimates of the relative rates at which polyploidization and speciation occur; they do not address how often they occur together. As a consequence, the extent of synchronization between changes in ploidy and the formation of species remains unclear. Furthermore, these analyses use data from within genera, and thus only estimate rates from the relatively recent past and do not account for polyploidization rate variation over time. Indeed, it has been argued that times of environmental stress may greatly increase the rate of polyploidization, with evidence of an excess of polyploidization events dating back to the Cretaceous–Paleogene boundary, a time of massive environmental upheaval and widespread extinction (Vanneste *et al.*, 2014).

Another open question is how often these polyploidization events involve hybridization between species versus arise within a species (i.e., allo- versus autopolyploidy). Of the above studies, only Scarpino *et al.* (2014) attempted to tease apart the nature of the polyploids (by assuming that some ploidy levels, e.g., hexaploids, would only be formed by hybridization between species); they found that allopolyploidy was roughly four times more common than autopolyploidy. This inference conforms to the traditional view that autopolyploid species should form less frequently because they suffer reduced fertility due to multivalent formation during meiosis (**Figure 2(c)**; Clausen *et al.*, 1945; Stebbins, 1971). It is also consistent with phylogenetic studies of groups with lots of polyploids, which tend to find a preponderance of allo- versus autopolyploids (e.g., Doyle *et al.*, 2003; Brysting *et al.*, 2007; Rothfels *et al.*, 2014). On the other hand, estimates of autopolyploid speciation may be biased downwards because autopolyploids are frequently overlooked as unique species due to their morphological similarity to diploid progenitors, even if they satisfy the conditions of most species concepts (Soltis *et al.*, 2007). Indeed, recent studies suggest that autopolyploids may form and establish at high rates (Ramsey and Schemske, 1998; Ramsey and Schemske, 2002), and autopolyploid speciation may be more common than previously thought (Parisod *et al.*, 2010b). Future studies are needed to quantify more precisely the contribution of allopolyploidy and autopolyploidy to polyploid speciation.

The Role of Polyploidization in the Formation of Species

In the previous subsection, we discussed estimates of the rate at which polyploid species arise. Here, we tackle the more difficult question: to what extent is the change in ploidy, itself, responsible for the formation of new species?

Because newly formed polyploids can be reproductively isolated from their diploid progenitor species, as exemplified by radicole, and because many closely related species differ in ploidy level (Wood *et al.*, 2009), it is often assumed that polyploidization drove speciation for all species pairs that differ in ploidy. For example, in the fern genus *Pteris* (Pteridaceae), a recent study found that 40 out of 106 studied species were polyploid and concluded that these were the result of polyploid speciation (Chao *et al.*, 2012). An alternative, however, is that new species form via mechanisms that are not associated with ploidy shifts (e.g., the accumulation of genetic incompatibilities in isolated populations), with the ploidy shifts occurring independently over evolutionary time.

Ideally, we would learn about the role of polyploidization in the generation of new species by directly observing the process of speciation. Unfortunately, we typically only have snapshots at different stages in different taxa. There have, however, been studies that explore very closely related taxa and measure the contributions of various features, including ploidy differences, to reproductive isolation. One study of diploid and tetraploid subspecies of fireweed, *Chamerion angustifolium*, found that the reproductive isolation between them was almost entirely (98%) due to mechanisms like pollinator differences and preferences for high versus low elevation habitats: little of the observed reproductive isolation was due to the hybrid sterility typically assumed to prevent gene flow between diploids and polyploids (Husband and Sabara, 2004; Martin and Husband, 2013).

This example illustrates many of the problems facing scientists investigating polyploid speciation. For one, it is difficult to know what mechanisms acting to separate species today were important in driving or facilitating their initial divergence. Did fireweed divide into high and low elevation habitats, and subsequently there happened to be a polyploidization event whose descendants came to dominate the lower elevation population, or did polyploidization facilitate the initial divergence?

A second problem is that, even if polyploidization was the first step toward speciation, it is hard to know which features of the new polyploids mattered most. It could be that the critical feature was an altered morphology or ecological tolerance of the polyploid, not its genetic incompatibility with the diploids. If polyploids form often enough (estimated at a frequency of 0.24% in fireweed; Husband and Sabara, 2004) and if they have an advantage over the diploids in certain habitats (e.g., at low elevations in the fireweed example), then eventually a self-sustaining population of polyploids may colonize sites beyond the range – and niche – of the diploid. Here, for example, polyploids may have established because they can better survive at lower elevations; the sterility of crosses between polyploids and diploids may have been largely irrelevant.

The view that polyploidy provides an 'instantaneous' reproductive barrier between species is based largely on the assumption that crosses between diploids and tetraploids will generate infertile triploids (the 'triploid block'). Having three sets of chromosomes reduces fertility, because meiosis either fails in the absence of paired chromosomes or proceeds but leads to gametes without a full set of chromosomes ('aneuploidy'; **Figure 2(b)**). Nevertheless, this view is now considered too absolute: inter-ploidy hybrids need not be completely sterile, and even if they are, other routes can allow

gene flow between populations of different ploidy levels (Soltis and Soltis, 1989).

In fact, rather than causing a block, triploids may provide an important genetic connection between different ploidy levels – a 'triploid bridge' – particularly in the early phases when a new tetraploid population is first establishing (Bever and Felber, 1992; Husband, 2004; Rieseberg and Willis, 2007). Triploids can facilitate tetraploid establishment by occasionally producing unreduced (triploid) gametes that fertilize a normal haploid gamete to produce a new tetraploid individual or by producing partially reduced (e.g., diploid) gametes that can combine with a diploid gamete produced by a tetraploid – in either case, genetic material can flow to the tetraploid population, reducing its reproductive isolation. An increasing number of empirical studies have documented gene flow between ploidy levels, including gene flow from diploids to both auto- and allopolyploids (Slotte *et al.*, 2008; Parisod *et al.*, 2010b).

Of course, even if reproductive isolation is initially incomplete, selection on new polyploid populations will favor stronger reproductive barriers to avoid the production of sterile (or partially sterile) triploid offspring. This process – selection favoring the evolution of greater degrees of reproductive isolation to avoid wasting gametes on low-fitness hybrids – is referred to as reinforcement and is expected to be particularly relevant to the establishment of new polyploids, which might otherwise breed repeatedly with their diploid progenitor until they go extinct ('minority cytotype exclusion'; Levin, 1975; Butlin, 1987).

While the above discussion considers reproductive isolation between a polyploid and its diploid progenitors, another consideration is how polyploids – specifically allopolyploids – affect gene flow between the two parental diploid species. The triploid bridge, for example, might allow introgression (via the polyploid) of genes between two parental species that are otherwise genetically isolated. The opposite is also possible, however, if polyploid hybrids replace inter-fertile diploid hybrids at points of contact between two species and reduce gene flow between them (e.g., through increased meiotic break down in triploid progeny). Both of these outcomes are theoretically possible, but whether allopolyploids tend to facilitate or hinder divergence between parental diploid species is an interesting open question.

Polyploid Speciation II: The Speciation of Polyploids

The Influence of Ploidy on Diversification Rates

Another way that polyploidy can impact speciation, aside from the formation of new species by ploidy changes, is by altering the rate of speciation (and extinction). In other words, do polyploid species themselves form new species more or less often than their non-polyploid relatives? This is a question with a rich and contentious history. Early evolutionary biologists tended to believe that, while polyploids may form frequently, they rarely themselves speciated and instead tended to go extinct: they were 'evolutionary dead-ends' (Stebbins, 1950; Wagner, 1970). This opinion was informed, in part, by the belief that the 'extra' genomes of polyploids would mask mutations from selection (because most mutations are recessive), reducing the efficacy of selection and ultimately making polyploids less adaptable (Stebbins, 1950). However, there are also theoretical arguments in favor of polyploids speciating more frequently or going extinct more slowly. For example, by uniting multiple genomes, polyploids often exhibit greater enzymatic variability (Roose and Gottlieb, 1976) and maintain higher levels of heterozygosity, which has the potential to increase evolutionary flexibility (Mable and Roberts, 1997; Petit and Thompson, 1999; Parisod *et al.*, 2010a,b) and promote diversification (Stebbins, 1985; Ricklefs and Renner, 1994). Polyploids may also benefit from the redundancy inherent in polyploidization in that they have 'back-up' copies of each gene if ever one is damaged (and thus they may go extinct more slowly) and because these 'extra' gene copies, even if initially identical, are available to be molded by selection for different uses (Ohno, 1970; Zhang, 2003; Des Marais and Rausher, 2008), potentially increasing speciation rates. For example, Hofberger *et al.* (2013) argue that polyploidy allowed the evolution of a key group of defensive compounds in the mustard plant family, and Málaga-Trillo and Meyer (2001) similarly link the extensive body plan variation in fish to rounds of ancestral polyploidy.

Polyploidy may also increase diversification rates directly by increasing the rate that reproductive isolation arises between populations. Because most mutations that affect fitness are deleterious, the probable fate of a duplicate gene pair is the silencing of one of its members. If different copies of an important gene are silenced in different populations, offspring of a cross between populations will have reduced fitness because some of their progeny will not inherit any functional copies. Because this 'reciprocal silencing' or 'divergent resolution' can happen at multiple loci, isolated polyploid populations may rapidly lose the ability to produce fertile hybrids (Werth and Windham, 1991; Taylor *et al.*, 2001).

These theoretical links between polyploidy and elevated diversification rates are seemingly supported by four main empirical observations. First, clades with a higher percentage of polyploids tend to contain more species (Petit and Thompson, 1999; Otto and Whitton, 2000; Vamosi and Dickinson, 2006), although this may simply reflect the fact that small young clades have not had time to accumulate polyploids or that diploids may produce polyploid daughter species at high rates in some clades (without these polyploids diversifying at high rates). Second, extant polyploids can be highly ecologically successful relative to their diploid relatives (Hahn *et al.*, 2012; Te Beest *et al.*, 2012), while their related diploids are rare, undiscovered, or extinct (e.g., Grusz *et al.*, 2009; Beck *et al.*, 2010). Third, studies of both paleontological and genomic data have inferred multiple 'paleopolyploidy' events in the history of most major lineages (e.g., Masterson, 1994; Sidow, 1996; Wolfe and Shields, 1997; Soltis, 2005). Some of these paleopolyploidy events appear to have occurred at the base of major radiations (for example, at the base of the angiosperms and the base of teleost fishes), suggesting that polyploidization may have elevated speciation rates in these lineages (Hoegg *et al.*, 2004; De Bodt *et al.*, 2005; Barker *et al.*, 2008; Santini *et al.*, 2009; Tank *et al.*, 2015).

However, additional investigations, mostly in the past decade, have cast doubt on the arguments that polyploids should have increased diversification rates. At a theoretical level, the model of Muir and Hahn (2015) shows that the conditions under which reciprocal silencing leads to speciation are very restrictive, requiring nearly complete geographical isolation. The empirical arguments, likewise, are not as compelling as they first appear. For example, while clades with polyploids do tend to have more species than clades composed entirely of diploids, that pattern appears to be driven by the diploids in the mixed clades speciating more (both by forming new diploids and by creating polyploids, Mayrose *et al.*, 2011); polyploid-only clades are no richer than their diploid-only relatives (Vamosi and Dickinson, 2006). And the few studies to systematically examine the ecological 'success' of polyploids (i.e., their ecological or geographic breadth in comparison with related diploids) fail to find any advantages for the polyploids (Petit and Thompson, 1999; Martin and Husband, 2009).

The paleopolyploidy arguments likewise are less convincing than they first appear. While there are numerous examples of paleopolyploidy, relatively few analyses have asked whether there are more such cases than expected given the high rate at which polyploidization occurs. Because diploids give rise to polyploids, but not vice versa, there is a ratchet-like process to increase ploidy levels, which can explain the prevalence of polyploidy and of paleopolyploidy events, without any need for polyploids to speciate more than diploids (Meyers and Levin, 2006). Indeed, a recent simulation study using empirical estimates of speciation, extinction, and polyploidy rates assuming that polyploids and diploids diversify at the same rates found that there should be approximately 4.6 to 8.9 paleopolyploidy events in the history of any given angiosperm species (Mayrose *et al.*, 2011), instead of the 1 to 4 such events thought to have occurred (Jiao *et al.*, 2011). Thus, if anything, the number of paleopolyploidization events in plants suggests that polyploids have diversified less than diploids.

The related argument – that polyploidy events tend to occur at the base of major clades – suffers from problems related to the effects of incomplete sampling and extinction. Jiao *et al.* (2011), for example, reconstruct a paleopolyploidy event at the base of the seed plants, but the dating is imprecise, with the event occurring sometime during the approximately 100 million years between the divergence of the lycophytes from the rest of vascular plants and the divergence of the ancestor of extant gymnosperms from that of the angiosperms (Smith *et al.*, 2010). Furthermore, if a polyploidization event leads to a number of dead-end species that go extinct before a subsequent event leads to a species-rich clade, polyploidy will appear to be at the base of the diverse clade, even though polyploidy did not cause higher speciation rates (Donoghue and Purnell, 2005). Overall, there is no strong evidence that paleopolyploidy events directly caused increased diversification.

Recent analyses, typically using phylogenetic approaches, reinforce the emerging picture that, on average, polyploid lineages diversify more slowly than their diploid relatives (at least in plants; Mayrose *et al.*, 2011; Husband *et al.*, 2013). This diversification trend is driven by polyploids having both reduced speciation rates and elevated rates of extinction (Mayrose *et al.*, 2011), which results in evolutionary trees where polyploids frequently arise but commonly go extinct, such that the majority of polyploids observed in the present are relatively young species that have yet to go extinct (e.g., see Beck *et al.*, 2011; Escudero *et al.*, 2014). Within this broad tendency, exceptions exist – for example, the Hawaiian silversword alliance, the New World cottons, and several species-rich clades of bamboos all appear to have radiated at the polyploid level (Carr *et al.*, 1996; Adams and Wendel, 2004; Triplett *et al.*, 2014). Furthermore, there is some evidence that polyploid fish may diversify more rapidly than their diploid relatives (Zhan *et al.*, 2014), in keeping with an increase in diversification associated with the genome duplication event at the base of the telost fishes (Santini *et al.*, 2009). In addition, much of the speciation advantage experienced by diploids may, ironically, be due to their greater ability to produce polyploid daughter species; by comparison, polyploids are relatively bad at polyploid speciation (Mayrose *et al.*, 2011; Scarpino *et al.*, 2014)!

Conclusions

Polyploidy has contributed to the rich diversity of life, with ancient polyploidization events (paleopolyploidization) inferred to have occurred early in the evolution of angiosperms, teleost fishes, vertebrates, and yeast, along with numerous recent events (neopolyploidization) in many groups of plants and in some animals. However, the prevalence of polyploids reflects the combination of two processes: the establishment of new polyploid populations and the diversification of these populations, corresponding to the two main sections of this article. The interaction of these processes can be thought of as a balance, whereby new polyploid individuals are constantly added to populations, due largely to errors in meiosis or fertilization. Many of these ploidy mutants are, however, unfit and fail to leave descendants. Occasionally newly formed polyploids are successful and establish new populations. Once established, many of these new polyploid populations form their own species, but these new species are also generally unfit (at least in plants); only rarely are they able to avoid extinction and themselves speciate. That the ultimate fate of most polyploid individuals and populations is extinction does not preclude the potential for rare advantageous polyploids to have important long-term evolutionary consequences, including establishing major branches of the tree of life (Mayrose *et al.*, 2014; Arrigo and Barker, 2012).

Much remains to be learned about the impact of polyploidization on speciation, at both these levels. At the first level, it is clear that polyploids often differ phenotypically from their parent species in ecologically important ways as well as having a degree of chromosome-based reproductive isolation, potentially providing them with an easy route to speciation (Coyne and Orr, 2004). Accumulating data suggest that this route, however, is often not 'instantaneous.' Indeed, the prevailing view is that a period of gene flow between diploids and recently formed polyploids assists in polyploid establishment, both by increasing genetic variation in the polyploids and by increasing the number of potential mates

for the polyploid individuals. Even in those cases where isolation is strong and rapid, it is unclear whether it is the typically invoked chromosomal incompatibilities or other phenotypic differences that are most responsible for the isolation between the new polyploid and its progenitors.

While there is a strengthening consensus that polyploid plant species tend to diversify more slowly than their diploid relatives (Mayrose *et al.*, 2011; Arrigo and Barker, 2012; Escudero *et al.*, 2014; Mayrose *et al.*, 2014; Scarpino *et al.*, 2014; but see Tank *et al.*, 2015), it is unclear how widely applicable these results are to other taxonomic groups; the opposite pattern, for example, is suggested for polyploid fish (Santini *et al.*, 2009; Zhan *et al.*, 2014). In addition, why some polyploid lineages can persist and even proliferate, while others are lost, remains unknown.

Future research promises to clarify the role that hybridization (allopolyploidy) and environmental perturbation (Vanneste *et al.*, 2014) play in the success or failure of polyploid lineages. Another promising area of research is to confirm the tantalizing finding that previous rounds of polyploidization inhibit subsequent rounds (Mayrose *et al.*, 2011; Scarpino *et al.*, 2014). Is this because rising chromosome numbers cause increasingly severe meiotic problems or because the advantages of genome doubling are stronger in small genomes, which may be more constrained with fewer genes to take on new functions? Finally, as this review emphasizes, future research is needed to determine whether polyploid transitions are concentrated in time at speciation events, and if so, whether polyploidization plays an early and/or major role in the development of reproductive isolation.

See also: Hybrid Speciation. Seedless Land Plants, Evolution and Diversification of

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Population Structure and Gene Flow

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Glossary

Anadromous It describes fish species that lay eggs that hatch in fresh water, but after hatching the fish migrates to the sea to feed and grow to reproductive maturity.

Philopatry The process of an adult returning to the region or habitat of its birth for mating and reproduction.

A frequently used term in biology is 'structure' – the organization and relation of parts. It may refer to communities, it may refer to morphology; in evolutionary biology, it is often used to refer to population structure. This type of structure ;is invoked when individuals of a study species comprise more than one population, defined by the diversity within each. A population evolves primarily through the reproductive success of its members, and (for most eukaryotic organisms) who they mate with. When, for whatever reason, there are groups of individuals that are less likely to mate with other groups of individuals, we set them apart because these distinct populations, harboring distinct diversity, may be responding to different environments (Slatkin, 1987).

The primary information about diversity in a population involves the number of genetic variants from a region of the genome, or alleles, found in a sample of individuals. First, we may just count the number of types sampled and assess the frequency of each, and a simple frame of reference comes from the Hardy–Weinberg (HW) model. This model is generally used as a null hypothesis in evolutionary studies, so that rejection of this model leads us to understand what mechanism(s) of evolution (change in genetic diversity) may be acting on that population (Hartl, 2000). The basis for testing this hypothesis involves the frequencies of alleles, and the frequencies of diploid genotypes comprised of these alleles, assuming independent assortment and no evolutionary mechanisms acting. Of course, we expect the diversity of almost any population to be influenced by immigration, finite population size, mutation, selection, or nonrandom mating. Only when we reject this simple model can we begin to identify which are acting, and whether they lead to identifiable structure.

So, the next consideration is space: where were the samples taken from? As a set of genetic samples includes more locations, we can ask whether the allele and genotype frequencies for the sample at any location are consistent (statistically probable) with the allele and genotype frequencies at another location being the same. If not, we may start to see that these frequencies have diverged across locations, and a first question to ask is whether this is the result of nonrandom mating. Here this simply means that individuals at one location are more likely to have mated with another individual from the same location than an individual from the other location. This is one way to define population structure – that the sampled individuals from distinct locations are not often mating with one another. There may be reasons why individuals at the same location mate nonrandomly as well (**Figure 1**).

This nonrandom mating is a form of inbreeding, as it involves reproduction between individuals that tend to be somewhat more closely related to one another. This in turn leads to an excess of genotype homozygosity relative to all other spatial samples, in the terms of the HW model, and is a means of statistically testing for population structure. Some of these statistical tests are detailed below.

It might also seem as though the DNA sequence divergence of particular alleles would tell us about population structure; two individuals that carry mitochondrial haplotypes that are different at 5 out of every 100 nucleotides seem more likely to be from different populations, in an evolutionary sense, than if their haplotypes are nearly identical. However, even alleles that are quite divergent may be found in the same population if individuals still mate with one another. Famously, alleles at the major histocompatibility complex in mammals may be much older than the species they are found in (Hughes and Nei, 1992)! Any single locus can have an unusual history or dynamics that lead it to carry ancient diversity. To understand this diversity we require the context of other loci, to see if

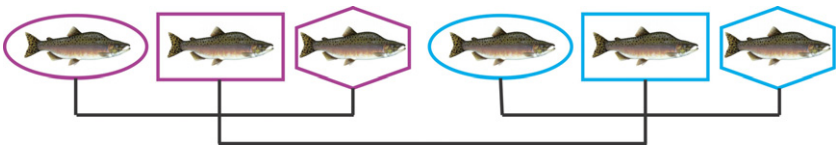


Figure 1 Visualizing population structure. There are two types of population genetic structure in pink salmon (*Oncorhynchus gorbuscha*). Branching of highly simplified genealogy identifies relatedness of individuals from population samples. Regional samples (represented as distinct shapes) exhibit high within-sample relatedness because of philopatry, and could help identify adaptation to distinct environments. However, the same spatial samples are split temporally because of the 2-year maturation cycle of this anadromous fish (Beacham *et al.*, 2012): the 'odd' years (pink) have evolved distinct allele and genotype frequencies from the 'even' years (blue), and have different patterns of abundance and demography. Thus, structure results from both spatial limits on gene flow, as well as the temporal effect of alternating maturation cycles.

individuals are clearly distinct at multiple loci, suggesting again that they are not mating with one another. The cause of reproductive isolation may be extrinsic – a barrier between populations – or intrinsic, when the populations do not recognize one another as good mates or when there are fitness consequences for crosses between the populations (Bewick and Dyer, 2014).

Leaving the biological, intrinsic mechanisms alone for now, we can see that an important way in which populations form is from simple limits on individuals encountering one another. So, organisms that move very little in their lifetime would be expected to only encounter a mate locally; organisms that have at least some stage in their life of high motility will expand the spatial range of possible mates (Slatkin, 1987). If an individual moves from one area to another, and successfully mates, we now have gene flow – literally, the movement of alleles from one location to another. If this happens often, it is likely that the locations are part of the same population (we cannot statistically differentiate the individuals from different locations), and if it is rare then we begin to posit 'structure.'

It is important to note that this is a quantitative and continuous designation. It may seem odd, but rejecting a hypothesis of a single population does not necessarily mean there are two, or more, populations. A phenomenon known as 'isolation by distance' refers to situations in which the level of gene flow is sufficient that neighboring locations may appear to be consistent with one population, but as the domain is expanded to include more geographically or ecologically distinct samples, the probability of sufficient dispersal and gene flow diminishes, and allele frequencies, genotype frequencies, or pattern of substitutions become consistent with populations that are demographically isolated from one another (Wright, 1943). So, the number of populations inferred may depend on the scale and sampling for the analysis.

Inference of Structure

Analytically, our understanding of structure follows the logic identified above. There are many ways to approach this problem; here we focus first on the diversity within and among sample locations – in other words, examples in which the location of the sampled individual influences the analysis – and then on approaches that assess the fit of the data to a series of models of population structure to determine the most probable description of structure given the data; these latter approaches do not require spatial information, and can help identify patterns arising through other mechanisms.

As noted above, when individuals are sampled from multiple locations, we can measure the genetic diversity both within locations as well as inclusive of multiple locations. Traditionally, the complete data set is considered as the 'total' diversity, and diversity from individual 'sites' are evaluated for how much of the total diversity they contain. By symbolizing the site-level diversity as S, and the total diversity as T, we can evaluate a family of statistics that quantify structure as X_{ST}, where S and T are as defined and X is a symbol that varies depending on assumptions about the data. Though this now sounds complicated, our most general model for quantifying population structure can be expressed in simple terms:

$$X_{ST} = \frac{(\text{total diversity}) - (\text{mean within-site diversity})}{(\text{total diversity})}$$

where diversity may be measured as heterozygosity or related measures of genetic diversity (where X is replaced by F or G), variance in microsatellite allele size (where we assume the stepwise mutation model, and use R_{ST}), nucleotide diversity (which may be evaluated using Φ_{ST}), and other 'flavors' of this approach (Excoffier *et al.*, 1992; Hartl and Clark, 1997). Typically, researchers will refer to F_{ST}, which was the first common 'fixation index' for such studies and can be estimated using a variety of computational approaches (Whitlock, 2011).

Often these indices are calculated in a hierarchical 'analysis of molecular variance,' mirroring a standard ANOVA statistical framework. If there is no structure – no correlation of allele frequencies by site, no increase in genotypic similarity by site – then there is as much diversity at the 'site' level of diversity as the 'total' level and the statistic X_{ST} is close to zero. At the extreme where every single site sampled harbors low diversity, but allelic diversity is distinct from all other sites (high divergence among regional groups of alleles), the statistic approaches 1 (**Figure 2**).

In recent years, effort has gone toward recognizing the limits of this class of statistics that are caused by how much variation is found at the site level, limiting the comparability of these indices among taxa. Improved (but slightly more complicated) statistics may be appropriate for some considerations, and may greatly improve our capacity to explore structure at multiple hierarchies of biodiversity (Jost, 2008; Whitlock, 2011; Smouse *et al.*, 2015). In almost all cases, this family of statistics is assessed for statistical significance through permutation testing, where genotypes are randomly assigned to a spatial site to generate a null distribution of divergence.

The other way in which population structure is often assessed is explicitly through finding the best fit of genotype data from individuals to inferred populations, where it is assumed that a true population will have minimal deviations from Hardy–Weinberg expectations on allele and genotype frequencies. As discussed above, to the extent two locations are connected by immigrants, the allele and genotype frequencies will not be divergent from one another; if gene flow is limiting, then by the stochastic process of variation in reproductive success (drift) these frequencies diverge from one another at different locations. When there are populations with heterogeneous allele and genotype frequencies, whether the location of the samples are considered or not, the overall fit to Hardy–Weinberg expectations will be poor.

A way to identify populations and which individuals belong to each is implemented in programs like structure (Pritchard *et al.*, 2000). A type of 'clustering analysis,' this approach identifies how well data fit Hardy–Weinberg expectations when 1, 2, 3, or more evolutionary populations are assumed – in each case, the likelihood of this analysis comes from the fit (lack of deviation) of genotype data in each cluster to HW expectations and thus the number of populations can be inferred in some circumstances (**Figure 3**). Other clustering approaches (Gao *et al.*, 2007) may be used when this

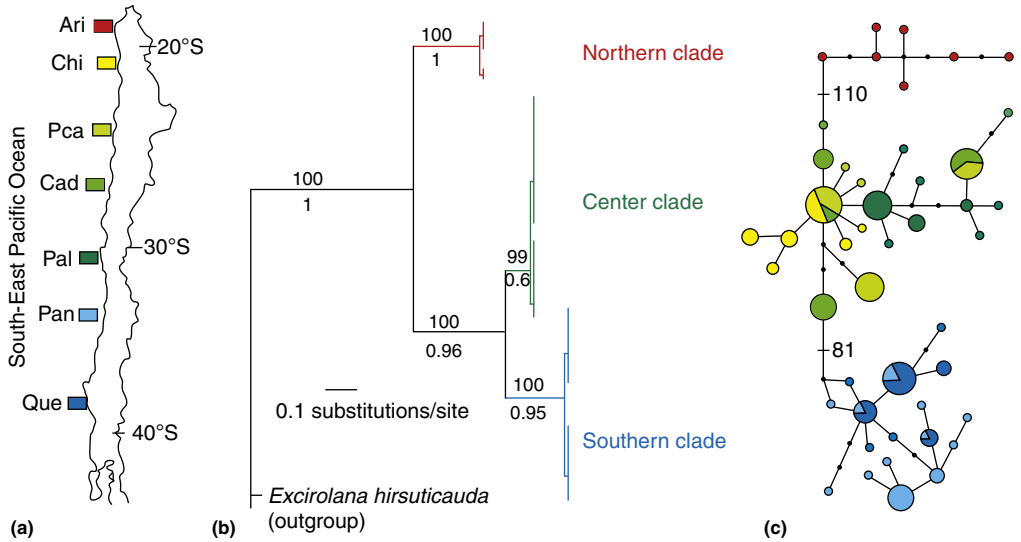
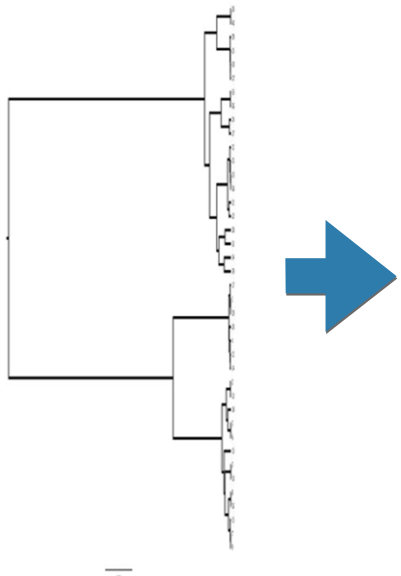


Figure 2 Illustrating hierarchical population structure. Mitochondrial haplotypes were sequenced from the isopod *Excirolana braziliensis* (Varela and Haye, 2012), from sites indicated in part (a). In (b), a phylogram illustrates the relationships among these haplotypes; the genetic relationship between particular haplotypes as well as the locations at which they were collected are then summarized in (c). From this network it can clearly be seen that the total amount of sequence variation in Excirolana is large (the numbers along the haplotype network indicate the number of nucleotide substitutions between clades), and at particular sites (or within sub-regions) the variation is less (e.g., only 7 distinct haplotypes in the Northern Clade). For sequence data, it is typical to calculate Φ_{ST} which uses the sum of squared differences among DNA sequences as a metric within an ANOVA framework. Φ_{ST} values among locations in the Center Clade, for example, range from 0.158 to 0.444, while values between sites in the Center Clade and other regions consistently present Φ_{ST} greater than 0.95.



Population	Allele 1	Allele 2
1	96	93
1	96	96
1	94	94
1	95	95
1	95	96
1	96	93
1	94	96
1	95	95
1	96	94
1	96	96
2	95	95
2	93	97
2	96	96
2	96	95
2	96	96
2	94	93
2	94	93
2	93	94
2	96	93
2	91	96

Figure 3 Clustering analyses. A genealogy illustrates the temporal relationship among alleles in two sites with limited gene flow. Simulated diploid microsatellite genotypes at a single locus present data that, when a single population is assumed, exhibit homozygote excess relative to the allele frequencies and the relationships of the HW model. When two populations (blue and green) are assumed, the fit of genotypes to HW is much better. This is a model-fitting approach to inference of population structure.

underlying genetic model is likely to be violated (e.g., in selfing plants where assumptions of HW are known to be false).

Of course, while it is known that migration and gene flow are the forces that will homogenize allele and genotype frequencies among locations, it is important to recognize that migration is not always (or often) omni-directional and symmetric among sites. From fundamental papers in population genetics to more contemporary efforts, we know that in these instances the upstream, or source, population diversity will end up driving the diversity in the recipient sites (Nagylaki, 1978; Wares and Pringle, 2008). This has numerous

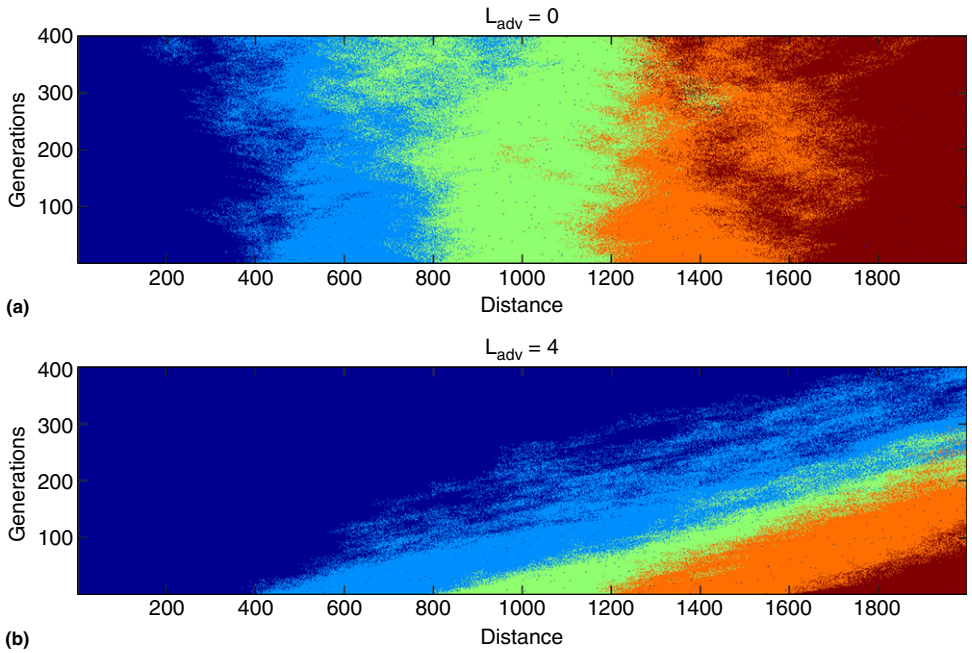


Figure 4 Effects of asymmetric gene flow. When dispersal is biased by wind, currents, or other environmental features, the genetic diversity of a system may be homogenized with the diversity of the 'upstream' region (source region) dominating the entire system. In both panel (a) and panel (b), a simulation model is carried out where the domain is initialized with haploid individuals and 5 distinct alleles, each geographically isolated to 1/5 of the domain. The model is run for 400 generations. In (a), there is no advection (asymmetric gene flow) and diffusion is the primary mechanism of dispersal. In (b), advection is approximately 40% as strong as random diffusion of offspring, and the offspring preferentially disperse toward the right. The upstream allele (indicated in blue) quickly dominates the entire domain. Reproduced from Wares, J.P. and Pringle, J.M., 2008. Drift by drift: Effective population size is limited by advection. *BMC Evolutionary Biology*, **8**, 235.

effects on the maintenance of diversity throughout the domain of a species (Figure 4). The most direct way to identify source–sink demographic systems, where the composition of the 'sink' populations may be entirely reflective of the composition of the 'source' is to use multi-locus genetic data to identify parentage of recruits by site (Peery *et al.*, 2008).

The Context of Population Structure

At some level the structure of a 'species' into 'populations' may seem academic, but 'species' are not responding to environmental change – the populations they are comprised of are. Repeatedly, researchers have found that individuals sampled from distinct sites – and ultimately shown to be in distinct populations – have distinct environmental tolerances, reaction norms, and levels of additive genetic diversity allowing response to change (Sanford and Kelly, 2011; Crozier and Hutchings, 2014; Evans *et al.*, 2015). Understanding population structure gives us great insight into the frequency and mechanisms of movement between sites, as well as the variation in the environment – some of it abiotic, some biotic – that maintains population structure. Much of what we know of trait variation – even the trait of reproductive isolation – is not necessarily at the level of species; instead, the values of traits may differ by geographic location and the genetic composition of those populations (Cutter, 2012; Mandeville *et al.*, 2015). The extent to which even subtle traits – coloration and other quantitative measures – vary among populations *could* be driven by local adaptation at associated genes, but is often undirected change associated with genetic structure or population structure of the focal organism instead. The context of structure is thus necessary for interpreting how evolution is changing natural patterns of diversity. Population genetics was founded on a statistical necessity; it is now necessary for exploring diversity at finer scales than can be named.

See also: Genetic Variation in Populations. Hardy–Weinberg Equilibrium and Random Mating. Inbreeding and Nonrandom Mating. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Ring Species

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Predation and Parasitism

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Introduction

Predator–prey and parasite–host interactions are nearly ubiquitous in ecological communities. These two interaction types comprise a subset of the broader victim–exploiter interaction in which one consumer species benefits at the cost of its partner through a direct trophic (consumptive) interaction. Predation and parasitism differ from one another in the lethality of the interaction for the victim species and the number of individuals involved on either side of the interaction. In a predatory interaction, the consumer species kills individuals of the prey species. Typically, this involves an individual predator consuming multiple prey individuals over the course of its life cycle. In a parasitic interaction, the parasite's consumption of the host is not necessarily lethal and often involves a reversal of the relative number of individuals participating on either side of the interaction: many parasites consuming a single host. A single individual parasite may interact with a single host individual, while hosts are likely to interact with many individual parasites.

A third class of consumers, parasitoids, shares a mix of traits between predators and parasites. Parasitoids function like parasites in that they usually interact with a single victim over the course of their lives. But like predators, the parasitoid's victim dies as a result of the interaction. Parasitoid–host systems can often be extremely specialized. Here, we will treat parasitoid–host interactions as a particularly intimate form of predator–prey interactions. Some of the traits involved in these systems, such as manipulation of host gene expression by parasitoids and the host's immune response are certainly more reminiscent of a gut parasite's interaction with its host than a lion killing an impala. However, the lethality that defines parasitoid life histories is an important distinction (Figure 1).

The outcomes of either predation or parasitism can have crucial fitness consequences for both interacting species. Natural selection is expected to act on variation in the ability of predators and parasites to efficiently consume their victims as well as on variation in the ability of victim species to either avoid or fend off aggressors. The intrinsic differences between predation and parasitism lead to different predictions regarding coevolutionary dynamics in these systems. However, the central premise is similar for both types of interactions. A simple Lotka–Volterra model of predator–prey dynamics is useful for illustrating the general premise of predator–prey coevolution (Abrams, 2001). This model predicts rates of change in a prey population of size V and a predator population of size P over a period of time t, and may be written as:

$$\frac{dV}{dt} = V(r - cP)$$

$$\frac{dP}{dt} = P(bcV - d)$$

where r is the intrinsic growth rate of the prey population, d is the mortality rate of the predator population, c is the capture rate for the average predator, and b is the conversion rate of captured victims into predator offspring. Although this is a somewhat simplistic model, more complex extrapolations that incorporate density-dependence, more realistic functional responses, and discrete generation time still incorporate the basic components of the Lotka–Volterra framework involved in coevolution.

The value of c (capture rate) is determined by traits in both predator and prey populations, and highlights how trait evolution in one species can have an immediate effect on the fitness landscape of the other species. Given intraspecific genetic variation, prey species are expected to evolve trait values which decrease the magnitude of c, while natural selection should favor predator traits that increase c (Abrams, 2001). The strength of this antagonistic coevolution around c

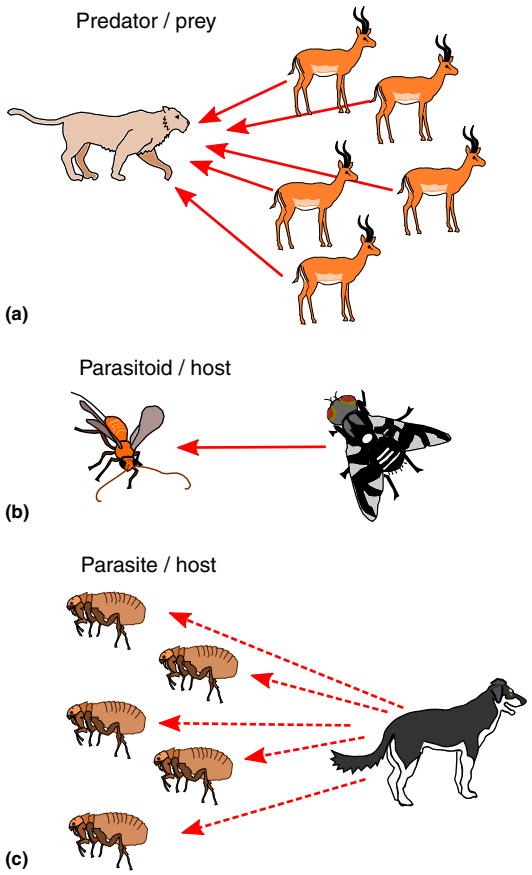

Figure 1 Diagram of basic demographic and lethality relationships for three types of exploiter/victim ecological relationships: (a) a single predator killing and consuming multiple prey individuals over its lifetime, (b) a single parasitoid killing and consuming a single host individual over its lifetime, and (c) many parasite individuals consuming (but not killing) a single host individual.

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is necessarily limited by the tradeoffs evolving traits may impose on other components of fitness. For instance, a defensive trait that may reduce c for a prey species may be energetically costly, decreasing r (fecundity). Similarly, in parasite–host systems natural selection should act on traits of both species affecting the rate of transmission of parasites between hosts, the parasite's ability to draw resources from the host, and the host's resistance to the parasite.

Footprints of coevolutionary dynamics in predator–prey and parasite–host systems may be seen in two different patterns in nature: coevolutionary arms races and codiversification. A third pattern restricted to parasite–host coevolution is the mitigation of virulence. Each of these is discussed in the following sections.

Coevolutionary Arms Races

Alongside tightly specialized mutualisms, evolutionary arms races are probably the phenomena most commonly associated with the term coevolution. The concept of two species locked in a never-ending struggle to gain the evolutionary upper hand is a powerful one, and it fits well with the widely held notion of nature as 'red in tooth and claw.' This scenario, in which an evolutionary advantage gained in one species is met with a compensatory change in the other, is both straightforward and plausible. This sort of constant coevolutionary struggle has even been implicated as a major reason behind the advantage of sex. However, empirical evidence for arms races can be difficult to find in nature, given that our view of current ecological communities is often limited to only their present state, revealing only a single time point in a dynamic process. The best evidence for on-going arms races comes from predator–prey or parasite–host populations whose traits differ in a correlated manner over geographic space (Thompson, 2005). The geographic mosaic theory of coevolution predicts variation in the strength and specifics of the coevolutionary process among different local populations of interacting species. Thus, predator or parasite populations that are well matched to the traits of their local victim populations, and vice versa, provide strong evidence for on-going coevolutionary arms races.

Perhaps the best studied example of this is the classic case of rough-skinned newts and garter snakes in the western United States (Brodie, 2011). In the 1960s, otherwise harmless rough-skinned newts (*Taricha granulosa*) in Pacific Northwest were found to be highly toxic. The newts produce a compound called tetrodotoxin, which binds to and blocks sodium channels in neurons. The level of toxicity produced by these animals was surprising; with individual newts weighing only a few grams producing doses capable of killing 25 000 mice. Moreover, at the time there were few known predators of these newts, and in particular no large vertebrate predators that would require such a formidable defense. Further ecological surveys of the newts identified a reason for the toxicity: high levels of predation by the common garter snake (*Thamnophis sirtalis*), which is resistant to the newt's toxins. Decades of work in this system, led by Edmund Brodie Jr. and Edmund Brodie III, has succeeded in painting a clear picture of the coevolutionary arms race between newts and garter snakes.

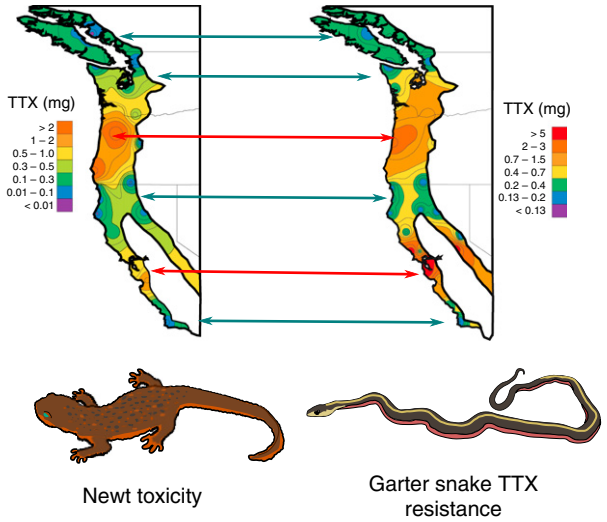

Figure 2 Depiction of the geographic mosaic in the newt/garter snake coevolutionary arms race. The map on the left shows how newt toxicity (amount of TTX produced) varies over space. The map on the right shows the geographic variation in snake resistance (dose of TTX needed to hinder snake performance). The broad correspondence of geographic variation in the traits of these two species are highlighted with the red (high toxicity and resistance) and blue-green (low toxicity and resistance) arrows. Adapted from Hanifin, C.T., Brodie Jr., E.D., Brodie III, E.D., 2008. Phenotypic mismatches reveal escape from arms-race coevolution. PLoS Biology 6, 0471–0482. Originally published under the Creative Commons Attribution license.

Considerable variation exists in both newt toxicity and snake resistance in different geographic locations. Congruent with the geographic mosaic model of coevolution, there is also strong geographic correlation in trait values of newt and snake populations, with more toxic newts generally found within areas with more resistant snakes (**Figure 2**; Brodie *et al.*, 2002; Hanifin *et al.*, 2008). The molecular basis for both newt toxin production and snake resistance is also now well understood. Newts increase production of tetrodotoxin, while resistant garter snakes possess mutations which change the conformation of sodium channel proteins, which decreases the binding affinity between the toxin and the channels (Geffeney *et al.*, 2002). Experiments have also demonstrated a clear fitness tradeoff in resistance for the snakes. The sodium channel mutations that reduce the toxicity of tetrodotoxin on the snakes also have a negative effect on snake locomotor capacity. Resistant snakes are slower and clumsier than nonresistant snakes (Brodie and Brodie, 1999). This helps explain why the highly resistant alleles have not spread through all the snake populations; the locomotor costs are not worth the benefit when the local newts are less poisonous. This classic garter snake/newt system represents an elegant case study highlighting the potential power of coevolutionary arms races in predator–prey systems in driving trait evolution.

In addition to directional escalation of trait values, frequency dependent selection dynamics during coevolutionary arms races may lead to long-term maintenance of genetic diversity in populations. Selection should favor parasite phenotypes that are best suited to attacking the most common phenotype in the host population. As those parasite phenotypes

increase in number, the fitness of the most common host phenotype will decline, and fitness increases for previously rare host phenotypes that can avoid or fend off the now-common parasite phenotypes. The outcome of these dynamics may be to favor host populations that are heterogeneous with respect to defensive traits. In our own immune systems, the tremendous diversity of our MHC genes may partly be explained by our coevolutionary history with parasites.

Codiversification

Another potential outcome of coevolution in predator or parasite systems is codiversification. Instead of the back-and-forth struggle of trait evolution in an arms race, reciprocal selection pressures imposed by predators or parasites and their prey or hosts may lead to reciprocal diversification in both trophic levels. One method of reducing predation or parasitism pressure may be for a prey or host species to escape from their enemies by adopting new habitats. Divergent adaptation to novel habitats or niches is the basic premise underlying ecological speciation. The relatively enemy-free habitat of the derived population may not last long, however, as new enemies may move in to take advantage of the flourishing new resource. These new enemies may be derived from the previous enemy population, now undergoing divergent adaptation in lock-step with its old coevolutionary partner or an entirely new set of enemies may move in.

Just as selection may favor shifts of prey species into new habitats, selection may favor predators and parasites that can find new victims. If a prey species gains a strong upper hand in defense, there may not be sufficient genetic variation in the predator population for an adequate coevolutionary counter move. The resulting fitness landscape may favor predator populations improving their capture rate (parameter c in the model above) by exploiting different, more susceptible prey species. In systems involving a high degree of specialization, like parasitoid insects, these shifts may also trigger ecological speciation events. Lock-step codiversification is expected to produce matching phylogenies in the species of both trophic levels. However, more complex patterns of host shifting may result in discordant phylogenetic patterns that are nonetheless driven by coevolution. For example, even if a novel enemy species came from outside the ancestral system, evolutionary changes in one species are still having a direct effect on the fitness landscape of the other.

One prime example of codiversification in action is the case of the apple maggot fly, *Rhagoletis pomonella*, and the specialist parasitoid wasps that attack it. The apple maggot fly was originally a specialist attacking the fruit of hawthorn trees (*Crataegus* sp.) throughout much of North America. Domestic apples were introduced to North America from Eurasia in the seventeenth century. About 150 years after this introduction, a population of these flies shifted and began attacking apples instead of hawthorn fruit (Bush, 1969). Genetic and phenotypic evidence has confirmed that these two populations are now strongly reproductively isolated and are on the path to becoming separate species. The apple and hawthorn flies differ in their chemosensory response to host fruit volatiles and in their life history timing (coinciding with differences in the timing of fruit ripening of their respective hosts). Four parasitoid wasp species specialize in attacking the apple maggot fly, and predation by these wasps may have been a key factor in the selective advantage of the shift to apples. Parasitoid rates in the hawthorn race can be quite high (sometimes in excess of 50%). Apples are considerably larger than hawthorns, with much more room for fly larvae to hide from the ovipositors of wasps on the fruit surface. This enemy-free space is thought to be a major reason behind the shift to apples (Feder, 1995).

However, the apple-infesting populations of *R. pomonella* are not entirely free of parasitoid enemies. The incipient speciation of the apple flies has led to incipient speciation in the wasps as well. The most prevalent of the four parasitoids, *Diachasma alloeum*, shows strikingly similar patterns of ecological divergence and reproductive isolation (Forbes *et al.*, 2009). These wasps lay eggs into late instar fly larvae tunneling through the fruit. The wasps infesting different races of flies have differentiated in the same key ecological traits that separate the flies: response to fruit olfactory cues, the first crucial step in finding their larval quarry and life history timing specifically in synch with the life cycle of apple flies. Interestingly, genetic evidence suggests that this is not a simple case of lock-step codiversification. Rather, the proximate ancestor of the apple fly-infesting wasps appears to be *D. alloeum* populations specialized to attacking the blueberry maggot fly, *Rhagoletis mendax*. Thus, the coevolutionary consequences of divergent adaptation in one species are not restricted to the original interaction partner but can ripple further out into the ecological community.

Parasite Transmission and the Attenuation of Virulence

In many parasite–host systems, effective transmission of parasite offspring to new hosts is a critical component of parasite success. It is likely that tradeoffs between efficient use of host resources (conferring greater fitness costs to the host) and transmission among hosts are common. Parasite transmission may require close contact between host individuals. Parasites that grow and reproduce quickly by efficiently pillaging the host's body may render their hosts too ill to pass their progeny on to new hosts. An aggressive parasite could make a host too weak to move widely and interact with conspecifics, or could kill the host before any opportunity for transmission.

One solution to this dilemma is for parasites to alter their host's phenotypes specifically to maximize transmission. This has important consequences for human disease. Many of the symptoms induced by parasites directly impact the success of parasite transmission. It is likely no coincidence that the main symptoms of infections by waterborne parasites such as *Vibrio cholera* (the pathogen responsible for cholera) or *Giardia lamblia* involve severe digestive distress, a surefire way to quickly reinfest water used by hosts. Similarly, the violent behavioral symptoms of rabies infections maximize the transmission of the rabies virus through saliva.

Instead of entirely hijacking host phenotypes, some parasites may increase transmission success by minimizing the costs imposed on their hosts. Healthier hosts may live longer,

move further, and interact with more conspecifics, ultimately leading to greater transmission and dispersal for the parasite. Thus, in situations where transmission is limiting, natural selection may favor reduced virulence in the parasite populations. A famous example of this phenomenon occurred during an attempt at biocontrol of invasive rabbits in Australia (Fenner, 1983). European rabbits (*Oryctolagus cuniculus*) were introduced to Australia during the mid-nineteenth century. The combination of their rapid reproduction rate and a relatively depauperate predator community in Australia led to an explosion of the rabbit population, causing widespread environmental and economic damage. A solution to the rabbit invasion appeared when an extremely virulent Myxoma virus was discovered in a population of captive rabbits in South America. The virus was intentionally introduced to the Australian rabbit population in 1950. The plan appeared to be a great success in the first couple of years. The virus was nearly always lethal, and the rabbit population declined by more than 75%. However, as the density of the rabbit population plummeted, transmission became an important selective force for the virus. Aggressively virulent strains of the virus caused rabbits (and the viruses they harbored) to die before they were likely to come into contact with other rabbits, while less virulent strains were more likely to be transmitted and survive. By 1952, less virulent strains of the virus began to proliferate and the rabbit population rebounded. The coevolutionary combination of attenuated virulence by the virus and genetic resistance to infection by the rabbits has led to the long-term persistence of Myxoma-infected rabbit populations in Australia.

The above example illustrated how parasitic interactions may evolve to become less costly to host species. The difference between parasitism, commensalism, and mutualism is determined by the net result of the costs and benefits to the symbiont partners. Thus, a sufficient decrease in virulence could tip the entire balance of the interaction into a commensal or even mutualistic relationship. Such shifts in the net result of species interactions may occur in both directions. In a mutualism, if one species starts to cheat by failing to provide a benefit to its partner, the interaction may immediately become parasitic in nature. Similarly, if selection on transmission success by parasites leads to minimizing fitness costs for hosts, it is not difficult to imagine a scenario where the net fitness costs to the host are minimized by the parasite providing some offsetting benefit, which could tip the balance into mutualist territory. A striking example of this phenomenon occurred serendipitously in the laboratory of Dr. Kwang Jeon of the University of Tennessee (**Figure 3**; Jeon, 1972). Jeon was conducting cyto-nuclear transplant experiments with lab lines of the amoeba *Amoeba discoides*. In 1966, some of the amoeba cultures became infected with very high numbers of an intracellular bacterial parasite. The bacteria appeared to be highly virulent; most newly infected cells died. Those that survived showed markedly slower generation time, smaller size, and greater susceptibility to starvation and mechanical stress. Because of the amount of work that had gone into this project, the amoeba lines were kept alive and nursed along for 5 years. During that time, the infection persisted, but the negative effects completely disappeared. But this was not a straightforward case of attenuation of virulence. In that 5-year span, Jeon's amoebae not only became resistant to the effects of the bacteria but they also became dependent on them. Nuclei from the now resistant infected lines performed very poorly when transferred to cells from uninfected lines. These infected-into-uninfected transfers resulted in a greater than 10-fold reduction in survivorship compared to transfers within infected or uninfected cells. Similarly, the bacteria could not be cultured outside of the amoebae, indicating that like many endoparasites, they were obligatorily dependent on their hosts. Thus, in only 5 years ($\sim$1000 amoeba generations) a clearly parasitic interaction evolved toward a nearly obligatory mutualism.

Coevolution and Ecological Dynamics

Coevolutionary responses have important implications for community dynamics in predator–prey and parasite–host systems. Population dynamics and evolutionary change act at the same time scale: generational time. It was once common for biologists to make a clear distinction between biological processes operating in 'ecological time' or 'evolutionary time.' However, this old dichotomy is neither correct nor useful. Both population demographics and evolutionary change constantly inform and affect one another. The fitness effects of particular alleles depend on the specific ecological and demographic contexts of the population. Likewise, shifts in allele frequencies between generations can alter the outcome of ecological interactions. In the simple Lotka–Volterra model presented above, selective deaths of both predator and prey species may instantaneously change the trait values that control the capture rate parameter (c). Explicity considering this additional dimension of change in models of species interactions demonstrates that on-going coevolution may strongly affect the overall dynamics of predator–prey systems (Abrams, 2001).

Interestingly, coevolutionary responses have the potential to stabilize otherwise unstable systems or destabilize otherwise stable systems (Abrams, 2001). One of the important determinants of this appears to be whether the traits involved in prey defense are limited in the potential directionality of their evolutionary response (Abrams and Matsuda, 1997; Gavrilets, 1997). That is, are prey traits likely to evolve in a single direction or can advantageous mismatches be achieved by moving bidirectionally along the trait axis? Unidirectional defensive trait evolution tends to make systems more stable, while bidirectional defensive trait evolution tends to induce cycles in previously stable systems or increase the amplitude of existing cycles.

Coevolution may alter population dynamics, but population dynamics may also influence the outcome of coevolution. Whether arms races continue to escalate may be determined by the particular mathematical relationship between predator and prey populations. For instance, the functional response of predators to greater prey population densities is a key consideration (Abrams, 1986). Stronger prey defense may result in a lower capture rate (c) for predators, but the resulting higher prey densities may compensate for this change. This suggests that selection imposed by prey defensive traits on predator traits may generally be weaker than the

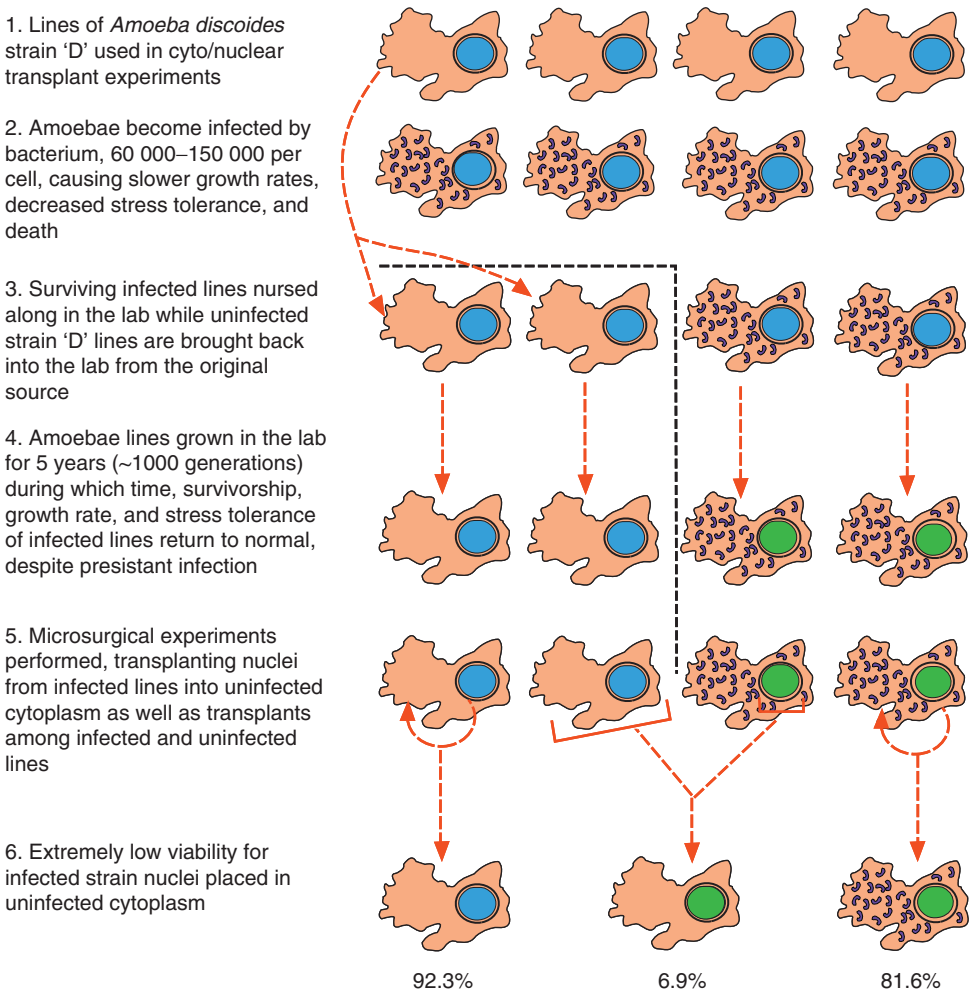

Figure 3 Schematic of coevolutionary shift from parasitism to mutualism between *Amoeba discoides* and a bacterial endosymbiont in the lab of Dr. Kwang Jeon at the University of Tennessee in the late 1960s. Diagram based on description of events, methods, and results by Jeon, K., 1972. Development of cellular dependence on infective organisms: Micurgical studies in amoebas. Science 176, 1122–1123.

reverse, with stronger predator attack traits resulting in both higher per capita capture rates and higher predator densities. This reciprocal feedback between coevolution and community demographic dynamics in predation and parasitism systems calls attention to the continued need for greater integration between evolutionary biology and ecology.

Macroevolutionary Patterns

The examples above demonstrate the various ways that coevolution affects predator–prey and parasite–host systems. While they may be difficult to study empirically, the fingerprints of coevolution are ubiquitous in ecological communities. But what effect has coevolution between predators and prey and parasites and hosts had on broad scale patterns of biodiversity over geological time? Do we see a clear signature of persistent arms races or codiversification in the fossil record? The answer may be that coevolution does indeed drive macroevolutionary patterns, but perhaps in a diffuse and limited fashion. It is nearly impossible to study pairwise specialist interactions in the fossil record; organisms rarely leave behind precise records of who they ate and who ate them. However, paleontologists have been able to take the approach of studying patterns within ecological guilds that were likely to be broadly interacting.

Paleontologist Robert Bakker analyzed coevolutionary trends in morphology between cursorial (chasing) mammalian predators and their primary prey, ungulates, across the entire history of their shared existence from the beginning of the Cenozoic period until the present day (Bakker, 1983). This is a case where the primary trait affecting the parameter c is basically the same for both predator and prey: speed. With few exceptions, ungulate species rely on speed during the early stages of a chase to escape predators, and it is this fleetness of foot of both predator and prey that determines the outcome of most hunts. The basic expectation of coevolution between these guilds would be increased running speed through time. Directional change in skeletal morphology for both ungulates and predators indicates that both groups have become

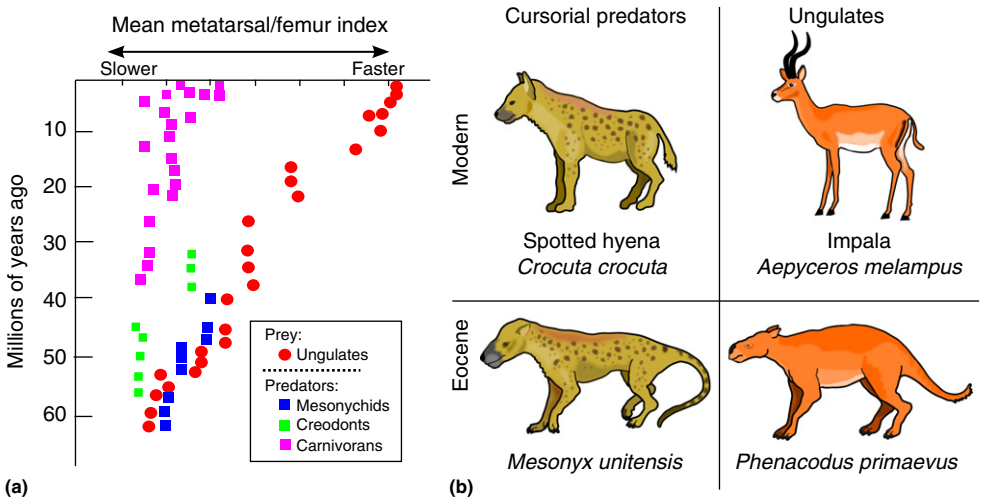

Figure 4 (a) Plot of inferred running speed (based on limb ratios) over time for ungulates (red circles), and three lineages of cursorial mammalian carnivores, mesonychids (blue squares), creodonts (green squares), and carnivorans (pink squares). These data highlight the continuous directional evolution of running speed in hoofed mammals, but not their predators, at the macroevolutionary scale. Based on data presented in Bakker, R.T., 1983. The deer flees, the wolf pursues: Incongruencies in predator–prey coevolution. In: Futuyma, D.J., Slatkin, M. (Eds.), Coevolution. Sunderland, MA: Sinauer. (b) Example cursorial predators (left) and ungulates (right) from two time along the graph, the Holocene (modern times) (top) and the late Eocene (~50 million years ago). These examples highlight the greater change in limb morphology for hoofed herbivores vs. running predators over the past 50 million years.

increasingly swifter. Several traits associated with greater speed including less acute limb angles, more constrained movement in ball joints, the reduction of side digits, and shorter phalanges show clear progressions in both groups. However, the pace of speed-related skeletal adaptation in ungulates far outpaces that of predators. Ungulates have consistently been getting faster, while cursorial predators have been unable to keep up (pun intended) (Figure 4). Bakker argues that basic trophic structure may be responsible for the widening gap in this arms race. The necessarily greater population size of the prey species makes them less prone to extinction and more likely to stumble upon evolutionary innovations for speed. Alternatively, this could be a case of stronger tradeoffs for the predator species; many carnivores use their hands and claws for digging, fighting, or manipulating prey, and such diverse functional requirements may prevent carnivores from evolving super-specialized running tools. In either case, the fossil record suggests that predator–prey coevolution can indeed drive important long-term evolutionary trends but that there are likely considerable limits to perpetual escalation in arms races.

See also: Commensalism, Amensalism, and Synnecrosis. Ecological Speciation and Its Consequences Geographic Mosaic of Coevolution. Intraspecific Coevolutionary Arms Races. Secondary Metabolites, the Role in Plant Diversification of

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Protein Biophysics and Evolution

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Glossary

Aggregation Proteins are said to 'aggregate' if they specifically or nonspecifically stick to other proteins and form large protein complexes without biological function.
Complex A macromolecular assembly of two or more proteins. When multiple proteins bind to each other, they are said to form a 'complex.'
Conformation The specific, three-dimensional arrangement of all atoms in a protein.
Enzyme A protein that catalyzes a chemical reaction.
Epistasis Interaction between two or more mutations in a protein, such that the fitness effect of one mutation is altered by the presence or absence of the other mutations. (Note that this definition differs from the standard genetical definition which considers functional or fitness interactions among loci rather than individual sites in a protein.)
Folded state A protein is said to be in its 'folded state' when it is folded into its native conformation.
Hydrophobic The biophysical property of molecules to segregate themselves from water. Hydrophobic amino acids tend to be found in the core of proteins, where they are protected from water. When hydrophobic amino acids are exposed on the protein surface, such proteins tend to 'aggregate.'
Misfolding Proteins are said to be 'misfolded' when they have taken on a conformation other than their native conformation. A misfolded protein is similar to an unfolded protein; the main difference is that the term 'misfolded' specifically carries the connotation of a negative effect. Misfolded proteins are expected to have deleterious effects on organism fitness, while unfolded proteins may or may not have such effects.
Native conformation The lowest energy conformation of a protein, also often referred to as 'ground state.' Most proteins carry out their biological function when they are folded into their native conformation.
Solvent accessibility The extent to which an amino acid in a protein is exposed to solvent (i.e., water). Amino acids on the surface of a protein tend to have high solvent accessibility, and amino acids in the core tend to have low solvent accessibility.
Stability The extent to which a protein can be found in its native conformation. The more stable a protein is, the more likely it is to assume and remain in its native conformation. Formally, stability is the difference in free energy between the native conformation and the ensemble of all unfolded states.
Unfolded state The opposite of folded state. A protein is said to be in its 'unfolded state' when it is not folded into its native conformation.

Introduction

Proteins are among the most fundamental components of living organisms. Proteins can act both as structural building blocks, for example, to build tissues such as hair, skin, nail, muscle, connective tissue, or bones, and as functional building blocks, where they serve as biochemical machines that carry out specific functions, such as digestion, sensing (e.g., of light, taste, or smell), synthesis or degradation of metabolites, and many others. The evolution of protein-coding genes is shaped by the biophysical and biochemical effects mutations have on the expressed proteins. In particular, mutations that negatively affect a protein's ability to carry out its native function will, in general, be deleterious and selected against. By contrast, mutations that have no measurable effect on the structure and function of a protein will generally be neutral. Such mutations will accumulate in genomes in proportion to the rate at which they are generated. Finally, mutations that alter a protein's function can occasionally be beneficial to the organism. An example could be a viral protein that acquires a mutation that reduces the virus's susceptibility to an antiviral drug. Such beneficial mutations can often accumulate quite rapidly, in particular in organisms that experience high mutation rates.

In the next section, we will describe the main mechanisms shaping protein biophysics. The subsequent section connects these mechanisms to the effects of individual mutations. The final section elaborates on how these mechanisms may interact to shape sequence evolution. For a more in-depth treatment of these and related topics, see e.g., Liberles *et al.* (2012), Sikosek and Chan (2014).

Biophysical Mechanisms

There are three broad categories of mechanisms that connect protein biophysics to organism fitness: First, proteins need to fold stably into their native structure. Second, proteins need to carry out their specific function. Third, proteins must not be toxic, i.e., they must not harm the organism expressing them.

Protein Folding and Protein Stability

Protein folding and protein stability are governed by basic thermodynamics. A protein is a polymer (a long molecule composed of many repeated subunits) that can take on many different conformations. It is useful to think of a protein as a

string of beads, where the beads represent the individual amino-acid residues. There are myriad ways in which such a string of beads can be arranged in space, and we refer to each individual arrangement as a 'conformation.' In the case of proteins, the beads (residues) have different chemical properties: some attract each other, some repel each other, and some try to avoid water (are hydrophobic). As a result, different conformations have different energies. For example, if two residues repel each other, then conformations in which the residues are in physical contact will tend to have higher energies. In contrast, if two residues attract each other, then conformations in which the residues are in physical contact will tend to have lower energies. Note, however, that because the residues are connected in a fixed polymer chain, there may not be a conformation that forms every favorable contact and avoids every unfavorable one – however, there will always be some conformations that have more favorable and less unfavorable contacts (and so lower energies) than others.

Given the simple energetic picture of a protein as a string of beads, one might think that proteins simply collapse into the conformation that minimizes their energy and then stay there. However, things are not quite that simple, because proteins generally exist in environments that have a temperature greater than absolute zero, meaning that the atoms and residues in a protein are always in motion. As a consequence, a protein doesn't just remain fixed in one low-energy conformation; instead, over time it takes on many different conformations.

Thermodynamics allow us to calculate the likelihood that a protein will be found in a given conformation. Assume we could enumerate all possible conformations a protein can assume and wanted to know the probability that we will find the protein in conformation i. At equilibrium, that probability is given by the Boltzmann distribution

$$P_i = e^{-E_i/kT}/Z$$

where E_i is the energy of the conformation i, T is the temperature, k is the Boltzmann constant (a numerical constant converting temperature into energy), and Z is the partition function

$$Z = \sum_j e^{-E_j/kT}$$

As the equation for P_i clearly shows, the lower (more negative) the energy of a conformation the more likely it is that the protein will assume that conformation. However, this effect is counteracted by temperature. The higher the temperature, the more often we will find a protein in a higher-energy conformation.

In practice, enumerating all possible conformations that a protein can assume is not very useful. First, there are a vast number of possible conformations. Second, many of these conformations are quite similar to each other, and therefore it is preferable to group them into more broadly defined conformational categories. Third, many of the theoretically possible conformations are highly unlikely to ever occur due to their high energy, and therefore can be disregarded. In fact, in practice we are often only interested in two broad classes of conformations. The first, which we usually call the 'native conformation' or 'folded state,' corresponds to the set of (usually very similar) conformations the protein will typically be found in under physiologic conditions in an organism. In general, a protein has to be in its folded state to carry out its biological function. The second, which we usually call the 'unfolded' or 'misfolded' state, comprises all other possible conformations.

It turns out that the Boltzmann formula applies to these broad categories of conformations, as long as we replace the energy E in the formula with the Gibbs free energy G. Thus, we can write

$$\frac{P_{\text{folded}}}{P_{\text{unfolded}}} = \frac{e^{-G_{\text{folded}}/kT}}{e^{-G_{\text{unfolded}}/kT}} = e^{-\Delta G/kT}$$

where $\Delta G = G_{\text{folded}} - G_{\text{unfolded}}$ is the difference in free energy between the folded and the unfolded state. ΔG is commonly referred to simply as the 'stability' of the protein. The lower (more negative) the ΔG, the more likely the protein is going to be found in its native conformation. In fact, since we defined the unfolded state as all possible conformations that do not belong to the folded state, we have $P_{\text{folded}} = 1 - P_{\text{unfolded}}$, and hence we find that

$$P_{\text{folded}} = \frac{1}{1 + e^{\Delta G/kT}}$$

This simple formula shows us that a protein with $\Delta G = 0$ has a probability of being folded of 0.5, i.e., it can be found in its native conformation exactly half of the time. More negative ΔG values imply the protein is more likely to be folded than unfolded, and positive ΔG values imply the protein is more likely to be unfolded than folded (see also **Figure 1**).

Typical ΔG values for functional proteins tend to be on the order of -5 to -15 kcal mol^{-1}. These values tell us that at room temperature ($T \approx 294$ K), proteins are typically unfolded less than 0.02% of the time, and the more stable proteins are

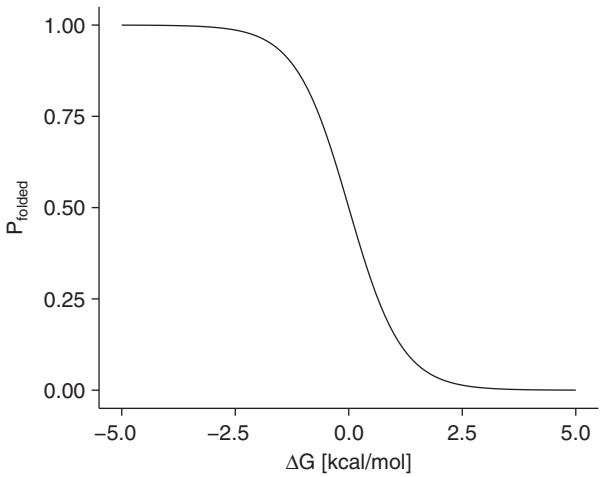

Figure 1 Probability of finding a protein in its native conformation at room temperature ($T = 294$ K), as a function of the protein's stability ΔG. At typical stabilities of $\Delta G = -5$ to -15 kcal mol^{-1}, proteins are virtually always properly folded. However, as ΔG increases, the proportion of time that a protein spends in its native conformation declines rapidly, reaching 50% at $\Delta G = 0$ kcal mol^{-1} and declining to nearly 0% around $\Delta G = 2.5$ kcal mol^{-1}. This figure assumes the protein conformations are at equilibrium.

virtually never unfolded. (The Boltzmann constant is $k = 0.001\ 987$ kcal mol^{-1} K^{-1}.)

Protein Function: Catalytic Sites, Protein–Protein Interactions, Ligand Binding

In addition to folding into a sufficiently stable native conformation, proteins often perform specific biochemical functions such as catalyzing chemical reactions, engaging in protein–protein interactions, or binding to ligands.

Catalysis usually requires two ingredients: (1) The protein's native conformation needs to form an active site with a size and shape suitable to accommodate the target substrate. (2) In the active site, there need to be a few key amino-acid side chains that catalyze the actual enzymatic reaction. Often a protein containing hundreds of residues may have only a handful of amino acids that are directly involved in catalysis.

Protein–protein interactions and/or ligand binding generally require suitable patches on the surface of the 3D structure where the protein partner or ligand can bind. There is no simple rule to what these patches may look like; their properties are largely determined by the biophysical properties of the binding partner.

Toxic Functions and Protein Misfolding

One might think that the worst that can happen to a protein, in terms of organism fitness, is the loss of its function. However, sometimes proteins do worse than just losing their function. Sometimes they actually become toxic, and exert deleterious effects that exceed those expected from the simple waste of resources on a nonfunctional protein.

The most common form of protein toxicity arises through misfolding. Misfolded proteins frequently expose hydrophobic residues on their surface, and these residues can bind to other proteins. As a result, the misfolded proteins aggregate, and these aggregates interfere with proper functioning of the cellular machinery. In humans, many diseases, in particular neurodegenerative diseases such as Alzheimer's and Huntington's, are associated with aggregated misfolded proteins. However, it seems that this is not just an issue for humans. In fact, evidence of selection against protein misfolding can be found throughout the tree of life (Drummond and Wilke, 2009, 2008).

Biophysical Effects of Mutations

The previous section has introduced several of the key mechanisms needed to understand protein biophysics. We now discuss how these mechanisms relate to mutations.

Mutations and Stability Changes

The stability ΔG of a protein is a property of the protein's specific amino-acid sequence. If we make a mutation somewhere in the protein, then ΔG will change. For this reason, we frequently are interested in the 'stability changes' associated with individual mutations. For example, assume the wild-type

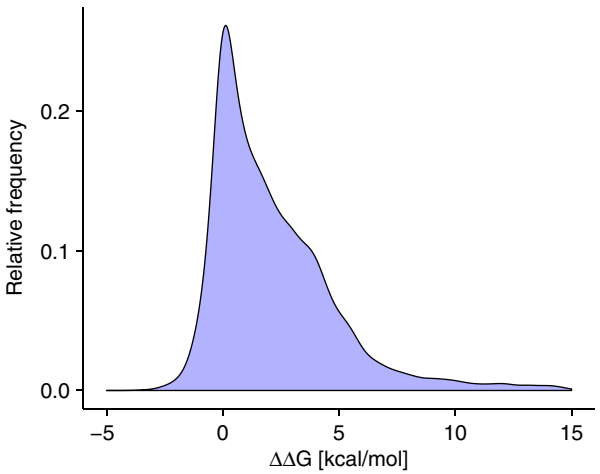

Figure 2 Predicted distribution of $\Delta\Delta G$ values for all single-point mutations in a protein. We see several characteristic features: (1) The majority of the mutations are destabilizing (have $\Delta\Delta G > 0$). (2) The mode of the distribution is near zero, so that a substantial fraction of mutations have only minor effects on stability. (3) Some mutations have destabilizing effects of 5 kcal mol^{-1} or more. Such mutations can, individually, cause a protein to entirely lose the ability to remain stably folded (see **Figure 1** for reference). The data presented are for the protein methionyl tRNA synthetase (PDB ID: 1a8h), as calculated by Echave *et al.* (2015). The $\Delta\Delta G$ values were computationally predicted using the FoldX method (Guerois *et al.*, 2002).

stability of a protein is ΔG_{wt} and the stability of a mutant is ΔG_{mutant}. Then we can define the stability change

$$\Delta\Delta G = \Delta G_{wt} - \Delta G_{mutant}$$

If $\Delta\Delta G < 0$, then the mutation is 'stabilizing,' i.e., it increases the stability of the protein. If $\Delta\Delta G > 0$ on the other hand, then the mutation is 'destabilizing,' i.e., it decreases the stability of the protein. Typical $\Delta\Delta G$ values fall between -5 and $+15$ kcal mol^{-1}, but $\Delta\Delta G$s are more likely to be positive than negative (**Figure 2**). Thus, the majority of mutations reduce the stability of a protein, and many mutations can destabilize a protein to an extent that it loses its ability to fold to its native conformation.

Epistasis

When multiple mutations occur in a protein, they can interact with each other, a situation termed 'epistasis.' Concrete examples would be a mutation that is beneficial in the presence of another mutation and deleterious otherwise, or two deleterious mutations that jointly become beneficial to the organism (**Figure 3**).

Epistasis arises naturally in the context of protein stability. For example, consider a pair of mutations A and B where A alone destabilizes the protein and renders it nonfunctional, but in the presence of B the protein can tolerate mutation A. This situation can arise, for example, if the stability change associated with A, $\Delta\Delta G_A$, is of a magnitude comparable to or exceeding the ΔG_{wt} of the wild-type protein while the stability change associated with B, $\Delta\Delta G_B$, is negative. Thus, in the

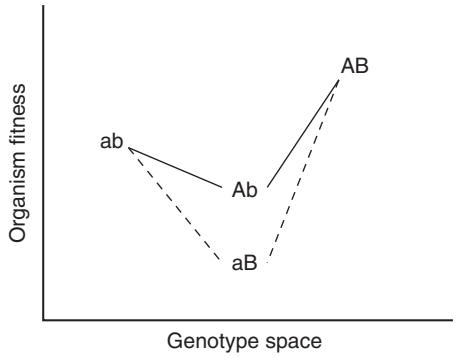

Figure 3 Illustration of interaction between two mutations (epistasis). An organism carrying the wild-type protein (labeled 'ab') has a given fitness. Two individual amino-acid substitutions both create protein variants with lower fitness (labeled 'Ab' and 'aB'). However, in combination, the two substitutions cause the organism to have higher fitness than it had with the wild-type protein. Adapted from Figure 2B of Covert, A.W., Lenski, R.E., Wilke, C.O., Ofria, C., 2013. Experiments on the role of deleterious mutations as stepping stones in adaptive evolution. Proceedings of the National Academy of Sciences of the United States of America 110, E3171–E3178. doi:10.1073/pnas.1313424110.

absence of B, the mutated protein will have stability $\Delta G_{\text{mutant A}=\Delta G\text{wt}} + \Delta\Delta G_A > 0$, but in the presence of B, the mutated protein will have stability $\Delta G_{\text{mutant A}+\text{B}} \approx \Delta G_{\text{wt}} + \Delta\Delta G_A + \Delta\Delta G_B < 0$. In the latter equation, we have used the approximately equals sign ($\approx$) because it is not necessarily the case that the stability changes of two mutations are perfectly additive. In principle, the occurrence of mutation A could alter the magnitude of $\Delta\Delta G_B$ and vice versa. However, in practice, it has been found that stability changes are frequently additive, or at least approximately additive, and substantial, useful insight into protein evolution can be obtained from models that assume $\Delta\Delta G$s are additive (Bloom *et al.*, 2005).

Epistasis can additionally arise through direct interactions between two amino-acid side chains in direct contact with each other in the protein structure. If one of the two amino acids is mutated, the second one may require a compensating mutation. For example, assume amino acid A is large and amino acid B is small. If A gets mutated to a small amino acid, then B may have to be mutated to a large amino acid so that the A–B pair continues to take up the same amount of space in the structure. In the absence of this compensating mutation, the mutation at A may disrupt the protein fold and hence be quite deleterious. These kinds of compensating interactions between contacting amino acids are so common that it is possible to infer 3D contacts in a protein structure from patterns of covariation between sites observed in evolving sequences (Jones *et al.*, 2012).

Protein Stability and Mutations that Generate Novel Function

There are a few general mechanisms of how a mutation may generate a novel function. First, a mutation to a catalytic amino acid in an enzyme may change the type of substrate that is generated by an enzyme-catalyzed reaction. Similarly, mutations in or near the active site may change the active site's shape or size and hence allow for different substrates to enter. Finally, a mutation in a surface patch involved in ligand or protein binding may change the specificity of that patch, and either bind a specific partner more or less tightly than previously, or alternatively may allow the patch to bind a new partner.

In all these cases, besides whatever effect a given mutation has on protein function, the mutation will also change protein stability. And since there is no particular reason why a functionally relevant mutation may have a specific stability effect $\Delta\Delta G$, we can assume that the mutations $\Delta\Delta G$ is simply chosen at random from the $\Delta\Delta G$ distribution. As Figure 2 shows, a randomly chosen $\Delta\Delta G$ will likely be destabilizing. Therefore, we can expect that most mutations that create novel function will also destabilize the protein. In line with this hypothesis, it has been observed that proteins are more likely to evolve novel function if they have previously experienced a stabilizing mutation (Bloom *et al.*, 2006; Gong *et al.*, 2013).

How Protein Biophysics Shapes Sequence Evolution

In this final section, we discuss some examples of larger-scale evolutionary trends that emerge from the biophysical mechanisms governing protein folding and function.

Purifying Selection in the Core of Proteins and in Protein–Protein Interfaces

Typical proteins are several hundred amino acids long. However, only a small fraction of these amino acids are directly involved in the function of the protein, by being catalytic residues or belonging to protein–protein interfaces. The other residues serve primarily as scaffolding, to place the key functional residues into the correct locations in space. Therefore, a majority of sites in a protein experience purifying selection; mutations at these sites are allowed as long as they do not disrupt the protein's stability or alter its native conformation. However, not all sites in a protein are equally important for the protein's stability or conformation. In particular, mutations at surface sites are on average less likely to disrupt the protein than mutations in the core of the protein (Franzosa and Xia, 2009). The simple explanation for this observation is that surface sites participate in fewer residue–residue interactions than core sites, and hence are less likely to be constrained in the types of amino acids they can tolerate.

Similarly, one finds that surface sites in protein–protein interfaces are more conserved than surface sites outside such interfaces. Interface sites experience additional purifying selection to maintain the protein–protein interaction (Franzosa and Xia, 2009). A mutation in a protein–protein interface is deleterious if it destabilizes its protein, if it destabilizes the protein–protein interaction, or both. By contrast, a mutation outside an interface region is deleterious only if it directly affects the stability or fold of its protein.

Positive Selection in Viral Immune Escape

Positive selection, i.e., selection for proteins with modified or novel function, can be observed most readily in viral evolution. Many viruses experience significant selection

pressure to escape their host's immune system, or to be able to cause infections despite vaccination or antiviral treatments.

As an example of immune escape, consider the case of influenza virus. The influenza virus causes annual, seasonal outbreaks in humans. People who successfully fight of an influenza infection acquire immunity to the influenza strain with which they were infected. Similarly, people who get vaccinated tend to be protected against the specific strains against which the vaccine was targeted. However, in both cases, immunity tends to wear off within a few years, and people become again susceptible to the circulating influenza strains. This waning of immunity occurs because every year, the virus accumulates a few additional mutations that make it look different to the human immune system.

Biophysically, this immune escape evolution is similar to the evolution of protein–protein interfaces, only that now selection prefers mutations that eliminate – rather than maintain – binding. The human immune system's primary defense against influenza infections are antibodies that bind proteins exposed on the viral surface, and in particular the protein hemagglutinin (the H in influenza strain classifications such as H1N1 or H3N2). If hemagglutinin experiences a mutation that makes it less of a target to antibodies currently present in the human population, then the virus carrying that mutation will have a selective advantage over viruses not carrying that mutation, and hence the mutation spreads through the viral population. In fact, the most variable regions in influenza hemagglutinin are the regions of the proteins at which antibodies commonly bind (Bush *et al.*, 1999).

See also: Ancestral Reconstruction: Theory and Practice. Codon Usage and Translational Selection. Compensatory Evolution. Gene Origin, Sex Chromosomes and. Molecular Evolution, Functional Synthesis of. Molecular Evolution, History of. Robustness and Evolvability in Molecular Evolution

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Protist Diversification

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Glossary

Algae Term of convenience for protists that are photosynthetic (or are very closely related to photosynthetic protists).

Amoeba (plural Amoebae) A cell that lacks flagella and produces some kind of pseudopodia, almost always used for locomotion, or feeding, or both.

Bacterivory The ability to feed on prokaryotic cells, almost invariably via phagotrophy.

BYA Billion Years Ago.

Complex plastids Plastids surrounded by more than two membranes, resulting from a higher-order endosymbiosis (e.g., secondary endosymbiosis or tertiary endosymbiosis).

Endosymbiosis Symbiosis where one of the partners (the symbiont) resides inside the cells of the other (the host). The origins of mitochondria and plastids involved a symbiosis of this kind. Also referred to as 'endocytobiosis.'

Flagellate A eukaryotic cell with flagella.

LECA Last eukaryotic common ancestor. The most recent-living population/species from which all extant (i.e., living) eukaryotes are descended.

Macroalgae Term of convenience for algae that are macroscopic and multicellular, or at least have a large, differentiated thallus, for example, 'seaweeds.' Contrast with 'microalgae.'

Microalgae Term of convenience for algae that are unicellular or colonial. Contrast with 'macroalgae.'

Mixotrophy As used here, the ability of an organism to perform both photosynthesis and heterotrophy, specifically via phagotrophy. Note that this term is often used slightly differently in the bacteriological and botanical literature, there referring to photosynthesis combined with heterotrophy of any kind.

MYA Million Years Ago.

Nucleomorph The highly reduced nucleus of the eukaryotic symbiont that gave rise to a complex plastid, still retained within the plastid (in the periplastid compartment). Only a few groups with complex plastids have retained nucleomorphs (cryptophytes and chlorarachniophytes, in particular), whereas the symbiont nucleus has been completely lost in most complex plastids.

Periplastid compartment Also known as 'periplastidial compartment,' or 'PPC.' In complex plastids, the space between the inner two membranes on one side, and outer 1–2 membranes on the other. Represents the cytoplasmic compartment of the eukaryotic symbiont that gave rise to the plastid. Contains the nucleomorph, as well as eukaryotic-type ribosomes, in nucleomorph-bearing plastids.

Phagotrophy The ability of a cell to feed by ingesting large particles, usually other cells.

Plastid General term for the photosynthetic organelles of eukaryotes (e.g., the chloroplasts of green algae) and related non-photosynthetic organelles (e.g., the apicoplasts of many apicomplexan parasites).

Primary alga A protist with a plastid that descends directly from an event of primary endosymbiosis, in other words, with a plastid that is directly descended from a photosynthetic bacterium.

Primary endosymbiosis Endosymbiosis involving a prokaryotic symbiont, rather than eukaryotic symbiont. Used with reference to the origins of organelles of endosymbiotic origin, especially plastids.

Protists Eukaryotes that are not animals (Metazoa), land plants, or true fungi.

Protozoa Term of convenience for protists that are heterotrophic, but not fungus-like.

Secondary alga A protist with a (photosynthetic) plastid that directly descends from an event of secondary endosymbiosis, in other words, with a plastid that is most immediately descended from a primary alga, rather than from a photosynthetic bacterium.

Secondary endosymbiosis A term used with reference to the origins of 'complex' plastids. Refers to an endosymbiosis where the symbiont was a primary alga (i.e., had a plastid because of descent from an event of primary endosymbiosis).

Tertiary endosymbiosis A term used with reference to the origins of 'complex' plastids. Refers to an endosymbiosis where the symbiont was a secondary alga (i.e., had a plastid because of descent from an event of secondary endosymbiosis).

What are Protists? Evolutionary and Ecological Importance

Eukaryotic organisms are distinguished from prokaryotes by numerous of cellular and genomic traits, including the possession of distinct nuclei containing a genome that is distributed across multiple linear chromosomes, many-armed endomembrane systems, organelles that arose through endosymbiosis (e.g., mitochondria and, frequently, plastids), and more complex cytoskeletal systems. Eukaryotes are also remarkable because they include the large many-celled organisms that seem, at first glance, to dominate the Earth – principally animals (Metazoa), land plants, and fungi. However, animals, plants, and fungi are actually only three highly specialized and unusual lineages amongst the very broad diversity of eukaryotic life forms. The other eukaryotic organisms are collectively called protists. These are mostly unicellular and microscopic, with some important and spectacular exceptions.

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The existence of protists is well known in the abstract, but their tremendous significance for understanding the evolution and biodiversity of eukaryotes is routinely underappreciated. They are also of great ecological importance and take on a huge variety of roles in natural systems.

According to the most widely used definition, protists are simply those eukaryotes that are not true animals, land plants, or true fungi. 'Protists' does not represent a phylogenetically cohesive grouping of closely related organisms, neither do protists share any biological features other than those that are common to all eukaryotes. Most subgroups of protists are loosely categorized as either algae or protozoa. Algae are 'plant-like' in that they are photosynthetic (or are closely related to photosynthetic forms). They range from small unicellular forms, many of which are motile, through to multicellular macroalgae that can rival large land plants in size and complexity. Unicellular and colonial algae are responsible for a large proportion of aquatic primary production, while macroalgae of various kinds are dominant components of ecosystems of regional significance, especially in coastal waters (examples include kelp forests and the algal components of tropical reefs). Protozoa are heterotrophic, and most are 'animal-like' in that they ingest solid food particles, using phagotrophy. The great majority of protozoa are unicellular or exist as simple many-celled forms (e.g., colonies of similar cells), and motility is a common trait. Many protozoa are free-living, and most of these prey on prokaryotes ('bacterivory') or other microbial eukaryotes. A substantial proportion of protozoa are instead parasites of animals, plants, or other protists. Diseases of humans caused by protozoa include malaria, amoebic dysentery, sleeping sickness, Chagas' disease, leishmaniasis, giardiasis ('beaver fever'), toxoplasmosis, and the sexually transmitted infection trichomoniasis.

Both algae and protozoa are rough terms of convenience that do not refer either to phylogenetically coherent groups, or to clear-cut ecological types. For example, many unicellular photosynthetic algae are 'mixotrophs' that will prey upon other microorganisms (McKie-Krisberg and Sanders, 2014; Sanders, 1991; Stoecker, 1999). Predation upon prokaryotes by mixotrophic algae can actually exceed bacterivory by heterotrophic protozoa in some systems (see Unrein *et al.*, 2007). There are also several heterotrophic protist groups that are unrelated to true fungi, but nonetheless resemble fungi in some aspect of their life cycle, for example, in existing primarily as a mycelium of hyphae and/or by producing a fruiting body as a reproductive or dispersal structure (examples include oomycetes and slime molds – see below). Such groups are often not considered 'protozoa,' and historically at least, have typically been studied by mycologists (see, e.g., Stephenson, 2011).

The Origin of Eukaryotes – A Brief Overview

It is more-or-less universally assumed that the first members of the eukaryote lineage were unicellular and that the last common ancestor of living eukaryotes (last eukaryotic common ancestor, or 'LECA') was also a single-celled organism, as are many of the eukaryotic lineages alive today (see below). In other words, it is clear that questions of how eukaryote cells evolved and diversified are, to a large extent, questions about protists, and that protists are a crucial source of the information required to answer them.

Ancient History of Eukaryotes

Most researchers who study eukaryote evolution (though not all) assume that eukaryotic cells evolved from prokaryotic ancestors. The closest relatives of eukaryotes among the prokaryotes are the Archaea. Recent sophisticated phylogenetic analyses support the hypothesis that eukaryotes actually evolved from within the diversity of living archaea (i.e., that archaea are paraphyletic), and this is further supported by the discovery of various mixtures of a few 'eukaryotic' genes in the genomes of the archaeal groups inferred to be most closely related to eukaryotes (Williams *et al.*, 2012, 2013; Spang *et al.*, 2015). This inference is depicted in cartoon form in **Figure 1(a)**. Nonetheless, irrespective of the exact relationship between the Archaea and eukaryotes, there is still a huge gulf in cellular organization between Archaea (or at least those that have been well studied to date – see Williams and Embley, 2015) and living protists. Comparative cell structure and genome analyses across eukaryotes, especially protists, has demonstrated that this distinction extends back to the common ancestors of living eukaryotes. In other words, LECA was already a eukaryotic cell of essentially 'fully developed' complexity (Koumandou *et al.*, 2013). The attempt to understand the actual evolution of the 'full eukaryotic cell' prior to LECA is an extremely vibrant, if contentious, discipline, but mostly beyond the scope of this article (though see comments on the mitochondrion, below). This topic is covered in detail in at least two excellent recent collections of review articles (Keeling and Koonin, 2014; Williams and Embley, 2015).

From the current understanding of the phylogenetic tree of living eukaryotes (see below), it is routinely inferred that LECA was a heterotrophic protozoan, most probably a single-celled flagellate (i.e., with eukaryotic flagella). Examinations of the cytoskeletons of living eukaryotes, combined with current phylogenetic estimates, suggest that LECA probably had two dissimilar flagella, and a complex microtubular cytoskeleton that defined the cell shape, including supporting a specialized region of the cell adapted for feeding by phagocytosis (Cavalier-Smith, 2013; Heiss *et al.*, 2011, 2013; Yubuki and Leander, 2013).

There are tremendous uncertainties concerning the timeline of eukaryote diversification. The fossil record of eukaryotes beyond ~750 MYA remains extremely sparse and limited in detail. The earliest fossils considered to potentially represent eukaryotic organisms reach back to more than 2 BYA (and possibly more than 3 BYA), but are identified on very limited criteria, primarily that their size and/or complexity is more characteristic of living eukaryotes than of living prokaryotes (Butterfield, 2015; Javaux *et al.*, 2010). The probable eukaryotes most solidly identified by these criteria date to ~1.6 BYA and younger, while fossils that can be assigned with high confidence to particular living eukaryote groups are all 1.2 BYA and younger (Butterfield, 2000, 2015; Butterfield *et al.*, 1990; Javaux *et al.*, 2004; Pang *et al.*, 2013; Porter and Knoll, 2000). Molecular dating approaches (based on multigene datasets assembled from living eukaryotes) that are calibrated using this latter part of the fossil record have also been

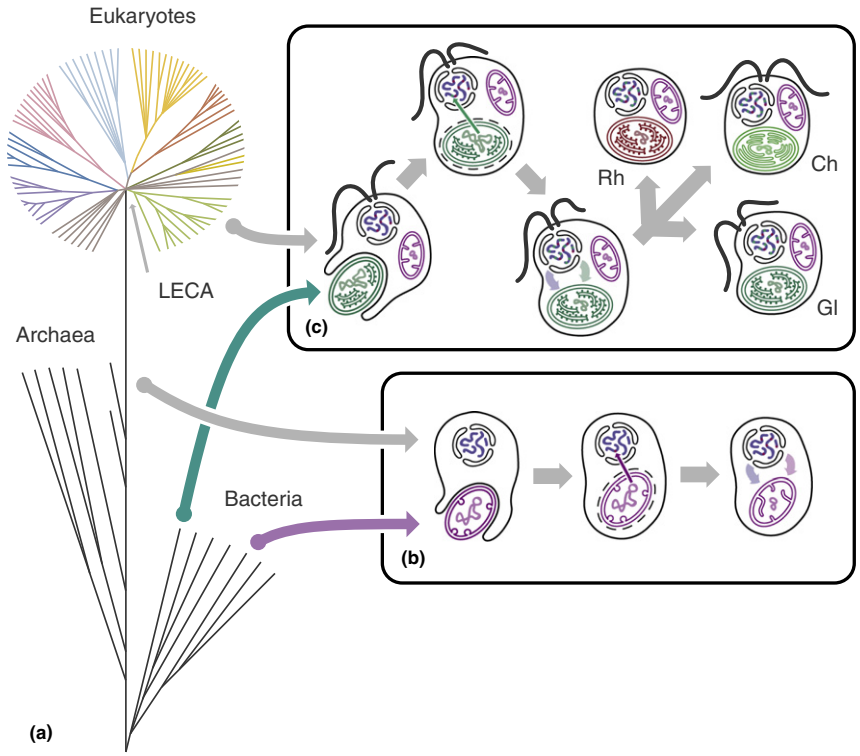

Figure 1 Early history of eukaryotic cells. (a) Simplified view of the Tree of Life, indicating the likely relationship between eukaryotes and a paraphyletic archaea group, and contextualizing the events of (primary) endosymbiosis leading to mitochondria and plastids in eukaryotic cells, respectively, before and after the last eukaryotic common ancestor (LECA). The colored branches within the eukaryote subtree represent major groupings, detailed in **Figure 3**. (b) Mitochondrial symbiosis between a member of the eukaryote lineage, and an alphaproteobacterium. Note that there is debate as to whether the nucleus was present in the eukaryote-lineage host prior to the mitochondrial symbiosis, and also whether the alphaproteobacterial symbiont was acquired by phagocytosis (see text); both are shown here for simplicity. The host vacuolar membrane surrounding the symbiont (if ever present) was lost, while both the cell membrane and outer membrane of the bacterium were retained. The evolutionary transformation of the bacterial symbiont into an organelle involved genome reduction, including endosymbiotic gene transfer to the nuclear/pre-nuclear genome (narrow purple arrow), and also the targeting to the mitochondrion of nucleus-encoded proteins, including some originally of endosymbiont origin (broad purple arrow) but also others originating from the nuclear lineage or elsewhere (broad blue arrow). Implied homology between alphaproteobacterial cell membrane invaginations and mitochondrial cristae follows Muñoz-Gómez *et al.* (2015). (c) Primary plastid endosymbiosis between a eukaryote ancestral to Archaeplastida and a cyanobacterium engulfed via phagocytosis. Early in the symbiosis, the host vacuolar membrane was lost, but both cyanobacterial membranes were retained. As with mitochondria, the evolutionary transformation of the symbiont into an organelle included genome reduction, endosymbiotic gene transfer to the nucleus (narrow green arrow), and the targeting to the plastid of nucleus-encoded proteins of cyanobacterial origin, and other origins (broad green and blue arrows). This primary plastid was inherited directly by the three archaeplastid groups: Rhodophyta (Rh), Chloroplastida (Ch), and Glaucophyta (Gl).

used to infer the ages of early eukaryotes. Such analyses show a lot of method-dependence and uncertainty, but have generally estimated ages for LECA between 1 and 2 BYA (with most estimates toward the lower end of this range), followed by a relatively rapid divergence of the major extant eukaryote groups discussed below (see Eme *et al.*, 2014). In summary, most researchers in this subdiscipline infer that eukaryote life had evolved by ~1.5 BYA (and possibly a long time before that), with LECA dating to some time before ~1 BYA.

Endosymbiosis; The Mitochondrion

The last eukaryotic common ancestor had mitochondria capable of aerobic respiration. The mitochondrion is one of the two major organelles of eukaryotes that are of endosymbiotic origin (plastids, such as chloroplasts, being the other). Both of these organelles were originally Gram-negative bacteria that became intracellular symbionts, and then lost their genetic independence over time (**Figures 1(b)** and **1(c)**). The great majority of the proteins required for the function of modern mitochondria and plastids are not encoded on their own genomes (though these genomes have almost always been retained, albeit in very reduced form), but are instead now encoded on the nuclear genome of the eukaryote 'host' (see Gray and Archibald, 2012). Transcripts specifying these proteins are translated on cytosolic ribosomes, and the polypeptides are then imported into the organelle by complex translocation machineries. In both mitochondria and plastids, some of these nucleus-encoded organelle-targeted proteins trace their origins back to the endosymbiont's genome, and the genes encoding them were moved to the nuclear genome through the process of 'endosymbiotic gene transfer,' however, a surprisingly large proportion seem to be derived from other

eukaryotic genes or other prokaryotic sources (Gray and Archibald, 2012).

The mitochondrion is descended from an alphaproteobacterium and was acquired once in eukaryotic history, prior to the time of LECA (Gray *et al.*, 1999; Gray and Archibald, 2012; **Figure 1(b)**). This organelle has been retained by all living eukaryote groups that have been studied in detail, even the many taxa (primarily of protists) that live in low oxygen environments and have no capacity for aerobic respiration (Müller *et al.*, 2012; Stairs *et al.*, 2015). There is a long-standing and ongoing debate as to whether the mitochondrial organelle was amongst the last eukaryotic innovations to be acquired (i.e., the original host was already a cell with a nucleus, complex endomembrane system, eukaryotic cytoskeleton, etc.), or whether it was one of the first (Gray and Archibald, 2012; Poole and Gribaldo, 2014). In particular, several hypotheses propose that a symbiosis between an archaeon (or Archaea-related prokaryote) and the bacterial ancestor of mitochondria was itself the seminal founding event of the eukaryotic lineage (see Embley and Martin, 2006; Martin *et al.*, 2015).

Primary Plastids

The history of the plastid endosymbiosis has important and complex impacts on our understanding of the biodiversity of living protistan eukaryotes. The original endosymbiotic event that ultimately lead to (almost) all of the organelles we call plastids occurred after the time of LECA, and clearly involved a fully developed eukaryotic cell as the host, and a cyanobacterium capable of oxygenic photosynthesis as the symbiont. The eventual outcome of this process of 'primary endosymbiosis' was a photosynthetic 'primary alga' with a plastid surrounded by two membranes that are derived from the cell membrane and outer membrane of the cyanobacterial symbiont (see Archibald, 2009, 2012; Gould *et al.*, 2008; Keeling, 2010; Reyes-Prieto *et al.*, 2007).

With the special exception of *Paulinella* (see 'Rhizaria' below), all of the living groups of primary algae appear to descend from a single primary endosymbiosis (**Figure 1(c)**). The evidence for this shared primary endosymbiosis includes phylogenies that show that plastid genes and genomes form a monophyletic group relative to cyanobacteria, unique (or at least unusual) shared features of plastid genome organization, and the fact that plastids share a common protein import machinery (Archibald, 2012; Keeling, 2010; McFadden and van Dooren, 2004; Price *et al.*, 2012; Reyes-Prieto *et al.*, 2007; Turner *et al.*, 1999). The large group of primary algae and land plants is called Archaeplastida, or sometimes simply 'Plantae,' although land plants themselves are a relatively recently derived subsection of this large and ancient lineage (see below).

Secondary Endosymbiosis

The real complexity in the history of plastid origins comes from the fact that multiple 'higher-order' endosymbioses have spread plastids to several lineages of protists outside of Archaeplastida. Secondary endosymbiosis refers to an event in which a eukaryotic cell (typically, but not necessarily, a heterotroph) acquires a primary alga as an endosymbiont, and over time the endosymbiont is reduced to a genetically dependent organelle that imports most of the proteins required for plastid function (**Figure 2(a)**). Plastids acquired through secondary endosymbiosis characteristically are surrounded by more bounding membranes than primary plastids, often four in total. The two inner membranes are the same two that surround a primary plastid. The outermost membrane hails from the host's endomembrane system, while the second-from-outermost is (generally inferred to be) descended from the cell membrane of the primary algal endosymbiont (Archibald, 2012; Keeling, 2010; though see Gould *et al.*, 2015). The usual presumption is that the primary alga that gave rise to the secondary plastid was originally retained intact and was enclosed in a vacuole, i.e., part of the host's endomembrane system. This accords with the observation that contemporary algal endosymbionts (which are relatively common in protists) typically reside inside host vacuoles (see Nowack and Melkonian, 2010). In several secondary algae the outer membrane of the plastid is actually physically continuous with the endoplasmic reticulum and nuclear envelope system of the host (Keeling, 2010: examples, haptophytes, cryptophytes, many stramenopiles – see below and **Figure 2(b)**). In a minority of cases (phototrophic euglenids, typical dinoflagellates), there are just three membranes surrounding the plastid, with the second-from-outside membrane (i.e., inferred descendant of the primary algal cell membrane) being lost, or perhaps never present (Keeling, 2010; **Figures 2(a)** and **2(b)**). These organelles with three or more membranes derived through serial endosymbiotic events, can be referred to collectively as complex plastids (e.g., Gould *et al.*, 2008; Archibald, 2015). The protein import systems of complex plastids are fascinating and complicated sets of interconnected mechanisms, but beyond the scope of this article (see Gould *et al.*, 2008; Keeling, 2010).

In most (presumed) secondary algae, there is very little left of the primary algal endosymbiont except the plastid itself, and (where still present) the membrane descended from the cell membrane of the primary alga. The equivalent of the algal symbiont's cytoplasm is the material immediately outside the inner two plastid membranes. This compartment, termed the periplastid compartment (or periplastidial compartment), has little volume, and usually lacks distinct organelles. However, there are two major algal lineages – cryptophytes and chlorarachniophytes (see below) – in which the nucleus of the primary algal endosymbiont is retained in the periplastid compartment in highly reduced form, lying between the inner pair and outer pair of membranes (see Gould *et al.*, 2008). In both groups this remnant nucleus, known as the nucleomorph, is surrounded by a nuclear envelope, and contains small linear chromosomes (always three, curiously) that possess fewer than 500 protein-coding genes in total (Douglas *et al.*, 2001; Gilson *et al.*, 2006; Moore and Archibald, 2009). Transcripts encoded by genes from the nucleomorph genome are translated on eukaryote-type ribosomes within the periplastid compartment. The existence of nucleomorphs provides very strong evidence of the reality of secondary endosymbiosis (Archibald, 2015), especially since phylogenies estimated for nucleomorph genes confirm that nucleomorphs are indeed most closely related to primary algae (e.g., Cavalier-Smith *et al.*, 1996).

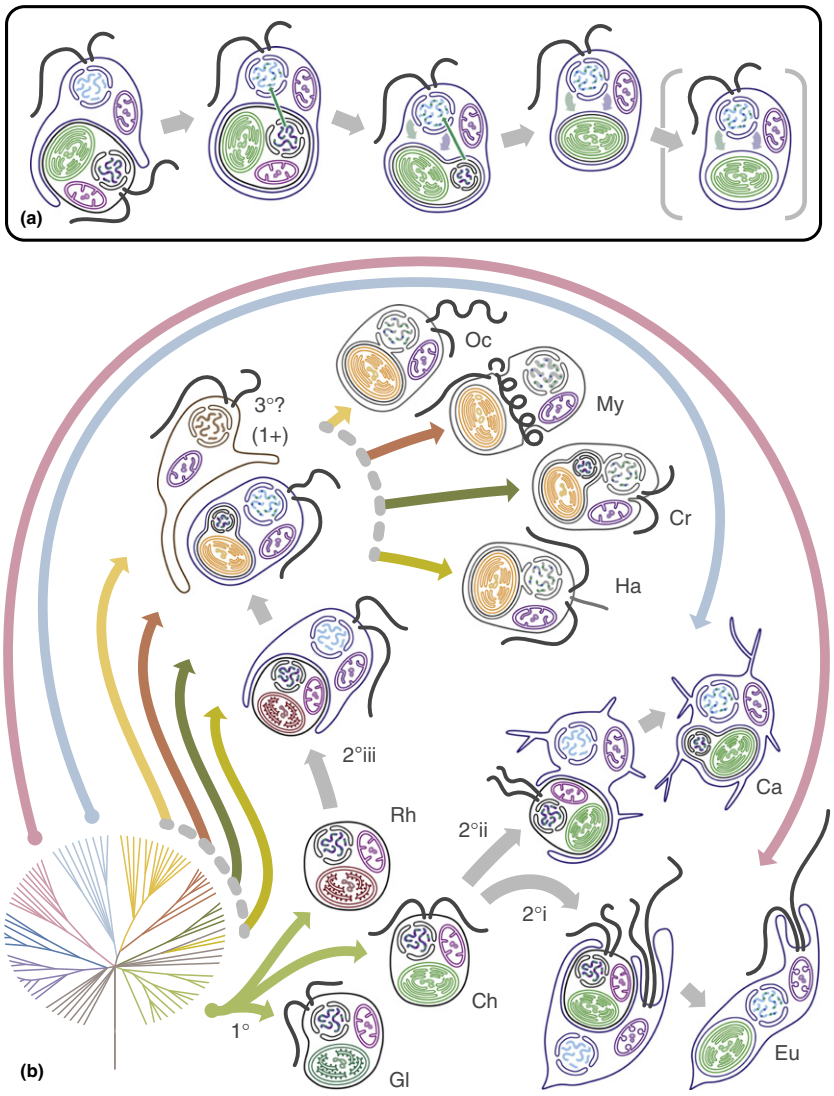

Figure 2 Higher-order endosymbioses and origins of the major groups of algae. (a) Diagrammatic representation of secondary endosymbiosis. The precise order of events shown is representative only; other sequences are plausible. A eukaryote, likely without its own plastid, phagocytosed a primary alga. Over evolutionary time there was a loss of genes from the nuclear genome of the algal symbiont, including endosymbiotic gene transfer to the nuclear genome of the host (narrow green arrow), and the symbiont cell itself became reduced, for example, by loss of its mitochondrion. As with primary endosymbiosis, the transformation of the symbiont into an organelle was cemented by the evolution of a translocation system for the import of proteins necessary for the maintenance of the symbiont, including its photosynthetic capabilities (broad green and blue arrows). In some cases, the organelle has continued to harbor a reduced form of the primary algal nucleus, the 'nucleomorph,' but this nucleus was completely lost in most cases, leaving what is essentially a plastid surrounded by four membranes. In a few cases, the second-from-the-outside of these four membranes was also lost, leaving a plastid with three membranes (right-most cell, in parentheses). (b) Phylogenetic context for the evolution of photosynthetic eukaryotes via multiple endosymbiotic events. The colored branches within the eukaryote subtree represent major groupings, detailed in **Figure 3**. Archaeplastida, the chief group of algae derived directly from primary endosymbiosis (1°), includes Rhodophyta (Rh), Chloroplastida (Ch) and Glaucophyta (Gl). Two separate events of secondary endosymbiosis involving chloroplastid symbionts (2°i; 2°ii) resulted in the advent of photosynthetic euglenids (euglenophytes; Eu), and chloroarachniophytes (Ca), which belong to Excavata and Rhizaria respectively. A third secondary endosymbiosis (2°iii), involving a rhodophyte as the symbiont, gave rise ultimately to the plastids of four groups of chlorophyll *c*-containing algae; Cryptophyta (Cr); Haptophyta (Ha), the myzozoan alveolates (My), the best-known algal representatives of which are dinoflagellates (depicted here), and the photosynthetic stramenopiles, also known as Ochrophytes (Oc). However, the dissemination of plastids to these groups probably also involved one or more events of tertiary endosymbiosis ('3°?'), involving secondary algae with rhodophyte-derived plastids as the symbionts. Possible quaternary endosymbiotic events have also been postulated (not shown).

Complex Plastids, Complex History

Secondary endosymbiosis then is a mechanism by which unrelated groups of eukaryotes can end up sharing the same basic trait of photosynthesis. Furthermore it is clear that secondary endosymbiosis has happened multiple times (**Figure 2(b)**). For example, there are two main groups of secondary algae – chlorarachniophytes and phototrophic euglenids – that have

chlorophylls *a* and *b*, like green algae and land plants (see below); however, phylogenies of plastid genome sequences show that chlorarachniophyte and euglenid plastids are related to the plastids of different subgroups of green algae (Rogers *et al.*, 2007; Turmel *et al.*, 2009). This demonstrates that chlorarachniophytes and euglenids acquired their plastids through separate events of secondary endosymbiosis.

The situation in other groups of algae with complex plastids is more complicated. Four important groups of algae – cryptophytes, haptophytes, photosynthetic dinoflagellates, and photosynthetic stramenopiles (also known as ochrophytes, or heterokont algae) – typically have complex plastids that contain a distinctive chlorophyll, chlorophyll *c*, in addition to chlorophyll *a* (see Andersen, 2004; Graham *et al.*, 2009). Phylogenies based on sequence data from the plastid genome show that these chlorophyll *c*-containing organelles are related to red algal plastids (Bachvaroff *et al.*, 2005; Janouskovec *et al.*, 2010; Yoon *et al.*, 2002), demonstrating that they have a separate endosymbiotic ancestry from the chloroplastid-derived complex plastids of chlorarachniophytes and euglenids discussed above. Furthermore, these plastid gene phylogenies also indicate that the plastids of chlorophyll *c*-containing groups stem ultimately from a single event of secondary endosymbiosis (Bachvaroff *et al.*, 2005; Yoon *et al.*, 2002). However, attempts to estimate the evolutionary trees of the algae themselves, using genes from their nuclear genomes, do not demonstrate that these organisms as closely related. For example, as discussed below, dinoflagellates are more closely related to ciliates (which never have their own plastids), than they are to stramenopiles, haptophytes, or cryptophytes (e.g., Burki *et al.*, 2012; Van de Peer and De Wachter, 1997).

There are two main scenarios that would resolve this apparent contradiction. One possibility is that all of these algae indeed stem directly from a single event of secondary endosymbiosis in a common ancestor, but this photosynthetic ability (and in most cases, the entire plastid) was subsequently lost by ancestors of several major groups of heterotrophs, at a minimum ciliates, Rhizaria, several groups of heterotrophic stramenopiles, and some obscure taxa related to plastid-bearing cryptophytes. The second possibility is that there was a single event of secondary endosymbiosis that gave rise to the chlorophyll *c*-containing plastid, but this plastid was subsequently acquired by one or more unrelated groups through additional symbiotic events (Bodyl *et al.*, 2009; Cavalier-Smith, 1999; Gould *et al.*, 2008; Keeling, 2013). These additional events could have involved a heterotrophic eukaryote acquiring a secondary alga as a symbiont, and over time, this symbiont becoming reduced to just a plastid, in other words 'tertiary endosymbiosis' (Archibald, 2009; Keeling 2013). Tertiary endosymbiosis is actually an independently documented phenomenon: There are some dinoflagellates that have plastids that seem to be derived relatively recently from different secondary algae. In the clearest case, the donor of the plastid was a haptophyte, as evident from both plastid pigments, and phylogenetic analysis of plastid genes (Tengs *et al.*, 2000).

Unfortunately, it is not straightforward to distinguish between secondary and tertiary endosymbioses that happened a long time in the past, and at present it is not clearly resolved which of the two scenarios outlined above best corresponds to the actual evolutionary history (see Archibald, 2015; Keeling, 2013; Gould *et al.*, 2015; Petersen *et al.*, 2014; **Figure 2(b)**). What is not disputed is that resolving the histories of red-alga-derived complex plastids, and of their host algae, remain amongst the most important questions in the study of the evolution of extant eukaryotes.

Uncovering Protist Diversity and Phylogeny

Our current understanding of the biodiversity and phylogenetics of protists is based on more than two centuries of research, but has undergone a profound evolution in the last couple of decades. Much of the known higher-lineage-level diversity of protists had been encountered by light microscopists by the end of the nineteenth century and some major groups that are recognized today were well circumscribed during this era, mostly larger forms and/or those with complex morphologies. By contrast, small heterotrophic flagellates and amoebae (in particular) tended to be grouped into high-level taxa by simple criteria, such as the number of flagella or the form of pseudopodia, that turned out not to define evolutionarily cohesive assemblages. The widespread application of high-quality electron microscopy since the early 1960s led to considerable improvement in our understanding of the diversity of form of protist cells (and related to this, there was eventually a greater tendency to consider protists as a whole, rather than treating 'plant-like' and 'animal-like' cells separately, e.g., Taylor, 1976; see Patterson, 1994). On one hand, this helped identify or highlight many artificial taxonomic groupings, and on the other, it demonstrated a number of previously unsuspected (or poorly supported) phylogenetic groupings (Patterson, 1999). The diverse-but-unified taxon now known as Euglenozoa (see below) is a classic example of a major grouping identified primarily using electron microscopy data (Cavalier-Smith, 1981; Kivic and Walne, 1984; Taylor, 1976).

As with prokaryotes, the application of molecular phylogenetics has transformed our understanding of the protist portion of the tree of life. Briefly, molecular phylogenetics of protists began in the 1970s, but the introduction of small subunit ribosomal RNA (SSU rRNA) as a molecular marker in the 1980s proved to be a crucial innovation (e.g., McCarroll *et al.*, 1983; Sogin *et al.*, 1986), especially after analysis methodologies and taxon sampling improved during the 1990s. Much of what we understand now about the higher-level relationships amongst eukaryotes was established or confirmed during the 1990s and 2000s, primarily through phylogenetic analyses of SSU rRNA sequences, and the common use of multigene phylogenies, mostly based on slowly evolving, protein-encoding nuclear genes (e.g., Baldauf *et al.*, 2000; Gajadhar *et al.*, 1991; Nikolaev *et al.*, 2004; Van de Peer and De Wachter, 1997; Wainright *et al.*, 1993; Parfrey *et al.*, 2010). In the early 2000s, the accumulation of whole genome sequences and surveys of mRNA sequences (EST surveys, later transcriptome sequencing) led to 'phylogenomic analyses' incorporating dozens-to-hundreds genes (Bapteste *et al.*, 2002; Rodriguez-Ezpeleta *et al.*, 2005; Philippe *et al.*, 2005; note that the term 'phylogenomics' is also applied to very different analyses in other contexts). Since ~2007,

phylogenomics has become the major tool for inferring the deepest-level structure of the eukaryote tree, and current 'supergroups' such as Sar and Obazoa were identified primarily on the basis of phylogenomic analyses (see below). At the same time, the application of environmental sequencing approaches over the last 15 years has greatly improved our understanding of the diversity of protists within major lineages (in particular), and in many cases has profoundly altered our knowledge about their distributions across habitats (e.g., Massana *et al.*, 2014).

Nonetheless, despite the massive availability of sequence data, the eukaryote tree of life remains incompletely resolved. Phylogenetic inference is complicated by methodological problems, the existence of lateral gene transfer, and the fact that taxon sampling (i.e., the number and variety of species for which there are sequence data available) is still very limited for many protist groups of particular phylogenetic importance (see Brown *et al.*, 2013; Burki *et al.*, 2012; Yabuki *et al.*, 2014; Zhao *et al.*, 2012). Technical difficulties are particularly hard-felt in the crucial question of the position of the 'root' of the eukaryote tree (i.e., identifying the earliest division amongst lineages represented by living eukaryotes), where the very large evolutionary distances between most prokaryotic sequences and their eukaryotic homologs make phylogenetic inference extremely challenging. Recent studies have estimated phylogenies for gene sets where these distances are unusually low, or considered other molecular data such as gene duplication and loss information, or the taxonomic distribution of important cellular machineries (e.g., Cavalier-Smith, 2010; Derelle and Lang, 2012; Derelle *et al.*, 2015; He *et al.*, 2014; Katz *et al.*, 2012; Wideman *et al.*, 2013); regardless, these have not yet given a consensus on the position of the root (Williams, 2014). Furthermore (and excitingly), new major lineages of protists are still being discovered and characterized at a high rate, both through isolation and cultivation methods, and the use of environmental molecular approaches (e.g., Glücksman *et al.*, 2011; Kim *et al.*, 2011; Not *et al.*, 2007; Seenivasan *et al.*, 2013; Yabuki *et al.*, 2010, 2011).

The Diversity of Living Eukaryotes

At present, the great majority of living eukaryotes can be assigned to one or other of four 'supergroups': Archaeplastida, Sar, Excavata, and Amorphea. Sar brings together three already impressively diverse groups; Stramenopiles, Alveolates, and Rhizaria, while Amorphea unites at least two major taxa; Amoebozoa and Obazoa (with the latter containing the better-known subgroup Opisthokonta). Each of these is likely to represent a monophyletic group, although proposals have been forwarded that the 'root' of the eukaryote tree (see above) might fall within Excavata or Amorphea. The best-known taxa that do not belong to one of these supergroups are the cryptophyte and haptophyte algae discussed briefly above. There is, however, a longer list of heterotrophic organisms that may also belong to phylogenetically distinct major lineages outside of the current supergroups (see 'other groups,' below). A summary tree depicting the major phylogenetic groups of eukaryotes in shown in **Figure 3**.

Archaeplastida

Archaeplastida ('ancient plastids') are the group containing essentially all of the primary algae (i.e., with plastids of primary endosymbiotic origin – see above), and the great majority of living species in this assemblage are photosynthetic. Their history likely dates back more than 1 billion years, based on scant early fossils (Butterfield *et al.*, 1990). Archaeplastida consists of three groups: Chloroplastida, Rhodophyta, and Glaucophyta.

Chloroplastida (also known as Viridiplantae or Chlorobionta) includes the various green algae, that is, all the primary algae with chlorophyll *b* in addition to chlorophyll *a*, and the land plants. Green algae range from numerous small unicellular flagellates, which likely represent the ancestral form of Chloroplastida (**Figure 4(a)**), through various colonial and filamentous types (**Figure 4(b)**), to complex, differentiated macroalgae. Large size and complexity has evolved independently within green algae on multiple occasions. For example, the major group of green seaweeds (Ulvophyceae) is not closely related to the major freshwater macroalgae or to land plants (Leliaert *et al.*, 2012). The land plants evolved from within the streptophyte/charophycean green algae, which are mostly freshwater forms. The precise relationships between land plants and the various streptophyte green algae are still incompletely resolved. Recent phylogenomic analyses of nuclear and plastid markers favor a close relationship with the unicellular or filamentous Zygnametales (Laurin-Lemay *et al.*, 2012; Leliaert *et al.*, 2012; Timme *et al.*, 2012; Wickett *et al.*, 2014; Zhong *et al.*, 2014; **Figure 4(b)**), rather than the previously preferred hypothesis of a closer affinity between the land plants and the complex macroalgae called Charales, or 'stoneworts' (see Lewis and McCourt, 2004).

Rhodophytes, or red algae, are mostly marine macroalgae (**Figure 1(c)**; see also **Figure 1(f)**), although there are also unicellular forms. Glaucophytes are a very obscure lineage of freshwater unicellular or colonial algae (**Figure 1(d)**). The plastids of both rhodophytes and glaucophytes lack the chlorophyll *b* that is the main accessory pigment of Chloroplastida, but instead retain the phycobilisome accessory pigment system present in the cyanobacterial ancestor of plastids (Graham *et al.*, 2009; Jackson *et al.*, 2015); the red color typical of red algae, for example, is primarily due to an abundance of the phycobilin phycoerythrin (Graham *et al.*, 2009). Remarkably, the plastids of glaucophytes also retain a vestige of the bacterial peptidoglycan cell wall between inner and outer plastid membranes (Löffelhardt and Bohnert, 1994; Jackson *et al.*, 2015).

Sar

The taxon Sar (also known as SAR, or Harosa) is a large and complex assemblage that draws together a wide variety of organisms that were not previously considered as specially related, but have been grouped by multigene phylogenetics or phylogenomic studies (Burki *et al.*, 2007; Hackett *et al.*, 2007; Rodriguez-Ezpeleta *et al.*, 2007). The name Sar/SAR was originally an acronym of its three subclades: Stramenopiles, Alveolata, and Rhizaria (Adl *et al.*, 2012; Burki *et al.*, 2007), each of which is already very diverse.

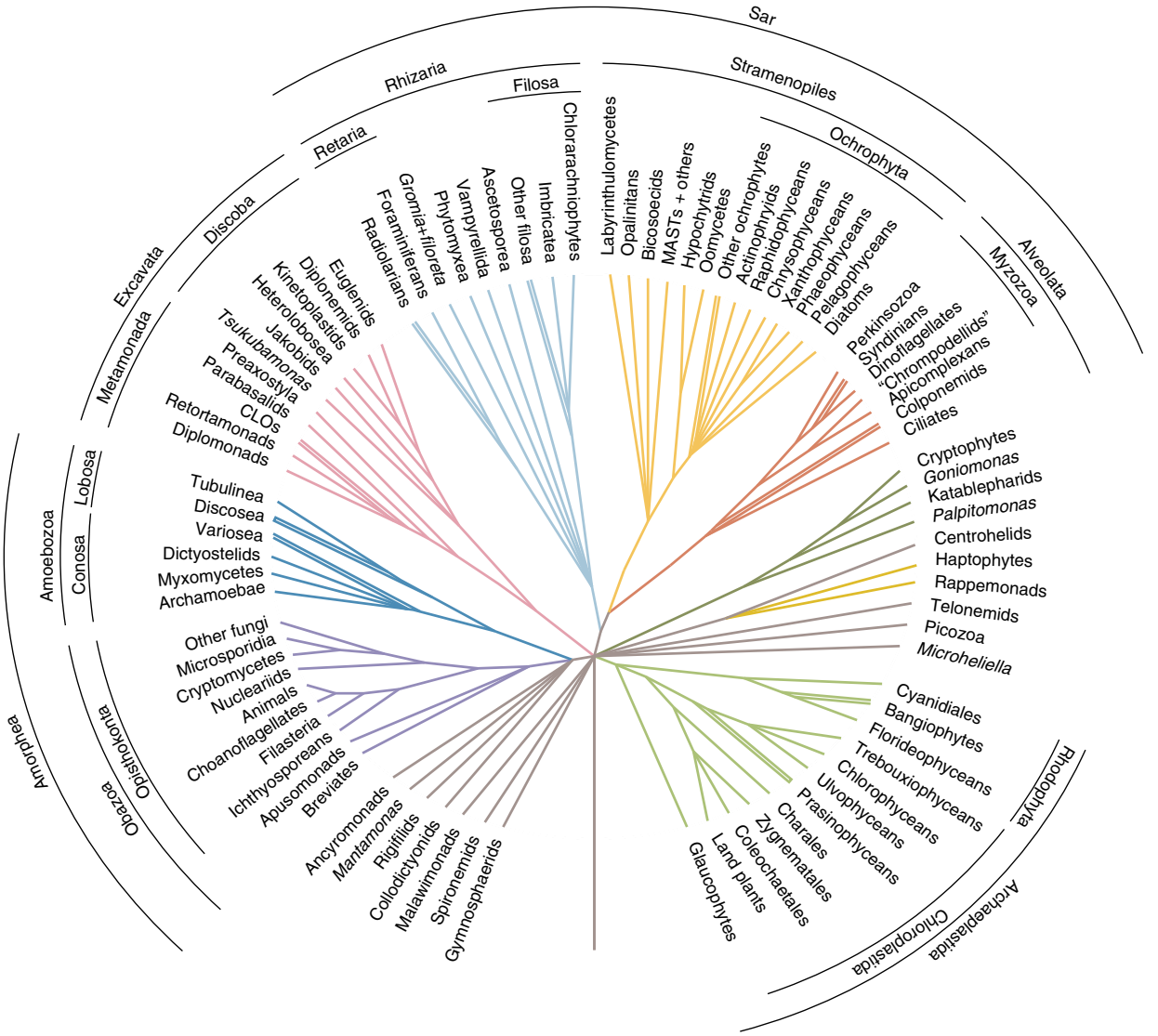

Figure 3 Summary tree of extant eukaryote groups. Branching order is based primarily on molecular phylogenetic and phylogenomic analyses. Some smaller taxa are not shown. Double lines indicate groupings that are likely paraphyletic rather than monophyletic. CLO – *Carpediemonas*-Like Organisms; MASTs – Marine Stramenopiles.

Stramenopiles

Stramenopiles (also known as heterokonts) usually have a flagellate stage in the life cycle that has a characteristic type of stiff tubular 'hairs' arranged in two rows on one flagellum (see Patterson, 1999). These flagellates swim in the direction the hair-bearing flagellum is pointing. Stramenopiles include a particularly wide variety of algae with chlorophyll *c*-containing complex plastids (see above), which are often now known as ochrophytes. These range from unicells and colonial microalgae, both flagellated and nonmotile (e.g., diatoms, Chrysophyceae, Pelagophyceae, Raphidophyceae, and many others; **Figures 4(e)** and **4(g)**) to large macroalgae, such as kelps and wracks (Andersen, 2004; Graham *et al.*, 2009; **Figure 4(f)**). A substantial proportion of microalgal stramenopiles produce elaborate cell coverings made of silica (diatoms and many chrysophyceans, e.g.). Ochrophytes of different kinds are frequently amongst the most important algae within a given aquatic system: for example, diatoms, together with dinoflagellates (see below), are the dominant larger microalgae in ocean waters. However, the stramenopile clade also contains numerous lineages of protozoa, most of which are small bacterivorous flagellates (see Patterson, 1999; Massana *et al.*, 2014; **Figure 4(h)**). These protozoan stramenopiles actually exceed the algal stramenopiles in molecular sequence diversity (Massana *et al.*, 2014; Shiratori *et al.*, 2015), but are more poorly studied: for example, some of the most abundant protozoa in the oceans are stramenopiles that belong to major groups which were discovered by environmental sequencing efforts within the last 15 years ('MASTs'; Massana *et al.*, 2004, 2014). There are also some important fungal-like groups among the heterotrophic stramenopiles. The most famous of these are the oomycetes, some of which are important

Figure 4 Archaeplastida, Stramenopiles, and Alveolata. Background boxes delineate major groupings, colored as in **Figure 3**. Sizes indicate maximum dimension of each cell (excluding flagella, thecae, etc.), unless otherwise noted. (a) *Zygnema* (Chloroplastid; individual cells 50 μm long). (b) *Haematococcus* (Chloroplastida; 30 μm). (c) *Pterosiphonia* (Rhodophyte; largest cells approximately 70 μm long). (d) *Gloeochaete* (Glaucophyte; 30 μm); image courtesy of Adrian Reyes Prieto. (e) *Pinnularia* (Diatom; 60 μm). (f) *Ascophyllum* (Phaeophycean) and *Vertebrata* (Rhodophyte); image courtesy of Eric Salomanki. (g) *Sarcinochrysis* (Pelagophycean; 6 μm); image courtesy of Robert (Bob) Andersen. (h) *Bicosoeca* (Bicosoecid; 10 μm excluding theca). (i) Undescribed euplotid (Ciliate; 45 μm). (j) *Nassulopsis* (Ciliate; 150 μm). (k) *Ceratium* (Dinoflagellate; 125 μm wide). (l) *Colpodella* ('Chrompodellid'; 12 μm). (m) *Toxoplasma* (Apicomplexan; each sporozoite is 5 μm); image courtesy of Jacqueline Leung and Ke Hu.

parasites of aquatic animals and land plants, including the causative agents of late blight in potatoes and sudden oak death (Thines, 2014).

Alveolata

The alveolates include the dinoflagellates, about half of which are algae with complex plastids, and two large and important groups of protozoa: the apicomplexan parasites, and the mostly free-living ciliates (**Figures 4(i)–4(m)**). Of these three groups, dinoflagellates and Apicomplexa are the most closely related; together with more obscure relatives these form a 'Myzozoa' grouping that appears to descend from a photosynthetic common ancestor (see below).

Dinoflagellates (**Figure 4(k)**) are one of the most abundant forms of larger microalgae, and the diversity of species in marine plankton is particularly high (Taylor *et al.*, 2008). They

are most famous for the formation of algal blooms, and the production of potent toxins that can harm or kill humans, usually through consumption of toxin-contaminated shellfish or finfish (though algae from several other groups can also be also bloom-forming and/or toxic). The non-algal dinoflagellates are mostly predators of other microorganisms, or parasites of animals or large protists. It has become increasingly clear that many, perhaps most, algal dinoflagellates are also predators of other microorganisms, and as such are textbook examples of mixotrophs (see above; Jeong *et al.*, 2010; Stoecker, 1999).

Apicomplexa are almost all parasites of animals, and the several thousand described species include the organisms that cause malaria, cryptosporidiosis, and toxoplasmosis in humans, as well as several major livestock diseases (**Figure 4(m)**). They typically have sexual life cycles that include multiple cell types, and that can involve multiple host species. Most are intracellular parasites that use a specialized 'apical complex,' which includes distinct kinds of secretory organelles, to actively invade host cells and subsequently modify them (Kemp *et al.*, 2013). Remarkably, most Apicomplexa also possess a complex plastid that is non-photosynthetic, but plays essential biosynthetic roles (see Gould *et al.*, 2008; Keeling, 2010). Apicomplexan parasites turn out to be most closely related to the 'chrompodellids' grouping (Janouskovec *et al.*, 2015) that includes both the colpodellid protozoa, which are predators or ectoparasites of other unicellular protists (**Figure 4(l)**), and a couple of recently discovered algae with photosynthetic complex plastids (*Chromera* and *Vitrella*; Moore *et al.*, 2008; Obornik *et al.*, 2011). Most of these, as well as deep-branching organisms from the dinoflagellate lineage (e.g., perkinsids), have structures that that are homologous to the apical complex, and are used in feeding or host invasion (Okamoto and Keeling, 2014).

The final major group of alveolates, the ciliates, usually have numerous cilia on the cell surface (Lynn, 2008; **Figures 4(i)** and **4(j)**). They are extremely diverse and abundant predators in many aquatic systems, often using closely packed arrays of specialized 'oral cilia' as a part of their feeding mechanism. They have an unusual genomic organization: each ciliate typically contains two types of nuclei. The 'somatic' macronuclei are the templates for essentially all transcription during the asexual phase of the life cycle, while the micronuclei participate in the sexual stage of the life cycle, and act as the germline (whereas macronuclei degenerate during and/or after the sexual process).

Rhizaria

Rhizaria (**Figures 5(a)–5(d)**), the final group within Sar, is a diverse collection primarily of free-living protozoan organisms, although the taxon also includes significant parasites of marine animals (e.g., ascetosporeans – haplosporidians and paramyxids), and of plants (many Phytomyxea, namely, plasmodiophorids; Burki and Keeling, 2014). Rhizaria also contains two small groups of photosynthetic organisms, one of which is the chlorarachniophyte secondary algae discussed above (**Figure 5(c)**), while the other is a tiny group of filose testate amoebae (see below) from the genus *Paulinella*, which have photosynthetic organelles that derived from cyanobacteria completely independently of the 'true' plastids discussed elsewhere in this article, and much more recently (Nowack, 2014). Many free-living Rhizaria produce thin, and often branching pseudopodia for feeding (e.g., **Figure 5(b)**). Some Rhizaria locomote using flagella (often using a gliding motility in preference to swimming; e.g., **Figure 5(d)**). Others are non-flagellated amoebae, which may use their pseudopodia for locomotion as well as feeding. The best-known rhizarians – Foraminifera (**Figure 5(a)**), the radiolarians, and the filose testate amoebae – are all amoebae of this kind, but in addition usually have self-mineralized shells (tests) or 'skeletons,' which are made of calcium carbonate, strontium sulfate or silica, depending on the group or subgroup (e.g., Febvre *et al.*, 2002). Foraminifera and radiolarians in particular are often very large cells (occasionally several millimeters or more in diameter), and are abundant in marine waters and sediments, and, in many cases, in the fossil record.

Excavata

Like Rhizaria, Excavata (**Figures 5(e)–5(j)**) are primarily a collection of protozoa, but also include a single group of secondary algae. The most recent common ancestor of excavates was a flagellate (probably a free-living bacterivore) with a characteristic broad feeding groove, and various extant free-living groups still have this basic cell form (Simpson, 2003; e.g., **Figure 5(f)**). Excavates are divided into two main subgroups, Metamonada and Discoba (Hampl *et al.*, 2009). Metamonads are descended from a common ancestor that had lost the ability to perform aerobic respiration, and consequently have highly modified mitochondria of various kinds that lack mitochondrial genomes (Cavalier-Smith, 2003; Stairs *et al.*, 2015). Many metamonads are free-living and inhabit oxygen-poor marine or freshwater environments (e.g., Kolisko *et al.*, 2010; **Figure 5(f)**), but several subgroups have become specialist parasites or symbionts of animals. The intestinal parasite *Giardia* and urogenital parasite *Trichomonas vaginalis* are highly prevalent human pathogens (e.g., Adam, 2001; Lehker and Alderete, 2000), however, there is also a spectacular diversity of symbiotic metamonads in the hindguts of many wood-eating termites (Brugerolle and Lee, 2002a,b; **Figure 5(e)**).

Most Discoba have aerobic mitochondria with organellar genomes, in fact, the small flagellate group Jakobida (e.g., *Reclinomonas* and *Andalucia*) have mitochondrial genomes that retain more unique genes, and more bacterial-like features, than those of any other eukaryote (Burger *et al.*, 2013; Lang *et al.*, 1997). Discoba also include Heterolobosea (**Figure 5(g)**), many of which have alternate amoeba and flagellate phases (the infamous 'brain-eating amoeba' *Naegleria fowleri* is a heterolobosean; see Visvesvara, 2013). The final major Discoba group, Euglenozoa, includes numerous protozoan flagellates that are abundant surface-associated bacterivores and predators of microbial eukaryotes (e.g., 'bodonid' kinetoplastids, and phagotrophic euglenids; Boenigk and Arndt, 2002; Larsen and Patterson, 1990; **Figure 5(j)**). It also includes the parasitic kinetoplastids that cause sleeping sickness, Chagas' disease, and leishmaniasis in humans (all caused by different trypanosomatids; **Figure 5(h)**), and several important diseases of animals and a few plants (Simpson *et al.*, 2006; Vickerman, 2002). Euglenozoa is

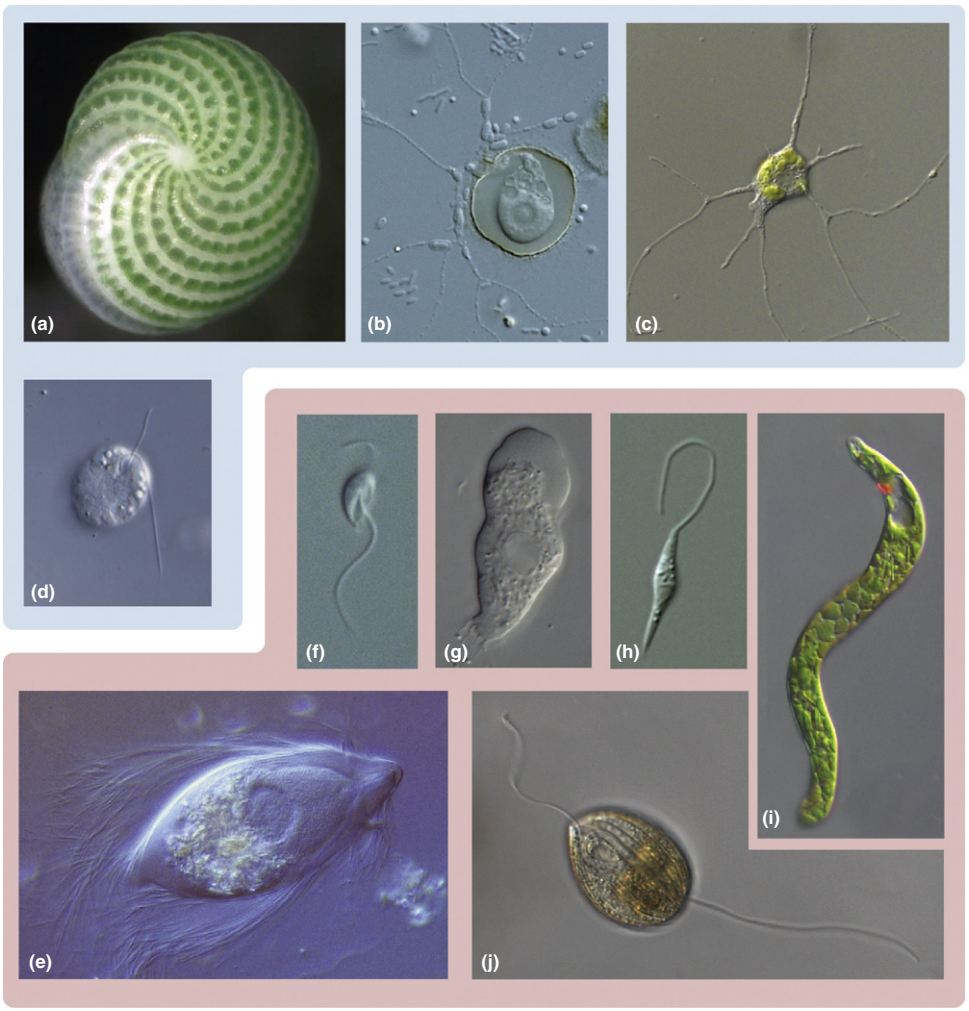

Figure 5 Rhizaria and Excavata. Background boxes delineate major groupings, colored as in **Figure 3**. Sizes indicate maximum dimension of each cell (excluding flagella, thecae, etc.), unless otherwise noted. (a) *Archaias angulatus* (Foraminiferan; 1.8 mm); image courtesy of Sam Bowser. (b) *Microgromia* sp. (Filosa; 12 μm excluding test). (c) *Chlorarachnion* (Chlorarachniophyte; ~15 μm); image courtesy of John Archibald. (d) *Protaspa*-like filosan (12 μm). (e) *Trichonympha* (Parabasalid; 75 μm); image courtesy of Patrick Keeling. (f) *Carpediemonas* ('CLO'; 5 μm). (g) *Selenaion* (Heterolobosean; 30 μm); image courtesy of Jong Soo Park. (h) *Leishmania* (Kinetoplastid; 13 μm). (i) *Euglena* (Euglenid; 60 μm); image courtesy of Richard Triemer. (j) *Anisonema* (Euglenid; 20 μm); image courtesy of Gordon Lax.

also the taxonomic home for the photosynthetic euglenids, which are secondary algae with a plastid of green algal origin (see above; **Figure 5(i)**).

Amorphea

The Amorphea supergroup (previously known as 'unikonts') unites Amoebozoa and Obazoa, two major taxa that are examined individually below (Adl *et al.*, 2012). A close relationship between Amoebozoa and Obazoa has been supported by many phylogenetic analyses (e.g., Baldauf *et al.*, 2000; Bapteste *et al.*, 2002; Hampl *et al.*, 2009; Derelle and Lang, 2012), albeit some analyses place the 'root' of the eukaryote tree within Amorphea (see above).

Amoebozoa

As the name suggests, most members of the taxon Amoebozoa are amoebae, and this is the group that includes famous large forms such as *Chaos*, and the genus *Amoeba* itself. Most produce lobose pseudopodia (**Figure 6(a)**), or lamellipodia, both of which are much broader than the fine pseudopodia typical of Rhizaria (see above; Smirnov *et al.*, 2011), however, there is also a substantial diversity of amoebozoa with fine, often reticulating pseudopodia (e.g., Berney *et al.*, 2015). Many amoebozoa are free-living amoebae (e.g., **Figure 6(a)**), although some are obligate or facultative parasites (e.g., *Acanthamoeba*; Visvesvara, 2013), and a few have a flagellate stage in their life cycle. They are especially common in freshwater and marine sediments. One clade, the lobose testate amoebae, inhabit shells (tests) with a single broad aperture to allow them to extend their pseudopodia (**Figure 6(b)**). Testates are common in freshwater and terrestrial habitats, including soil, where they can be present in very high abundances (Meisterfeld, 2002). Another unusual group within Amoebozoa, the archamoebae, are all anaerobes that have highly modified mitochondria (similar to the metamonads

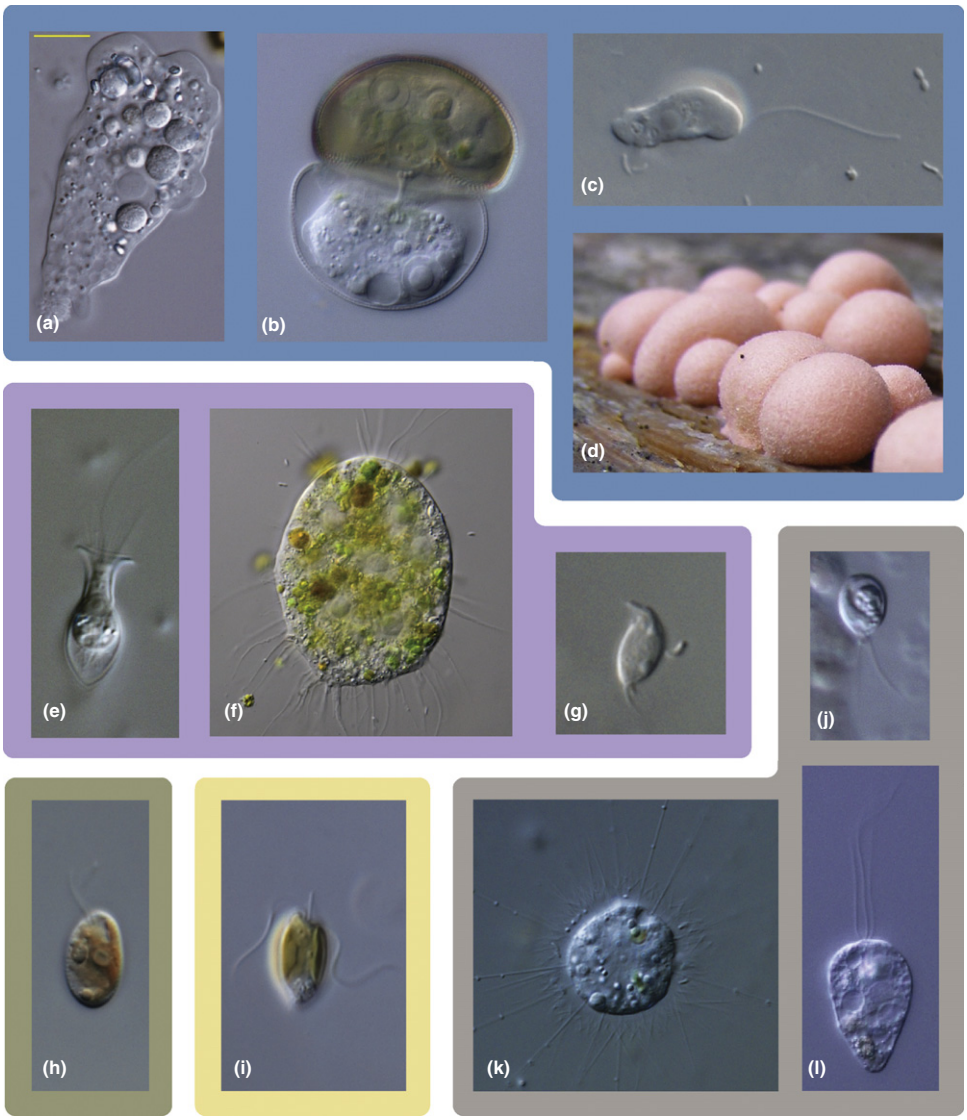

Figure 6 Amoebozoa, Obazoa, Cryptophyta, Haptophyta, and 'other taxa.' Background boxes delineate major groupings, colored as in **Figure 3**. Sizes indicate maximum dimension of each cell (excluding flagella, thecae, etc.), unless otherwise noted. (a) *Saccamoeba* (Tubulinea; 40 μm). (b) Dividing cell of *Arcella;* a lobose testate amoeba (Tubulinea; 60 μm). (c) *Mastigella* (Archamoeba; 12 μm) (d) Fruiting bodies of *Lycogala* (Myxomycete; macro image). (e) *Salpingoeca* (Choanoflagellate; 9 μm excluding theca). (f) *Nuclearia* (Nucleariid, 60 μm); image courtesy of Ferry Siemensma. (g) *Multimonas* (Apusomonad; 8 μm). (h) *Rhodomonas* (Cryptophyte; 10 μm). (i) *Prymnesium* (Haptophyte; 10 μm). (j) *Telonema* (Telonemid; 5 μm). (k) *Heterophrys* (Centrohelid; cell body 20 μm, not including radiating axopodia). (l) *Collodictyon* (Collodictyonid; 30 μm).

discussed above; see Stairs *et al.*, 2015). Archamoebae includes free-living amoebae, flagellated amoeboid organisms (**Figure 6(c)**), and commensals/parasites such as the major human pathogen *Entamoeba histolytica* (Ptáčková *et al.*, 2013; Stanley, 2003). Several distinct subgroups within Amoebozoa are 'slime molds': terrestrial organisms that exist as amoebae for at least one part of the life cycle, but also produce a fruiting body (sorocarp) that releases spores (Shadwick *et al.*, 2009; **Figure 6(d)**). Dictyostelid slime molds produce the fruiting body through the cooperative aggregation of many individual amoebae (Romeralo *et al.*, 2012), while the fruiting bodies of Myxomycetes (myxogastrids; **Figure 6(d)**) are reproductive structures generated by outgrowth from a single individual, a large multinucleate 'plasmodium' (Stephenson, 2011).

Obazoa (including opisthokonta)

The final major grouping of eukaryotes is the recently discriminated taxon Obazoa (Brown *et al.*, 2013), almost all of which fall into a slightly less inclusive but better-known group called Opisthokonta. Opisthokonta includes both animals (Metazoa) and true Fungi, which evolved from protistan ancestors independently of each other (Cavalier-Smith, 1987; Wainright *et al.*, 1993). Molecular phylogenies have demonstrated that animals are closely related to the choanoflagellates, a group of small solitary or colonial flagellates with a microvillar feeding collar, which are important bacterivores in ocean plankton (Leadbeater, 2015; **Figure 6(e)**), as well as several more obscure groups, such as the Ichthyosporean parasites, which mostly infect aquatic animals

(Lang *et al.*, 2002; Mendoza *et al.*, 2002; Shalchian-Tabrizi *et al.*, 2008; Steenkamp *et al.*, 2006). Fungi are related instead to nucleariids, a small group of amoebae with fine 'filose' pseudopodia (Amaral Zettler *et al.*, 2001; Steenkamp *et al.*, 2006; **Figure 6(f)**), although research over the last decade has identified a diverse clade of mostly uncultivated aquatic organisms, now called Cryptomycota (or Rozelliida), that is either the closest sister group to Fungi, or the deepest branch within Fungi, depending on exactly how Fungi are to be defined (Jones *et al.*, 2011; Lara *et al.*, 2010). Cryptomycota appear to be parasites of other aquatic organisms. They may produce chitinous cell walls similar to those of true fungi during some stages of the life cycle (e.g., in the formation of structures that allow them to infect their hosts), however, the feeding structures are not enclosed by these cell walls, unlike the absorptive hyphae of most mycelial fungi (James and Berbee, 2012; Jones *et al.*, 2011). The microsporidia, small animal-infecting intracellular parasites long considered related to fungi in some way (Van de Peer *et al.*, 2000), may well in fact belong to Cryptomycota, or at least be closely related to them (James *et al.*, 2013).

The grouping Obazoa reflects the recent confirmation that Opisthokonta is related to two small taxa of surface-dwelling bacterivorous flagellates, apusomonads (**Figure 6(g)**), and breviates (Brown *et al.*, 2013; Cavalier-Smith and Chao, 2010; Kim *et al.*, 2006). As a consequence of their phylogenetic position, these two previously obscure protist lineages are now of heightened importance for tracing the evolution of multicellularity in animals and fungi (Brown *et al.*, 2013).

Other Groups

The groupings listed above encompass most, but not all of the diversity of living eukaryotes. Firstly, they do not include two well-known groups of algae, cryptophytes and haptophytes, and their relatives. Cryptophytes in the strict sense (e.g., **Figure 6(h)**) are a group of chlorophyll *c*-containing unicellular flagellates (mostly; some have non-photosynthetic plastids), which are notable for retaining a nucleomorph within the plastid (see above). They are most closely related to a nested series of more obscure heterotrophic forms including goniomonads, katablepharids, and probably the recently described *Palpitomonas* (Yabuki *et al.*, 2010, 2014; Hackett *et al.*, 2007; Okamoto and Inouye, 2005; Okamoto *et al.*, 2009), but beyond this their phylogenetic affinities are still unclear (see below).

Haptophytes (**Figure 6(i)**) are also chlorophyll *c*-containing unicellular algae (see above) that are found in diverse environments, but are particularly important in the ocean. Many are flagellated (at least in some life cycle stages), and some are known to be mixotrophs (Andersen, 2004). The most famous haptophytes are the exclusively marine coccolithophorids, which produce a casing of often-elaborate calcium carbonate scales called 'coccoliths' (Young *et al.*, 2005). Some phylogenomic analyses favor a relationship with the taxon Sar (Burki *et al.*, 2012; though see below). Haptophtyes may also be related to 'rappemonads,' a recently discovered group of unicellular algae that can be abundant in ocean plankton, but have never been cultured (Kim *et al.*, 2011), and possibly also to centrohelid heliozoa (see below). Previous phylogenetic evidence that haptophytes are closely related to cryptophytes and their kin (Burki *et al.*, 2008; Hackett *et al.*, 2007; Patron *et al.*, 2007) has usually been only weakly supported, or not supported at all, by recent analyses (Burki *et al.*, 2012; Katz and Grant, 2015).

In addition, there are a greater number purely heterotrophic 'protozoa' for which there are reasonable molecular and high-resolution microscopy data, but whose phylogenetic positions are also unclear. Some appear to have affinities with Archaeplastida and/or Sar and/or cryptophytes and/or haptophytes (e.g., Burki *et al.*, 2009, 2012; Shalchian-Tabrizi *et al.*, 2006). These include the small flagellate group telonemids (**Figure 6(j)**), the best known of which are marine predators of other small protists (Klaveness *et al.*, 2005), and Picozoa, a recently discovered group of small flagellates that were originally inferred to be algae but are now known to be heterotrophs (Not *et al.*, 2007; Yoon *et al.*, 2011; Seenivasan *et al.*, 2013). Centrohelids (**Figure 6(k)**) are one of several kinds of 'heliozoan' amoebae that have a subspherical cell body supporting a radiating array of fine microtubule-supported pseudopodia (Zlatogursky, 2012; most other 'heliozoa' belong to Stramenopiles or Rhizaria). The best hypothesis at present is that centrohelids are related to haptophytes (Cavalier-Smith *et al.*, 2015), but this awaits further confirmation.

There are several other heterotrophic groups for whom multigene phylogenies typically suggest relationships with or within Amorphea (i.e., Amoebozoa and Obazoa). These include the collodictyonids, which are free-swimming flagellates (**Figure 6(l)**), ancyromonads and *Mantamonas*, which are very small gliding flagellates, and the amoeboid Rigifilida (Yabuki *et al.*, 2013; Brugerolle *et al.*, 2002; Cavalier-Smith *et al.*, 2014; Glücksman *et al.*, 2011, 2013; Mikrjukov and Mylnikov, 2001; Paps *et al.*, 2013; Zhao *et al.*, 2012). Perhaps the most puzzling protists of uncertain placement are the malawimonads, an otherwise very obscure group of small heterotrophic flagellates that is assigned to excavates by morphological data (Simpson, 2003), but shows closer affinities with Amoebozoa and Obazoa (and/or collodictyonids) in many multigene phylogenies, including most recent phylogenomic-scale analyses of mitochondrial and/or nuclear sequence data (Derelle and Lang, 2012; Derelle *et al.*, 2015; Hackett *et al.*, 2007; Zhao *et al.*, 2012).

Finally, there are still a few well-defined and morphologically distinct groups of living eukaryotes whose affinities remain uncertain, and for which there are no reported molecular sequence data. The best known of these are the spironemids, which are multiflagellated, probably predatory, cells that are occasionally reported in high abundance in soils and sediments (Foissner and Foissner, 1993).

Concluding Remarks

The broader-scale picture of the biodiversity and phylogenetic tree of living eukaryotes may well be largely resolved in the near future. The full extent of undescribed/undersampled protist diversity is not clear at present, however, most newly studied major lineages are likely to be rapidly integrated into a

reasonably accurate eukaryote phylogeny. This is due to the ongoing, even accelerating, efforts to broadly capture protist diversity using culturing approaches, as well as rapidly improving techniques for efficiently obtaining useful genomic/transcriptomic data from difficult-to-culture single cells and from environmental samples (Kolisko *et al.*, 2014; Yoon *et al.*, 2011). Hopefully, the ongoing taxonomic broadening of genomic datasets will also lead to resolution of the still-unclear history of higher-order plastid endosymbioses, especially complex plastids of red algal origin. It will be fascinating to see whether the position of the 'root' of eukaryotes can be pinpointed in the near future – recent evidence and approaches show promise, even though there is little evidence to date for convergence on a well-supported answer (Derelle *et al.*, 2015; Williams, 2014).

One area where there is still a particularly acute shortage of direct information is the early evolution of eukaryotic cells, prior to LECA, and shortly thereafter. While tremendous improvements in the understanding of the Proterozoic fossil record have been wrought in the last 2–3 decades (see Butterfield, 2015), it is surely the case that paleontologists are still scratching the surface. There are a few fossil finds that individually exert a huge influence on our current view of early eukaryote evolution (e.g., Butterfield *et al.*, 1990; Porter and Knoll, 2000), indicating that the next few important finds, whenever they emerge, will be similarly transformative. It is possible, however, that the next important revelations concerning the early history of eukaryotes will stem from microbiology rather than paleontology. New discoveries amongst the probable archaeal relatives of eukaryotes (Spang *et al.*, 2015) might reveal organisms that are genuinely intermediate in cell organization between typical prokaryotes and eukaryotes, ultimately telling us a lot more about the cells from which living protists, and all true eukaryotes, diversified.

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See also: Archaeplastida: Diversification of Red Algae and the Green Plant Lineage. Endosymbiotic Theory. Symbiogenesis, History of. Unikonts, Evolution and Diversification of (with Emphasis on Fungal-Like Forms). Endophytic Microbes, Evolution and Diversification of

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Q

Quantitative Genetics in Natural Populations

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Glossary

Adaptation Evolution of phenotypes conferring high fitness.
Additive genetic variance A measure of the variation in a phenotypic trait that is attributable to additive genetic effects.
Animal model A form of linear mixed effect model in which an individual's breeding value is fitted as a random effect. The most widely used statistical method for estimating quantitative genetic parameters in natural populations.
$\boldsymbol{\beta}$ Vector of directional selection, containing individual selection gradients (β) for a set of phenotypic traits.
Breeding value The expected (additive) effect of an individual's genotype on phenotype, usually expressed relative to the population mean phenotype. As a latent rather than directly observable variable it may be estimated by measuring offspring phenotype, or may be predicted using, for example, an animal model. Variance in breeding values is the additive genetic variance.
Directional selection Selection that favours an increase or decrease in the population mean phenotype.
Evolutionary constraint Broadly, any process that reduces the rate of adaptive evolution relative to naïve expectations. Constraint in **G** can be conceptualized as a relative lack of genetic variance in the direction of multivariate selection β.
G matrix Additive genetic variance–covariance matrix for a set of phenotypic traits.
Genetic correlation A standardized measure of the (additive) genetic covariance between two traits.
Genetic merit See breeding value.
Genome-wide association study (GWAS) Test of association between alleles and phenotypes using a genome-wide set of markers.
Genotype-by-environment interaction A phenomenon that occurs when environmental effects on phenotype depend on genotype (and vice versa).
Heritability A standardized measure of additive genetic variance for a trait. Formally estimated (in the narrow sense) as the proportion of total phenotypic variance explained by additive genetic variance.
Identical by descent (IBD) A DNA segment inherited by two or more individuals from a common ancestor.
Indirect genetic effect Causal dependence of one individual's phenotype on the genotype of another.
Latent variable In modeling, latent variables are those that are not directly observed but about which inferences can be made statistically. In quantitative genetic models, individual breeding values are often included as a latent variable.
Linkage disequilibrium (LD) The nonrandom association of alleles at different loci.
Maternal effect Causal influence of some characteristic(s) of mothers on the phenotype of their offspring (over and above the effect of genes inherited).
Molecular pedigree analysis The use of molecular data (e.g., microsatellite genotypes) to determine relationships and/or relatedness among individuals in a population.
Natural selection A causal dependence of fitness on phenotype.
Parent–offspring regression A statistical method for estimating heritabilities and other quantitative genetic parameters. For example, heritability can be estimated as the slope of a regression of offspring trait on midparent trait. Largely superseded by the animal model for studies of wild populations.
Quantitative trait locus (QTL) A locus influencing a quantitative trait.
Selection differential (S) A measure of (directional) selection on a trait. Formally the covariance between trait and fitness.
Selection gradient ($\boldsymbol{\beta}$) A measure of (directional) selection on a trait. Formally the partial regression of fitness on a trait.

Introduction

Quantitative genetics is a field of biological research that attempts to understand how genes affect the expression and evolution of complex phenotypic traits – that is, traits that are influenced by many different genes (as opposed to 'Mendelian traits' determined by a single gene). It is both a theoretical and empirical area of research. Theoretical models predict how

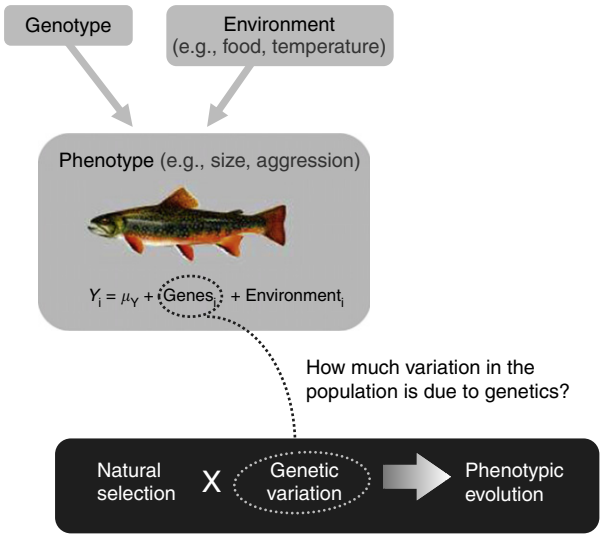

Figure 1 Quantitative genetic theory predicts phenotypic evolution as a function of natural selection and genetic variation (dark gray box). To use this predictive theory, empiricists must therefore estimate the genetic variation underpinning a trait (or traits) of interest. In wild populations this is most commonly done by modeling the individual phenotypes Y_i of animals of known relatedness to each other (see main text for detail). In the simplest case, Y_i is specified as the population mean, plus deviations attributable to genotype (the 'breeding value'), and (unknown) environmental effects experienced.

traits will respond to selection as a function of parameters, such as heritabilities and genetic correlations, that describe their patterns of inheritance and (co)variation in a population (Figure 1). Empirical quantitative geneticists use statistical methods to estimate these parameters, allowing hypotheses about phenotypic evolution to be tested. Because quantitative genetic theory is entirely general, it can be applied to studies of any trait (e.g., disease resistance, growth rate, and social behaviors) in any population. However, because of the type, and amount, of data required to estimate quantitative genetic parameters it is only relatively recently that these methods have been widely applied *in situ* in natural populations. In this article we provide a brief overview of this rapidly growing field of research, highlighting some ways in which quantitative genetic studies of natural populations are helping us to understand phenotypic evolution, and pointing some challenges and opportunities facing the field. Before progressing we first discuss the rationale for studying natural populations, and provide a (very) brief primer of some core concepts for readers with little (or no) prior exposure to quantitative genetics.

Why Study Wild Populations?

The rationale for taking evolutionary quantitative genetics from the lab to the field is simple. Theory tells us that the natural selection leads to adaptation, but only if phenotypic variation arises, at least in part from genetic effects. Field biologists have a long and successful tradition of studying natural selection, both through the optimality framework that characterizes much of behavioral ecology (Davies *et al.*, 2012), and through formal attempts to quantify (putatively causal) relationships between phenotype and fitness (Lande and Arnold, 1983; Endler, 1986; Kingsolver *et al.*, 2001). However, even the strongest directional selection can produce no evolutionary response in the absence of genetic variance. Ultimately the adaptive potential of any population thus depends on the presence of genetic variance for fitness itself (Fisher, 1930). So if we want to understand how traits evolve, and why sometimes they do not (Merilä and Sheldon, 2000), we need to know about genetics as well as selection. How much genetic variation is there in wild populations? How is it distributed within- and among-traits that effect fitness? Do genetic correlations among traits act as evolutionary constraints? Are there interactions between genetic and environmental effects on traits, and – more broadly – how do evolutionary and ecological processes influence each other under natural conditions? These are the sorts of questions that can be, and are being, addressed by researchers using quantitative genetic methods in natural populations.

Given the goal of understanding genetic variation in natural populations, researchers may use data collected from wild populations organisms animals *in situ* (i.e., in the field) or under artificial (or seminatural) conditions in the laboratory. The latter approach is not always feasible, but can be readily applied to studies of insects (Niemela *et al.*, 2013), rodents (Schroderus *et al.*, 2012), some fish (Dingemanse *et al.*, 2012), and many plant species (Pujol *et al.*, 2014). Lab-based studies using wild caught individuals (and their offspring) are sometimes criticized on the grounds that resulting quantitative genetic parameters estimates are less ecologically relevant. In particular, the risk is that, as a consequence of genotype-by-environment interactions, estimated patterns of genetic covariance among traits in the lab will not represent those in the natural environment. Nonetheless, we would argue this drawback will often be more than offset by the increase in experimental tractability. So while we focus primarily on *in situ* studies of wild populations in this article, we take the view that lab and field-based studies are much better seen as complementary, rather than competing, approaches.

Genetic Variation and Covariation: A Brief Primer

Classical quantitative genetics uses known relationships, or pedigree structures, to investigate genetic variation in, and covariation between, phenotypic traits of interest (Figure 1). If the statistical methods used can sometimes seem complex, at least the underlying principle is straightforward. If variation in genes is an important determinant of phenotypic variation then related pairs of individuals will tend to be phenotypically similar because they share alleles identically by descent (IBD). The more closely related individuals are, and so the more alleles they share, the more similar their phenotypes should be. Thus, by statistically quantifying the association between phenotypic and genetic similarity (i.e., relatedness) among individuals in a population we can estimate the genetic variance for a trait. In fact genetic variance itself can be further partitioned into additive and nonadditive components (the latter arising from dominance, epistasis, etc.; Falconer and McKay, 1996). In most wild quantitative genetic studies, the

additive genetic variance is of primary interest since it determines the predictable response to selection (and, more pragmatically, estimating other genetic components is difficult). The more additive the genetic variance, the more rapid a selection response can be. This idea is encapsulated in the univariate breeder's equation, $R = h^2S$. Here R is the per generation change in the mean phenotype (the 'response') which can be predicted as the product of S, a coefficient that measures the direction and strength of selection, and h^2, the narrow sense heritability. Heritability here is defined as the proportion of the phenotypic variance (V_P) attributable to the additive genetic variance (V_A). In reality, the breeder's equation is too simple to generally (if ever) be appropriate for predicting phenotypic change in the wild. This is because almost all the assumptions underpinning the model are likely to be violated in natural populations (Morrissey *et al.*, 2010). However, the point it illustrates nicely is that evolution (R) depends on both selection (S) and genetics (h^2).

In the same way that genetic effects can account for some of the observed trait variation, they may also explain covariation (or correlation) among traits. For instance, in many animals we find that larger individuals are (on average) more fecund. The positive covariance between body size and litter (or clutch) size may be due to environmental and/or genetic effects. If an individual finds itself in a good habitat patch it may acquire more resource than average, allowing it to invest heavily in both growth and reproduction. Here trait covariance is driven by environmental effects. However, if size is heritable, it is also possible that the same genotypes that cause large size also predispose to producing more offspring. One can estimate the contribution of (additive) genetic effects to the covariance COV_A, which is often standardized to a genetic correlation r_G.

For a pair of traits x, y we can define $r_{Gxy} = \frac{COV_{Axy}}{\sqrt{V_{Ax}V_{Ay}}}$.

Nonzero genetic correlations arise from underlying pleiotropy and/or linkage and mean that traits are not genetically independent of one another. As a consequence their selection responses will not be independent either (discussed below).

Development of Quantitative Genetics in the Wild

Applying quantitative genetic approaches to studies of natural populations is not completely straightforward, firstly because relationships among individuals are typically unknown, and secondly because datasets that from wild populations tend to be very different to those collected in carefully planned laboratory experiments. As such analyzing the data requires a somewhat different approach. The development of the field has been tightly linked to two methodological advances that have allowed empiricists to overcome these challenges. The first involved inferring relatedness using molecular markers (Garant and Kruuk, 2005). The second involved the adoption of advanced statistical techniques originally developed by animal breeders (Wilson *et al.*, 2010).

Inferring Relatedness

Estimating genetic variance requires knowing the relatedness among individuals (i.e., the proportion of the genome shared identically by descent). In wild populations where relatedness is typically unknown, two strategies are possible. The first consists in predicting relatedness from an inferred pedigree, with values of, for example, 0.5 between parents and their offspring, 0.25 between half siblings, and 0.125 between cousins. The second is to use estimates of pairwise relatedness made without an explicit pedigree structure.

To determine a pedigree structure it is sometimes possible to infer relationships from observations of social interactions (e.g., nestlings or mother–offspring pairs identified from suckling in mammals). However, this approach can only be used in a few taxa and can be inaccurate. For instance, assuming nestlings are full-siblings is problematic in many passerine birds because extra-pair paternity is common. This, in turn, can lead to biased quantitative genetic parameters and evolutionary inferences (Charmantier and Reale, 2005). The development of molecular markers, mainly microsatellite loci but increasingly single nucleotide polymorphism (SNP) has, broadly speaking, provided a solution to this issue. Given a relatively small set of genetic markers (typically 10–40 microsatellites), one can typically resolve close relationships within a population (e.g., parent–offspring and full-sibs) with satisfactory accuracy, allowing to reconstruct a 'molecular' pedigree even in the complete absence of behavioral information (Pemberton, 2008). Although applicable to a large number of species, molecular pedigree reconstruction is nonetheless dependant on the presence of close relatives, meaning that the approach is most useful in (usually small) populations where a fair number of close relative are likely to be sampled. It should also be noted that molecular pedigrees are generally still imperfect due to genotyping errors and incomplete sampling (Morrissey *et al.*, 2007; Morrissey and Wilson, 2010).

An alternative to using pedigrees to predict relatedness lies in estimating relatedness directly between all pairs of individuals using molecular markers (Wang, 2014). In theory this approach could be superior to pedigree-based methods because it estimates realized rather than expected relatedness (Visscher *et al.*, 2006; Speed and Balding, 2015). For example, under the pedigree approach all full-sibs pairs are assumed to share exactly 0.5 of their genome IBD, while in reality they vary around an expected population mean of 0.5 (Visscher *et al.*, 2006; Speed and Balding, 2015). However, to date the pedigree approach has been preferred in wild populations because estimating genome-wide relatedness accurately requires a very large number of markers. In particular, methods developed to take advantage of the small microsatellite datasets typical of molecular ecology studies over the last two decades tend to be statistically very 'noisy' (Ritland, 1996; Wilson *et al.*, 2003; Frentiu *et al.*, 2008). Note however that this issue can now be overcome by using very high densities of SNP markers (Bérénos *et al.*, 2014) and we anticipate a large growth in such 'pedigree-free' quantitative genetic studies in the very near future as a consequence of the adoption of next generation sequencing technologies.

Linear Mixed Effect Modeling and the 'Animal Model'

Heritabilities, genetic correlations, and other quantitative genetic parameters can be estimated from simple statistical

techniques including linear regression and ANOVA. In particular, parent–offspring regression was used in a large number of early field studies (Grant and Grant, 1995). However, these methods are not very well suited to analyzing the patchy and unbalanced datasets (i.e., lots of missing data and variable family sizes) or complex pedigree structures (i.e., pedigrees containing many classes of relationship, often spanning multiple generations) typical of studies in the wild. Nor do these methods easily allow a researcher to account for uncontrolled environmental effects, for instance spatial or temporal variation in habitat quality. For evolutionary ecologists testing environmental effects on phenotype is often an important aim in itself. In the context of quantitative genetic analyses, when relatives share environments (e.g., nest, maternal, or cohort effects) that are likely to influence phenotypes of interest, accounting for these 'common environment effects' is important to prevent environmentally driven resemblance among relatives from biasing estimates of genetic (co)variances (Kruuk and Hadfield, 2007).

The growth of quantitative genetics in the wild has been driven to a large degree by adoption of linear mixed effect models, and in particular the polygenic 'animal model,' in place of earlier statistical techniques. It is worth pointing out here that animal models are equally applicable to plants; the terminology simply reflects the method's development to allow analysis of complex pedigree structures as found in livestock systems. Early applications of animal models to natural systems immediately demonstrated its enormous potential (Réale *et al.*, 1999; Réale and Festa-Bianchet, 2000; Kruuk, 2004), and this is now by far the most widely used analytical approach. A detailed explanation of the animal model is beyond the scope of this article but see, for example, Kruuk (2004), Postma and Charmantier (2007), and Wilson *et al.* (2010) for treatments intended to be accessible for readers with limited statistical or genetic expertise. For present purposes, it suffices to say that this approach allows estimation of additive genetic (co)variance structures underpinning patterns of trait variation and covariation. To do this, animal models include, for each trait, an individual's genetic merit or 'breeding value' as a random effect. Breeding values are latent variables that represent the effect of an individual's genotype relative to the population mean phenotype. They are usually defined as coming from a normal distribution with mean zero and variance equal to V_A – the additive genetic variance. The expectation that breeding values will covary among individuals as a function of relatedness, means that we can estimate V_A provided we have pedigree (or relatedness) and phenotypic data for individuals in the population.

The animal model approach is equally applicable to single traits or multivariate phenotypes, and so can be used to estimate genetic covariances (COV_A). It is also highly flexible and can, for example, include additional fixed and random effects to account for nongenetic intrinsic sources of variation within and among individuals (e.g., age, date of capture, and sex) and environmental sources of resemblance among individuals (e.g., nest, spatial, or cohort effects). Models can be generalized to cope with non-Gaussian errors (e.g., if the trait of interest is binary or a count), and may be fitted using frequentist (e.g., restricted maximum likelihood) or Bayesian (e.g., Markov Chain Monte Carlo) inference methods in a range of statistical software applications (Gilmour *et al.*, 2006; Ovaskainen *et al.*, 2008; Hadfield, 2010). A brief overview of popular software and associated tutorials are provided in Wilson *et al.* (2010).

Lessons and Limitations

Quantitative genetic analyses have now been used to test specific evolutionary hypotheses about a wide range of phenomena in wild populations. Examples include, but are not limited to studies of maternal effects (McAdam *et al.*, 2002; Wilson *et al.*, 2005), parental care (MacColl and Hatchwell, 2003), mating systems (Reid *et al.*, 2014), phenotypic plasticity (Nussey *et al.*, 2005), senescence (Brommer *et al.*, 2007; Wilson *et al.*, 2008), sexual conflicts (Brommer and Rattiste, 2008; Poissant *et al.*, 2008), social evolution (Charmantier *et al.*, 2007), adaptation to climate change (Gienapp *et al.*, 2008), and predicting selection responses (Morrissey *et al.*, 2012a). This diversity of topics recapitulates the earlier point that quantitative genetic theory and methodology is very general. Space precludes detailed review of empirical findings here and the reader is referred elsewhere for more comprehensive treatments (see, e.g., Gienapp *et al.*, 2008; Kruuk *et al.*, 2008; Wilson *et al.*, 2008; Charmantier *et al.*, 2014). However, some general points emerging from studies to date are worth highlighting.

Genetic Variance Is Widespread and Abundant

A general conclusion emerging from the wealth of empirical studies is that natural populations harbor a good deal of additive genetic variance. This is often true for traits thought to be under strong selection (Kruuk *et al.*, 2008) and in some cases heritable variation has been found even for components of fitness itself (e.g., survival and reproduction; Wilson *et al.*, 2007a). Since selection is expected to reduce genetic variance by fixing beneficial alleles and purging deleterious ones, this has led to interest in the question of what maintains genetic variation (Hunt *et al.*, 2007; Walsh and Blows, 2009). In fact, while it has long been argued that there should be an inverse relationship between genetic variation (measured as, e.g., trait heritability) and the strength of selection, empirical evidence for this is mixed. Where heritability does decline with increasing selection, this pattern may be driven more by environmental contributions to V_P than by changes in V_A (e.g., Merilä and Sheldon, 2000; McCleery *et al.*, 2004, but see Teplitsky *et al.*, 2009 for a counter-example).

Evolutionary Change Is Hard to Predict and to Detect

The widespread finding of (within-population) additive genetic variance leads to an expectation of phenotypic change under the simplest evolutionary models (e.g., the univariate breeder's equation). This is because if traits are usually heritable, meta-analyses of natural selection studies indicate that directional selection (i.e., favouring phenotypic change) is also common (Kingsolver *et al.*, 2001). Conversely, the evidence for stabilising selection or selection that fluctuates in direction over temporal

or spatial heterogeneity appears less compelling (Kingsolver *et al.*, 2012; Morrissey and Hadfield, 2012; Siepielski *et al.*, 2013). While examples of rapid phenotypic change in wild populations are well documented (Hairston *et al.*, 2005), the extent to which these are explained by genetic responses to directional selection (i.e., evolutionary change) as opposed to, for instance, phenotypic plasticity and/or changing population demography (Ozgul *et al.*, 2010) is often less clear and represents a very active line of inquiry (Gienapp *et al.*, 2008).

In fact, for those systems where estimates of both selection and heritability are available, there often seems less change than the breeder's equation predicts (Merilä *et al.*, 2001). There are many possible reasons for mismatches between predicted evolution and observed phenotypic change (see, e.g., Merilä *et al.*, 2001; Walsh and Blows 2009; Morrissey *et al.*, 2010 for in-depth discussion). For instance, there could be evolutionary constraints arising from genetic correlations between a focal trait and other, correlated, traits under selection (Walsh and Blows, 2009); a point we return to below. Alternatively, we may have fundamentally misidentified the 'targets' of selection, interpreting trait-fitness correlations as causal (i.e., selection) when they are not (Morrissey *et al.*, 2010). It is also important to realize that quantitative genetic models predict responses to selection only. This can only reasonably be equated to total phenotypic change if environments are constant and genetic drift is negligible, conditions that will rarely (if ever) hold true. Separating genetic from nongenetic components of phenotypic change is possible using animal model-based approaches (Postma, 2006; Hadfield *et al.*, 2010). In simple terms, temporal trends in (predicted) breeding values over time (e.g., year of birth) should be indicative of genetic change in the population. However, while such trends have been widely claimed in the literature (Coltman *et al.*, 2003; Garant *et al.*, 2004; Wilson *et al.*, 2007b), they are no longer considered statistically robust (see Hadfield *et al.*, 2010 for an explanation of the problem but also a solution to it). This does not mean genetic responses to selection are not occurring in quantitative traits, but it does mean that unambiguously identifying and quantifying them is harder than was previously thought.

Trade-Offs May Not Be as Important as We Thought

Accepting that there is less evolutionary change than might, at least naively, be predicted from our estimates of selection and heritability, we must consider the question of what constrains adaptive evolution. Perhaps the most widely hypothesized form of evolutionary constraint is that of the trade-off between two traits (or fitness components) that 'compete' for resource allocation. An individual with limited resource can only allocate more to one trait (e.g., egg size) at a cost to the other (e.g., egg number). It is widely held that genetic correlations between traits involved in such resource allocation trade-offs should constrain evolution (Roff, 1997). With traits defined so as to be under positive selection (e.g., fecundity and survival), then a constraint arises from a negative genetic correlation. In this scenario, genotypes associated with high fecundity are not necessarily selectively advantageous because they come with the cost of reduced survival. In other words, selection is unable to optimize each trait independently, and the predicted outcome will be a compromise.

Interestingly, while trade-offs are central to many models of evolutionary outcomes in behavioral ecology and life history studies, quantitative genetic analyses of wild populations have often failed to document the negative genetic correlations predicted. In fact positive genetic correlations seem rather more prevalent (e.g., Coltman *et al.*, 2005; Kruuk *et al.*, 2008 for broader scale patterns). This suggests that wild populations can sometimes harbor genetic variance in individual 'quality' *sensu* Wilson and Nussey (2010), perhaps because high-quality genotypes predispose toward greater resource acquisition (de Jong and van Noordwijk, 1992; Wilson, 2014). This is not to say that trade-offs are not important, and there is clearly much work yet to be done. For instance, wider testing of the hypothesis that genetic correlations across environments constrain evolution (Sgrò, 2004; Robinson *et al.*, 2009) would be useful. However, taken at face value, the estimates of genetic correlations emerging from empirical quantitative genetic studies pose an interesting challenge to the pervasive view in evolutionary ecology that trade-offs routinely act as important sources of evolutionary constraint. They also support the view that bivariate (i.e., two trait) models will rarely be sufficient to identify evolutionary constraint (Walsh and Blows, 2009) such that fully multivariate studies are required (discussed further below).

Increasing the Range of Study Organisms and Focal Traits

The need for deep and well-connected pedigrees, combined with a strong interest toward systems were fitness metrics such as lifetime reproductive success are available has led to quantitative genetic studies in wild populations being disproportionately focused on a relatively small number of long-term ecological data sets in which individuals are marked and tracked across their lifetimes (Clutton-Brock and Sheldon, 2010; for specific examples see Kruuk *et al.*, 2002; McAdam *et al.*, 2002; Coltman *et al.*, 2005; Brommer *et al.*, 2007). These datasets are, of course, immensely valuable for all sorts of studies in ecology and evolutionary biology. However, they are also taxonomically biased toward animals, and in particular toward avian (especially passerine) and mammalian (notably ungulate) species frequently living under particular conditions (e.g., super-abundance of nesting sites in the form of nest boxes, predator free islands). Furthermore, the set of traits studied has, to a degree, been constrained by past data collection practices. As a consequence, in studies to date readily measured morphological and life history traits are better represented than, for example, behavioral or physiological ones.

If we hope to learn general lessons from quantitative genetic analyses of natural populations then obtaining suitable data on a greater diversity of taxa and traits should be prioritized. In fact trait diversity is already increasing, with growing interest in, for example, the genetic basis of variation in animal personality (Dingemanse *et al.*, 2009; Poissant *et al.*, 2013), dispersal (Charmantier *et al.*, 2011), disease resistance, and immune function (Graham *et al.*, 2010). Technological development is also rapidly increasing the number of traits that can be studied, for example, gene expression (Wolf, 2013), protein expression (Diz *et al.*, 2012) and (host) microbiomes (Spor *et al.*, 2011). Taxonomic diversity may also be increased through a variety of strategies including wider user of

molecular pedigree analysis in, for instance, fish (Wilson and Ferguson, 2002; Koch *et al.*, 2008) or invertebrates (Bretman *et al.*, 2011), and application of so called pedigree-free methods (Bérénos *et al.*, 2014). Additionally, efforts to better integrate the largely animal-focussed field of wild quantitative genetics, with the field of plant evolutionary ecology should be productive (Stinchcombe, 2014), particularly since plant systems often provide greater scope for experimentation.

Analytical and Statistical Challenges

The biological insights that can be obtained from any statistical modeling approach are properly limited by the quality and quantity of data, and by the validity of the model assumptions. It is perhaps justifiable to say that successfully applying quantitative genetic methods requires a degree of statistical competence (or at least perseverance) not always essential to other approaches. The rapid growth of wild quantitative genetics has been such that – perhaps inevitably – mistakes have been made along the way (e.g., inappropriate use of predicted breeding values to test selection responses as highlighted by Hadfield *et al.* (2010)). Nonetheless, development of models, analytical methods, and tools to help the empiricist has been rapid over the last decade and looks set to continue.

It is worth bearing in mind that while all quantitative genetic studies require a relatively large amount of data, studies in natural populations often suffer from small sample sizes, and so reduced statistical power, relative to those conducted in the lab. Often this simply reflects the intrinsic difficulty of sampling wild, free-living organisms. In addition, the lack of controlled conditions and (commonly) an absence of experimental manipulation can make statistical separation of environmental and genetic influences on phenotype unreliable. This is particularly true if close relatives share environments (Kruuk and Hadfield, 2007). Explicit modeling of environmental effects helps (see Stopher *et al.*, 2012 for an excellent example), especially if combined with experimental approaches such as cross-fostering of eggs or offspring (**Figure 2**) which is possible in some taxa (Hadfield *et al.*, 2013). Of course, models with more effects means estimating more model parameters from the same (limited) amount of data. What this ultimately means is that, while insightful findings emerge from individual case studies, there is a strong need for replication and meta-analyses. This combined with recent interest in making datasets public (Roche *et al.*, 2014) means that studies interrogating multiple datasets at once (Teplitsky *et al.*, 2014) are likely to increase in popularity.

Emerging Directions

Toward a Multivariate View of Adaptation and Constraint

Natural selection does not act on phenotypic traits in isolation from each other. Rather it is the process whereby differences in fitness arise (causally) from among individual variation in the multivariate phenotype (i.e., the set of all traits describing an individual's morphology, behavior, life history, and physiology). Many traits will be under selection, and genetic correlations among them mean that they are not free to evolve independently. Explicit recognition of this leads somewhat inescapably to the view that multivariate studies will be necessary to fully understand adaptation and evolutionary constraint (Blows, 2006; Blows and Walsh, 2009; Walsh and Blows, 2009). If empiricists have been a bit slow to embrace this, opting more commonly to focus on single traits or at best pairs of traits (e.g., homologous male and female traits; Poissant *et al.* (2010) or those putatively linked by trade-offs as outlined above), then there are at least sensible and pragmatic reasons for this. Firstly, it is difficult to obtain phenotypic data on all traits that might be important determinants of fitness, particularly in the field where sampling individuals at all can be challenging! Secondly, as the number of traits to be considered rises, the number of quantitative genetic parameters to be estimated from the data increases rapidly exacerbating the statistical challenges outlined earlier.

Nonetheless, failure to accurately predict change or explain stasis from univariate and bivariate studies of wild populations is leading researchers to model larger sets of traits. The starting point is usually to estimate the additive genetic variance–covariance matrix G among traits and a vector describing directional selection on each trait β, to predict the multivariate response (R) using the multivariate breeders equation, $R = G\beta$ (Lande, 1982). **Figure 3** illustrates this for the simplest (two traits) case, in which negative genetic covariance between positively selected traits constrains adaptation (i.e., the trade-off scenario discussed earlier). Importantly however, this multivariate approach to quantifying adaptive potential and constraint extends to any number of traits in a multivariate phenotype (Coltman *et al.*, 2005; Agrawal and Stinchcombe, 2009; Morrissey *et al.*, 2012b; Teplitsky *et al.*, 2014; Walling *et al.*, 2014).

Social Interactions and Shared Phenotypes

Quantitative genetics allows us to disentangle genetic from environmental influences on phenotype. However, environmental effects on a focal animal's phenotype (and fitness) often arise through social interactions with other individuals. For example, postnatal growth rates in mammals depend on the quantity and quality of milk provided by mothers. In this case, offspring phenotype (growth) depends on the 'environment' provided by a maternal phenotype (milk production). However, if maternal milk production is heritable, then there is a causal link between the genotype of one individual (the mother) and the phenotype of another (the offspring). This type of maternal effect represents one example of what is termed as indirect genetic effect (IGE; Moore *et al.*, 1997) or associative effect (Griffing, 1967, 1976). In the presence of IGE, genetic control of observed phenotypes is shared between interacting individuals, which could be parents and offspring (McAdam *et al.*, 2002; Wilson *et al.*, 2005), mating partners (Brommer and Rattiste, 2008; Teplitsky *et al.*, 2010), competitors (Wilson *et al.*, 2011), or cooperators (Charmantier *et al.*, 2007). Because individuals – and so their genotypes – determine the social environment, social environments can themselves evolve. This could have major implications for our understanding of trait evolutionary dynamics in wild populations. For example, if individuals differ (genetically) in

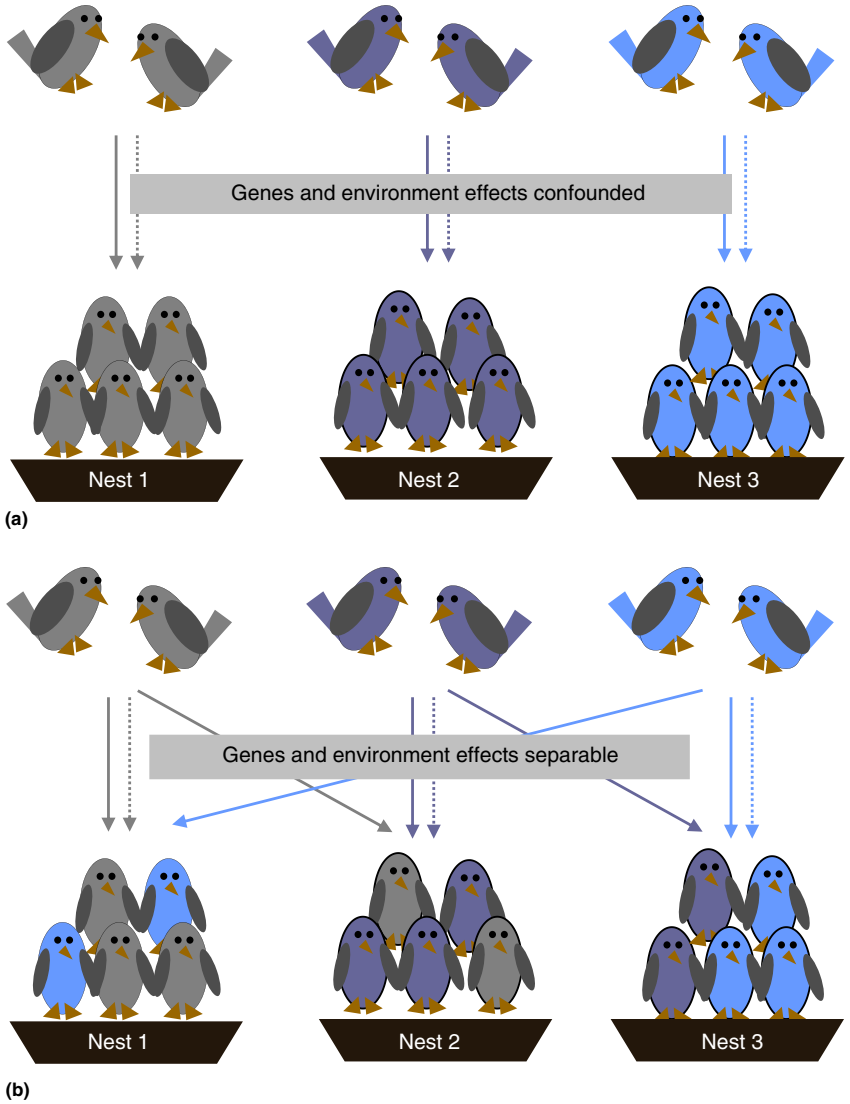

Figure 2 Cross-fostering is an experimental technique useful for disentangling genetic from environmental sources of variation in offspring phenotype. Parental birds influence offspring phenotype both via genetic inheritance (solid arrows) and through provision of the early life environment (dotted arrows, e.g., amount of food being provisioned to the nest). In the absence of experimental manipulation (a) these effects are completely confounded within each family (color) and so difficult to tease apart statistically. This problem is alleviated by cross-fostering designs in which hatchlings (or eggs) are exchanged between nests. Individuals now have genetic siblings elsewhere (i.e., sharing genes but not environment) and foster-siblings in their own nests (i.e., sharing environment but not genes). Cross-fostering is most widely used in wild bird studies but has also been successfully applied to mammals and insects in the lab.

competitive ability then we predict that IGE will act to constrain adaptation of resource dependent life history traits (Wolf, 2003; Hadfield *et al.*, 2011; Wilson, 2014). To date maternal effects have been the most widely studied form of IGE (McAdam *et al.*, 2014), but rapid development of theory and statistically tractable models (Bijma *et al.*, 2007; Bijma and Wade, 2008) means that the IGE framework will increasingly be used to model the quantitative genetics of social interactions.

Genetic Architecture of Quantitative Traits

Classical quantitative genetics approaches do a fairly good job at describing trait inheritance (Lynch and Walsh, 1998) and provide us with predictive models for adaptive phenotypic evolution (Morrissey *et al.*, 2010). However, they do not allow characterizing the loci underpinning trait variation, which considerably limits our understanding of the mechanisms and evolutionary processes involved (Mackay, 2001; Barrett and Schluter, 2008; Slate *et al.*, 2010). While substantial progress has been made toward describing the genetic architecture of quantitative traits in humans (Stranger *et al.*, 2011), agricultural species (Hayes and Goddard, 2001; Hayes *et al.*, 2010), and laboratory models such as *Drosophila melanogaster* and *Mus musculus* (Flint and Mackay, 2009), similar research in natural populations is still in its infancy (Slate *et al.*, 2010; Schielzeth and Husby, 2014). Mostly, this is due to past challenges in developing and genotyping large sets of markers

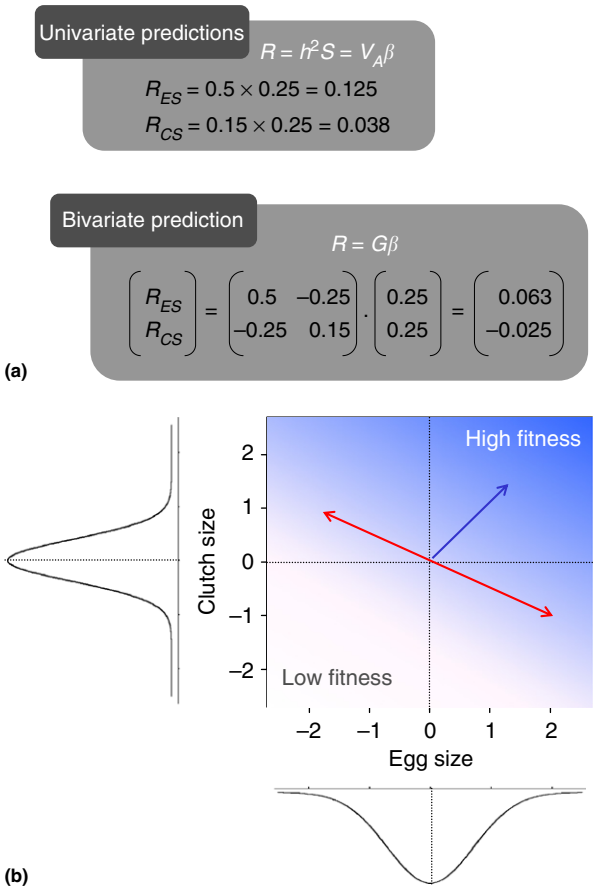

Figure 3 Trade-offs as a source of evolutionary constraint. Imagine a population in which clutch size (CS) and egg size (ES) are both under positive direction selection (with standardized selection gradients β equal to 0.25 on both traits) and genetically variable (with $V_{A(ES)} = 0.5$ and $V_{A(CS)} = 0.15$). While the univariate breeder's equation predicts a moderate increase in mean phenotype for each trait, the predictions responses under a bivariate model are much if there is a negative genetic correlation between them (here $COV_A = -0.25$ so $r_G = -0.91$). In fact, the bivariate model actually predicts a decrease in mean clutch size despite the positive selection on it. This scenario is depicted graphically in (b) where breeding values (black circles) for each trait are normally distributed but also covary negatively. This means that the major axis of genetic variation for the bivariate phenotype (red line) does not align with the direction of selection (blue arrow). Some adaption (i.e., evolution toward darker blue phenotypic space) is still possible so the constraint is not absolute. However, the rate of adaption is limited by the relative lack of genetic variation in the direction of selection.

in non-model organisms. However, as new technologies and approaches are now allowing rapid genotyping at tens to hundreds of thousands of SNP markers at reasonable cost in any species (Slate *et al.*, 2009; Davey *et al.*, 2011), research on the genetic architecture of quantitative traits in natural populations is set to blossom.

Traditionally, research on genetic architectures has focussed on mapping and characterizing loci responsible for phenotypic variation (Lynch and Walsh, 1998). The general principle behind quantitative trait locus (QTL) mapping is fairly simple. In brief, linkage disequilibrium (LD) between genetic markers and causative polymorphisms generates statistical associations between markers and phenotypes, and knowledge about the position of markers in the genome allows 'mapping' those associations (Lynch and Walsh, 1998). In unmanipulated natural populations, QTL are usually mapped using genome-wide pedigree linkage analysis (George *et al.*, 2000; Slate, 2005) or association studies (GWAS; McCarthy *et al.*, 2008). The first approach relies on LD generated by recombination events within pedigrees while the second relies on LD generated by historical recombination (Devlin and Risch, 1995). GWAS offer several advantages over pedigree linkage analysis, including much finer resolution (Devlin and Risch, 1995), increased power (Risch and Merikangas, 1996), and the ability to estimate allele-specific effects. GWAS also offer the practical advantages of not being dependent on the availability of genetic linkage maps, provided that marker positions can be inferred from a genome sequence, and of not requiring pedigree information (Slate *et al.*, 2010; Schielzeth and Husby, 2014). Nonetheless, so far pedigree linkage analysis has been favoured because it only requires a few hundred markers whereas efficient GWAS generally require tens if not hundreds of thousands of markers (Schielzeth and Husby, 2014). However, as large SNP datasets can now be generated with relative ease, GWAS are set to become more common in wild populations (Schielzeth and Husby, 2014).

In situ research in natural populations provides a unique opportunity to study naturally segregating adaptive standing genetic variation in an ecologically realistic setting (Ellegren and Sheldon, 2008). However, it must be stressed that researchers are facing important challenges (Slate *et al.*, 2010; Jensen *et al.*, 2014; Schielzeth and Husby, 2014). In particular, research in humans and other well-studied organisms indicates that QTL mapping often fails to identify many of the loci influencing quantitative traits (Manolio *et al.*, 2009). In studies of natural populations where sample sizes are usually comparatively modest this is likely to be an important issue (Santure *et al.*, 2013; Slate, 2013). This difficulty will also often be exacerbated by an inability to replicate or validate results due to the long-term and unique nature of some study systems and Genotype-by-environment interactions. In that context, new approaches aimed at describing general attributes of genetic architectures, rather than identifying individual loci *per se*, such as partitioning genetic variance among chromosomes or chromosome segments (Hayes *et al.*, 2010; Yang *et al.*, 2011; Robinson *et al.*, 2013), may prove to be particularly valuable. For example, information on the mean and variance of effect sizes across chromosomal segments could be combined with selection estimates to empirically test for a hypothesized relationship between genetic architectures and selection (Rajon and Plotkin, 2013).

Conclusions

Since the late 1990s we have witnessed a rapid increase in the number of quantitative genetic studies of wild populations *in situ* (Charmantier *et al.*, 2014). As developments in genotyping technologies and analytical techniques are now set to democratize this type of analysis to virtually any species, the

field will likely see an even more dramatic increase in popularity in the years to come. Although there are important limitations and challenges, if the goal is to understand phenotypic evolution in the wild as a dynamic and ongoing process, then quantitative genetic analyses provide clear benefits. This is because, in contrast to other approaches in evolutionary ecology (e.g., behavioral ecology and life history theory), it rejects the implicit assumption that adaptation can be studied while ignoring all genetic considerations. By recognizing that adaptive evolution depends on both natural selection and genetic (co)variation, quantitative genetic studies of wild populations provide understanding of both how natural selection has shaped phenotypes in the past, and how it will do so in the future.

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See also: Adaptive Landscapes. Genotype-by-Environment Interaction. Maternal Effects. Multivariate Quantitative Genetics. Natural Selection, Measuring. Quantitative Genetic Variation. Social Effects

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Quantitative Genetic Variation

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Natural populations exhibit variation for most measurable aspects of their phenotypes: morphology, physiology, behavior, and disease susceptibility. This phenotypic variation is typically continuously distributed and correspondingly these phenotypes are called 'quantitative traits' (Falconer and Mackay, 1996; Lynch and Walsh, 1998). The continuous variation arises from genetic complexity and environmental sensitivity: segregating alleles at multiple loci mean that many genotypes can give rise to the same phenotype, and the same genotype can have different phenotypic effects in different environments. Quantitative genetic variation is the substrate for phenotypic evolution in natural populations and for selective breeding of domestic crop and animal species. Quantitative genetic variation also underlies susceptibility to common complex diseases and behavioral disorders, as well as responses to pharmacological therapies. Knowledge of the detailed genetic underpinnings of variation for quantitative traits is thus critical for addressing unresolved evolutionary questions about the maintenance of genetic variation for quantitative traits within populations and the mechanisms of divergence of quantitative traits between populations and species; for increasing the rate of selective improvement of agriculturally important species; and for the development of novel and more personalized therapeutic interventions for improving human health.

The birth of genetics began with Mendel's discovery of the laws of segregation and independent assortment of alleles with large qualitative effects that can be tracked in pedigrees. However, these laws seemed to contradict the observation that many traits vary continuously and that offspring phenotypes were often near the average of both parents. Early in the twentieth century, Fisher resolved the apparent contradiction between the discrete nature of Mendelian inheritance with the continuous variation for quantitative traits, and provided the theoretical basis for understanding quantitative trait genetics (Fisher, 1918). Each locus affecting a quantitative trait has the same properties as a Mendelian locus. The differences are that the phenotypic effects are smaller and need to be measured (as opposed to categorized); there are many such loci acting together; the quantitative effects are sensitive to the environment; and the population allele frequencies need to be taken into account.

Properties of Quantitative Traits

To understand how individual loci with discrete allelic differences can give rise to patterns of trait variation, we can consider the simple case of a single diploid locus with two alleles, and thus three genotypes (A_1A_1, A_1A_2, A_2A_2; by convention, the A_2A_2 genotype is associated with decreased values of the trait). Building from this simple bi-allelic model is not meant to imply that traits in nature have such a simple genetic basis, but rather, provides the foundation upon which our understanding of multi-locus inheritance can be constructed. The mean value of each genotype is the population mean, averaged across all other segregating genotypes and the range of environments experienced by the population. These mean values, which are expressed in the units in which the phenotype is measured, can be assigned general genotypic values of $+a$ (A_1A_1), d (A_1A_2), and $-a$ (A_2A_2). These values are scaled relative to the midpoint between the two homozygotes, which is set to zero. Thus the additive effect, a, is one half of the difference in mean phenotype between the two homozygous genotypes; and the dominance effect, d, is the difference between the mean phenotypic value of the heterozygous genotype and the average of the two homozygous genotypes (Falconer and Mackay, 1996). d can vary to express any degree of dominance from $d = +a$ (the A_1 allele is dominant), $d = -a$ (the A_2 allele is dominant) to anything in between. If $d = 0$, we call the effect additive (or co-dominant) because the mean phenotype of heterozygotes is the same as the mean of the two homozygotes; $d > +a$ and $d < -a$ represent cases of over- and under-dominance, respectively.

Mendelian loci often exhibit a phenomenon called 'epistasis,' whereby the effects of one locus mask the effects of another and give rise to distorted segregation ratios in a cross of doubly heterozygous genotypes. Epistasis can be modeled for quantitative traits by considering the joint effects of two loci affecting the trait, A and B, each with two alleles. Thus, there are 9 possible two locus genotypes. If the effects of these genotypes can be predicted given the homozygous or dominance effects at each of the two loci (e.g., the effect of genotype $A_1A_2B_1B_1$ is $d_A + a_B$), then we say the two loci act additively. Note that we use the same word, additive, to refer to absence of dominance at a single locus and absence of epistasis for multiple loci. If, however, the effects of the two locus genotypes are not equal to those expected from the two individual loci, then there is epistasis, which is parameterized by additive by additive (aa_{AB}), additive by dominance (ad_{AB}, da_{AB}), and dominance by dominance interactions (dd_{AB}) (Falconer and Mackay, 1996; Mackay, 2014). These interaction effects are not restricted to the traditional epistatic effects detected for Mendelian loci, but can take on any quantitative value. The hallmark of epistasis for a quantitative trait is that the additive and/or dominance effect of one locus varies, depending on the genotype of the interacting locus (Mackay, 2014). Of course epistatic interactions are not constrained to occur between only two loci but can be of higher order and very difficult to parameterize.

Alleles affecting quantitative traits can take on a range of effects depending on the physical and social environment experienced by the individuals with the same genotype, a phenomenon called 'phenotypic plasticity.' These effects can be large, if the individuals are reared in very different environments (sometimes called macro-environments) or more subtle, when environmental conditions are tightly controlled. Thus, even genetically identical individuals reared in the same environments will have different phenotypes, called environmental

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noise or micro-environmental plasticity (Falconer and Mackay, 1996; Lynch and Walsh, 1998). To further complicate matters, different genotypes can have differential plastic responses to the same large environmental change (called genotype by environment interaction (GEI)) or the same range of micro-environments (Falconer and Mackay, 1996; Lynch and Walsh, 1998). In addition, different genotypes may experience different environments because of their phenotype (called genotype–environment correlation). Examples of the latter include feeding dairy cows according to their milk yield, training race horses according to their pedigree, and 'niche construction' in nature (Falconer and Mackay, 1996).

Finally, quantitative trait phenotypes can differ between different populations if the allele frequencies at the loci affecting the trait differ, even if the underlying genetic architecture is identical. This is easy to visualize by considering just one locus. If a population is fixed for the A_2A_2 genotype, then the population mean will be $-a$, whereas if the population is fixed for the A_1A_1 genotype, the population mean will be $+a$.

When we say that we wish to understand the genetic architecture of any quantitative trait, this is what we need to know (Mackay, 2001). (1) The numbers and identities of genes at which mutations affecting the trait arise, and the subset of these genes at which alleles affecting the trait segregate in natural populations. (2) The distribution of allelic effects of new mutations and segregating variants in nature. This could range from alleles of large effect segregating at a few loci to alleles of small effect segregating at many loci; or an exponential distribution of allelic effects with alleles with moderately large effects segregating at a few loci and alleles with small effects segregating at a large number of loci. (3) The effects of new mutations and segregating alleles on other quantitative traits (pleiotropy), including intermediate molecular phenotypes and reproductive fitness. (4) The manner in which alleles at multiple loci interact (additive or epistatic). (5) The answers to the above questions in a range of ecologically relevant environments, and the magnitude of GEI. (6) The identity, site class (i.e., regulatory or coding regions), and allele frequencies of causal molecular variants.

This seems like an impossible task, given the nonlinearity of the genotype–phenotype map for quantitative traits. In the pre-genome era, the general principles of genetic architecture have been worked out by realizing that although the loci cannot be individually distinguished, they collectively affect the trait variance. Further, relatives resemble each other for quantitative trait phenotypes; the extent of the resemblance depends on how close the relationship is. These two principles can be combined to infer the relative contribution of genetic and environmental variance to the phenotypic variance of a quantitative trait by measuring trait phenotypes on collections of related individuals (Falconer and Mackay, 1996; Lynch and Walsh, 1998). This allows the relative contributions to be estimated, and for the different sources of genetic variation to be distinguished by comparing different types of relatives, as described in more detail below.

Partitioning Variance Components

We begin with a description of the components of phenotypic variation (σ^2_P) for the trait; a quantity that can be readily estimated by measuring the trait on a sample of individuals from the population of interest. We know that we can broadly partition this into a component attributable to all sources of genetic (σ^2_G) and environmental (σ^2_E) variation. The genetic variance can be further partitioned into components consisting of additive genetic variance (σ^2_A), dominance variance (σ^2_D), and epistatic interaction variance (σ^2_I) (Falconer and Mackay, 1996; Lynch and Walsh, 1998). These genetic variance components are themselves complicated, and are functions of additive, dominance, and epistatic effects of all loci contributing to genetic variation of the trait and their allele frequencies. Genotype–environment correlation adds $2cov_{GE}$ (the correlation is parameterized by the covariance to retain the units of variation) to the phenotypic variance; and genotype–environment interaction adds σ^2_{GE} (Falconer and Mackay, 1996; Lynch and Walsh, 1998). In experimental quantitative genetic analyses, genotypes are randomized across standardized environmental conditions, so there should be no contribution of genotype–environment correlation or interaction to the phenotypic variance. However, this is not possible in natural settings. If cov_{GE} exists and we do not know about it, the practical consequence is that it is considered part of the genotype, and σ^2_G is increased by $2cov_{GE}$ (Falconer and Mackay, 1996). If we do not know the environments experienced by the individuals, σ^2_{GE} increases σ^2_E. σ^2_E can itself be partitioned into components due to any shared common spatial ($\sigma^2_{Ec(S)}$), temporal ($\sigma^2_{Ec(T)}$), or maternal ($\sigma^2_{Ec(M)}$) environments, which increase the phenotypic resemblance of related individuals over and above that due to genetic relatedness, and the residual, environmental variance (σ^2_{EW}) (Falconer and Mackay, 1996; Lynch and Walsh, 1998). In experimental quantitative genetic studies, $\sigma^2_{Ec(S)}$ and $\sigma^2_{Ec(T)}$ can usually be avoided or estimated; $\sigma^2_{Ec(M)}$ can be more problematic, especially for mammals.

Now let us consider the causes of phenotypic resemblance among relatives: this is because they share the same alleles and sometimes genotypes (called genetic covariance) and also because they share environments (common environmental variance). (Note that here we refer to the genetic covariance among individuals, not between traits as discussed in later chapters.) The probability that relatives share alleles due to having a common ancestor is the coefficient of relatedness, r; and the probability that relative share genotypes due to common ancestry is u. In a random breeding population, $r = ½$ for parents and offspring and full-siblings (both parents in common); $r = ¼$ for half-siblings (one parent in common), and $r = 1$ for monozygotic twins. Relatives in a random mating population can only share genotypes due to common ancestry if their pedigree can be traced back to the same individual(s). For example, $u = 0$ for parents and offspring and half-siblings and $u = ¼$ for full-siblings. The following expression gives the genetic covariance for any degree of relationship in a random mating population: $cov_G = r\sigma^2_D + u\sigma^2_D + r^2\sigma^2_{AA} + ru\sigma^2_{AD} + u^2\sigma^2_{DD} + r^3\sigma^2_{AAA} + r^2u\sigma^2_{AAD} + ru^2\sigma^2_{ADD} + u^3\sigma^2_{DDD}$ etc. (Falconer and Mackay, 1996; Lynch and Walsh, 1998). For most relatives, the largest coefficient is for the additive genetic variance, and coefficients on the epistatic variance terms are small (at most ¼). The exception is monozygotic twins, for which $r = u = 1$. However, it should be noted that all estimates of additive variance from data on relatives include

fractions of the higher order additive by additive interaction variances, and that for n loci there are n additive effects but of the order of n^2 pairwise interactions, n^3 three-way interactions, etc.

Inbreeding (mating of related individuals) causes a re-distribution of genetic variance between and within inbred lines. If all genetic variance is additive, the variance within inbred lines is $(1-F)\sigma_A^2$ and between inbred lines is $(1+F)\sigma_A^2$, where F, the inbreeding coefficient is the probability that alleles are identical by descent (i.e., from the same common ancestor) (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Thus, if lines are inbred to homozygosity, $F = 1$ and there is no genetic variance within each line, while the genetic variance between lines is double that in the outbred population from which they were derived. With dominance and epistasis, the re-distribution of genetic variance depends on allele frequencies of the underlying loci, so there is no general solution in terms of variance components in the base population until $F = 1$. At that point, the genetic variance between inbred lines is $2\sigma_A^2 + 4\sigma_{AA}^2 + 8\sigma_{AAA}^2 + \ldots$; i.e., pairwise and higher order epistatic interactions contribute to genetic divergence between fully inbred lines (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

We can readily estimate the regression of offspring phenotypic values on those of their parents, as well as estimate the intraclass correlation coefficient among full- or half-sib families. These simple classic designs serve to illustrate the complexities of estimating genetic variance components for quantitative traits. Regressions (b) or intraclass correlations (t) are, respectively, estimates of the phenotypic covariance between offspring and parents divided by the total phenotypic variance, or of the phenotypic covariance between full- (or half-) sib families divided by the total phenotypic variance. If we ignore three-way and higher epistatic interactions, the expected genetic and environmental contributions to each in an outbred population are as follows (Falconer and Mackay, 1996; Lynch and Walsh, 1998):

Regression of offspring on one parent:

$$b_{OP} = \frac{\frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_{AA}^2 + \sigma_{Ec(OP)}^2}{\sigma_P^2}$$

Intraclass correlation of full sibs:

$$t_{FS} = \frac{\frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{8}\sigma_{AD}^2 + \frac{1}{16}\sigma_{DD}^2 + \sigma_{Ec(FS)}^2}{\sigma_P^2}$$

Intraclass correlation of half sibs:

$$t_{HS} = \frac{\frac{1}{4}\sigma_A^2 + \frac{1}{16}\sigma_{AA}^2 + \sigma_{Ec(HS)}^2}{\sigma_P^2}$$

Thus, in each case, we have two estimates (the regression or intraclass correlation and the total phenotypic variance) and several unknowns (additive genetic variance plus other sources of genetic variance and any spatial, temporal or maternal common environment). Thus, we can estimate the joint contribution of all terms in the numerators of these expressions, but cannot partition them further. In principle, we could estimate some individual components by comparing genetic covariance for different degree relatives. However, this is difficult in practice because the estimates of variance components have high standard errors and require extremely large sample sizes. Organisms can be inbred to homozygosity as well as outcrossed, providing the opportunity to infer the relative contribution of additive genetic and interaction variance components and enable more sophisticated experimental designs, but these are not generally applicable to all organisms. Finally, common environment effects in organisms where they cannot be eliminated or accounted for will always bias the estimate of genetic covariance upwards.

Heritability

Of all the genetic variance components, σ_A^2, the additive genetic variance, is the most important for two reasons. First, it makes the largest contribution to the resemblance of most types of relatives. Second, the additive genetic variance is defined so that it represents the fraction of the genetic variance that is transmitted from parents to offspring. An important concept in quantitative genetics is the narrow sense heritability (h^2), which is defined as the ratio of additive genetic variance to the total phenotypic variance ($h^2 = \frac{\sigma_A^2}{\sigma_P^2}$) (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Put simply, the narrow sense heritability of a quantitative trait is the reliability of an individual's own phenotype as an indicator of its 'breeding value'; the proportion of the deviation of a parent's phenotype from the population mean that is expected to be transmitted to the progeny. If an individual's phenotype is x units above average, then the average phenotypic value of the offspring will be xh^2 units above average. This concept underpins the ability of a population to respond phenotypically to natural or artificial truncation selection: if parents are chosen based on the deviation of their average phenotypic value ($\overline{X}$) of a quantitative trait from the population mean (μ), then the average phenotype of their offspring will be $h^2(\overline{X} - \mu)$ units above the population mean. The narrow sense heritability can be readily estimated (with differing degrees of bias and precision) as $2b_{OP}$, $2t_{FS,}$ and $4t_{HS}$; from response to truncation selection; and using more elaborate experimental designs (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Heritability can be a slippery concept. The word suggests that it refers to whether or not a phenotype is inherited; this is not true. All phenotypes are inherited in the sense that developmental genetic programs are responsible for their manifestation. Heritability is a population concept and refers to the fraction of the variation in phenotypes among individuals that is due to additive genetic variance. Additive genetic variance in turn depends on allele frequencies at contributing loci and can be different in different populations if allele frequencies are different. Neither does additive genetic variance convey information about the gene action of contributing loci; additive variance is generated for loci with dominance/recessive gene action as well as epistasis over a wide range of allele frequencies. Heritability can also differ between populations if they experience different environments. Heritability therefore conveys no information about any difference in mean phenotype between populations (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Quantitative Trait Loci Mapping

Estimation of variance components, particularly the additive genetic variance, is critical for predicting short-term responses to natural or artificial selection. However, addressing the questions raised above regarding the genetic architecture of quantitative traits requires that we know the causal molecular polymorphisms, their gene action (additive, dominance, epistatic effects), and their allele frequencies. This information is required in order to predict individual phenotypes from genotypes (as opposed to mean offspring phenotypes). In this case, both additive as well as non-additive genotypic effects are important, even if they contribute little to the total additive variance of the trait. Mapping the quantitative trait loci (QTLs) affecting natural variation for quantitative traits is achieved using polymorphic marker loci with clear Mendelian segregation in linkage or association mapping populations (Falconer and Mackay, 1996; Lynch and Walsh, 1998). The principles of QTL mapping have been known since the early twentieth century; however, only recently has the discovery of abundant molecular markers, advances in rapid and cost-effective genotyping methods, including whole genome sequencing, and the development of sophisticated statistical and computational methods facilitated the molecular dissection of quantitative traits in a wide range of organisms.

QTL mapping requires a population for which there is genetic variation for the trait of interest, and in which individuals have been phenotyped for the trait and genotyped for a panel of molecular markers. In a classical linkage mapping population, correlations between the unknown variants affecting the quantitative trait and the molecular marker genotypes (this correlation is called linkage disequilibrium, LD) are generated by producing segregating progeny from two parental lines that are genetically divergent for the trait. In an association mapping population, LD between the causal variants affecting the trait and the molecular markers are generated by many generations of recombination during the population's history. In both cases, QTLs are detected if there is a difference in trait phenotype between the genotypes at a marker locus (Falconer and Mackay, 1996; Lynch and Walsh, 1998). This simple test is repeated for all markers (or pairs of markers) in a genome scan. Evidence for association is then evaluated after accounting for the multiple tests performed.

The choice of experimental design for QTL mapping is largely predicated by the biology of the organism being studied. Linkage mapping has the advantage that all alleles are at intermediate frequencies, which increases the power of mapping; and that LD is generated by the experimental design and not attributable to other causes that could give rise to spurious associations. However, linkage mapping interrogates a small sample of genetic diversity in the population (this can be partially ameliorated by constructing populations from four or eight parental lines); and the precision of mapping is limited by the numbers of recombination events that occur when creating the population. In contrast, association mapping can have greater mapping precision given the many generations of recombination experienced by most outbred populations and samples a wider range of genetic diversity. The drawbacks of association mapping are that very large numbers of marker loci are needed to account for the larger number of recombination events; the power to detect QTLs declines as the allele frequencies decrease (and individual rare alleles cannot be interrogated at all); and LD can be caused by population processes not related to genetic linkage (e.g., population structure that is not accounted for) that can cause spurious associations. Very large sample sizes are required in both linkage and association mapping studies to detect alleles with small to moderate effects with *P*-values that are low enough to pass multiple testing criteria (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Lessons Learned

Some general conclusions regarding the genetic architecture of quantitative traits have emerged from high-resolution QTL mapping studies in humans and especially in model organisms such as yeast, *Drosophila*, *Arabidopsis*, and mice (Mackay, 2001; Flint and Mackay, 2009; Mackay *et al.*, 2009; Mackay, 2014).

1. Most quantitative traits are indeed highly polygenic, with hundreds, if not more, contributing loci with additive effects. The corollary is that the individual additive effects are typically small, and together contribute only a small fraction of the total genetic variation in association mapping populations, especially in humans. This could be because the total additive genetic variance is overestimated due to the contribution of dominance and epistatic variance and/or because the effects of causal variants are underestimated when they are in LD with the marker locus.
2. The genetic basis of variation for quantitative traits inferred from mapping natural variants is typically novel and distinct from that inferred by studies of mutations with large effects. Possibly segregating variation may not be maintained in natural populations at loci that are required for wild type expression of the trait, and, conversely, mutagenesis screens miss (or ignore) loci at which mutations with subtle effects could affect quantitative traits.
3. Variants affecting quantitative traits have highly context-dependent effects; i.e., their effects differ in magnitude and/or direction in different genetic backgrounds (epistasis), different environments (GEI), and between males and females (genetic variation in sexual dimorphism). Thus, additive effects of alleles with highly context-dependent effects may not be detectable when averaged over multiple environments and/or genetic backgrounds. On the other hand, context-dependent effects are important. Epistatic interactions reveal genetic networks affecting quantitative traits, and genotype by environment variation and genetic variation in sexual dimorphism are potential mechanisms maintaining genetic variation for quantitative traits in natural populations.
4. Molecular variants, not genes, are the relevant unit of observation. Different variants in the same gene can have different effects on the same trait, and different variants in the same gene can independently affect different traits. Therefore, genes but not variants may be highly pleiotropic.

5. Most variation affecting quantitative traits does not cause amino acid changes in protein coding genes, but rather synonymous polymorphisms in coding regions (possibly associated with mRNA stability) and putative regulatory polymorphisms in promoters and introns that could affect transcription factor binding and mRNA splicing, and affect the amount, timing, or tissue-specific pattern of expression. Joint mapping of variants that affect both organismal phenotypes and intermediate molecular phenotypes such as networks of correlated gene expression traits, metabolites, and proteins will yield novel biological insights by which multiple polygenic perturbations affect quantitative traits.

Future Prospects

The genomics revolution and advances in computational power now enable us to perform genotype–phenotype mapping studies on the scale and with the density of molecular markers required to simultaneously identify many genes affecting variation for quantitative traits. In the foreseeable future, this will extend to complete genome sequences of all individuals in large mapping populations as well as measurements of gene expression and other molecular endophenotypes, giving unprecedented insights into the biological underpinnings of complex traits, and pleiotropic connections among traits. As the new technologies mature and become cost-effective, all organisms will become model organisms, enabling us to understand the genetic basis of wide-ranging ecological specializations and adaptations. Finally, we now have the tools to address long-standing unanswered questions in evolutionary quantitative genetics, including (but not limited to) the mechanisms maintaining quantitative genetic variation, the polygenic mutation rate, the molecular basis of GEI, the cause of limits to long-term selection, and phenotypic stability in the face of genetic and environmental variation. The future of evolutionary quantitative genetics is bright.

See also: Genetic Architecture. Multivariate Quantitative Genetics. Quantitative Genetic Variation, Comparing Patterns of. Quantitative Genetics in Natural Populations

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Quantitative Genetic Variation, Comparing Patterns of

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Glossary

Eigenvector A linear combination of the original variables (here, traits). The set of eigenvectors of a matrix (such as **G** or **P**) are themselves uncorrelated (mutually orthogonal), and completely describe the (phenotypic or genetic) 'space.'
Eigenvalue The scale factor describing the length of the associated, normalized, eigenvector. The eigenvalues of **P** describe the phenotypic variation for the associated linear combination of measured traits (eigenvector). Likewise, the eigenvalues of **G** describe the additive genetic variation along the axis defined by the corresponding eigenvector.
G The additive genetic variance–covariance matrix.
Genetic space The multidimensional (n dimensional where n is the number of traits considered) space in which the additive genetic breeding values of all individuals in the population occur. Eigenvectors of **G** describe axes (directions) of genetic space, and the associated eigenvalues describe the 'additive genetic' variation in those directions (for the specific population under consideration). When traits are highly genetically correlated with one another, the 'rank' of the genetic space will be less than n, with the trait combinations associated with genetic variance fewer than the total number of traits measured.
Linkage disequilibrium The nonrandom co-inheritance of alleles at different loci.
Matrix diagonalization Decomposition of a square matrix into its eigenvalues (a diagonal matrix) and eigenvectors (the new set of axes corresponding to the eigenvalues).
P The phenotypic variance–covariance matrix.
Phenotypic space The multidimensional (n dimensional where n is the number of traits considered) space in which the phenotypes of all individuals in the population occur. Eigenvectors of **P** describe axes (directions) of phenotypic space, and the associated eigenvalues describe the variation in those directions for the population under consideration. When traits are highly correlated with one another, the rank of the phenotypic space will be less than n. Different populations might occupy different regions of phenotypic space due to different trait values.
Pleiotropy The effect of an individual allele on more than one trait.
Rank The number of nonzero eigenvalues of a matrix, corresponding to the dimensionality of space.
Subspace The $(n-x)$-dimensional space described by a subset (x) of eigenvectors from an original $n \times n$ trait matrix. When traits covary, the population might be predominantly distributed in few dimensions of the phenotypic or genetic space.

Evolution, change through time, depends on a shift in the frequency distribution of genotypes between generations. For traits under a known selection pressure (S), we can predict the change in mean phenotype ($\Delta\bar{z}$) from one generation to the next using the breeder's equation: $\Delta\bar{z} = h^2 S$ (Lush, 1937). The extent to which trait values are transmitted from parent to offspring (i.e., trait heritability in the population, h^2) will determine how much of the difference in trait mean between selected individuals (μ^*) and the total population (μ: $S = \mu^* - \mu$) is transmitted to the next generation. However, natural selection is thought to typically act on functionally interrelated sets of traits, not on individual traits in isolation (Dobzhansky, 1956; Lewontin, 1970; Lande and Arnold, 1983; Phillips and Arnold, 1989), and co-inheritance of traits, due to pleiotropy or linkage disequilibrium, is also prevalent (Lande, 1980; Falconer, 1981; Paaby and Rockman, 2013). Therefore, the multivariate extension of the breeder's equation, is more typically used to predict phenotypic evolution. In the multivariate extension, the vector of change in trait mean ($\Delta\bar{z}$) depends on the scaling of the selection vector (β) by the additive genetic variance–covariance matrix, **G** (**Figure 1**). This symmetrical $n \times n$ matrix (where n is the number of traits being considered) (**Figure 1**), which summarizes the pattern of additive genetic (heritable) variation within the set of traits, is the primary parameter capturing the potential of populations for evolutionary change over ecological timescales.

The accuracy of evolutionary prediction depends on the constancy of **G** over the timescales that we might want to predict evolution. Unfortunately, the evolution of **G** cannot be theoretically predicted because change in **G** depends on the influence of many, typically unknown, factors such as the characteristics (frequency, effect size, and genomic location) of the alleles that underpin **G**, and on the shape of the adaptive landscape (Turelli, 1988; Jones *et al.*, 2003; Jones *et al.*, 2004; Guillaume and Whitlock, 2007; Revell, 2007; Arnold *et al.*, 2008). This theoretical uncertainty about the evolution of **G**, and the desire to know whether we could use the information on genetic variation currently present in a population to predict future or reconstruct historical responses to selection resulted in the birth of a new empirical field of research, comparative quantitative genetics, at the beginning of the twenty-first century (Steppan *et al.*, 2002).

The general pattern emerging from these empirical studies is that G-matrices estimated from different populations are likely to be broadly similar (Steppan *et al.*, 2002; McGuigan, 2006; Arnold *et al.*, 2008; Walsh and Lynch, 2014). However, examples of rapid, radical changes in **G** exist (Steppan *et al.*, 2002; Walsh and Lynch, 2014), and we cannot assume a

The multivariate breeder's equation

$$\begin{pmatrix} \Delta z_1 \\ \vdots \\ \Delta z_n \end{pmatrix} = \begin{pmatrix} G_{11} & \cdots & G_{1n} \\ \vdots & \ddots & \vdots \\ G_{n1} & \cdots & G_{nn} \end{pmatrix} \begin{pmatrix} \beta_1 \\ \vdots \\ \beta_n \end{pmatrix}$$

(a)

Example of application to real data

$$\begin{pmatrix} -0.05 \\ -0.17 \\ -0.28 \end{pmatrix} = \begin{pmatrix} 0.26 & 0.07 & 0.16 \\ 0.07 & 0.35 & 0.08 \\ 0.16 & 0.08 & 0.39 \end{pmatrix} \begin{pmatrix} 0.42 \\ -0.38 \\ -0.82 \end{pmatrix}$$

(b)

Eigenanalysis of G

	Eigenvector 1	Eigenvector 2	Eigenvector 3
Eigenvalue	0.553	0.296	0.151
Trait 1	0.51	–0.19	0.84
Trait 2	0.46	0.88	–0.08
Trait 3	0.73	–0.43	–0.54

(c)

Figure 1 Dissecting the parameters for predicting multivariate phenotypic evolution using the breeder's equation. (a) The multivariate breeder's equation predicts the evolution of a set of traits (vector of change in trait means, Δz) from the selection gradients (vector of selection coefficients for each trait, $\boldsymbol{\beta}$) and the additive genetic variance–covariance matrix (**G**). In **G**, the genetic variance associated with each trait appears on the diagonal, and the covariances between each pair of traits on the off-diagonal. The subscripts can be used to specify the traits associated with each entry, for example, G_{12} is the genetic covariance of trait 1 with trait 2; G-matrices are symmetrical – that is, the covariance between trait 1 and trait 2 is the same as the covariance between trait 2 and trait 1. (b) A three-trait example of the breeder's equation parameters. Here, all traits positively covary with one another (all off-diagonals of **G** are positive), but trait 1 is under positive selection while the other two traits are under negative selection (the selection coefficients in $\boldsymbol{\beta}$). The positive genetic covariance among all traits causes the response to multivariate selection (Δz) to be biased away from the direction of selection ($\boldsymbol{\beta}$), including a small response in Trait 1 against the direction of selection (i.e., a reduction in the phenotypic mean rather than an increase as predicted from the selection coefficient in $\boldsymbol{\beta}$). (c) The eigenanalysis of the example **G** presented in (b). Each eigenvector (reported in columns) is of unit length (**Box 2(A)**), with the corresponding eigenvalue giving the length (genetic variance in that direction). The values for each trait for a given eigenvector are called the trait loadings, and they reflect the relative contribution of that trait to the magnitude of the eigenvalue.

constant **G** under any conditions. Moreover, through well-designed experiments, including appropriate null hypotheses, comparative quantitative genetics has moved beyond proving or disproving the stability of **G**. These data are building our understanding of how the evolutionary processes of selection, drift, migration, and mutation drive changes in **G**, a field of research that is likely to increase in impact as advances in molecular genetics move us closer to characterizing the architecture underlying quantitative trait variation (Yang *et al.*, 2010; Scoville *et al.*, 2011). Here, we will first describe methods used to characterize variation among G-matrices, developing the language used to discuss **G**, and then briefly consider what comparative quantitative genetics has determined about the evolution of **G**.

How do We Describe G?

Organisms are made up of very many traits, and we expect that these traits are interdependent both in terms of their effects on fitness and in their genetic basis. As such, to understand the evolution of the genetic variance underlying these complex phenotypes, we need to think about higher dimensional spaces. Simple graphical approaches are effective for comparing two-trait (**Figures 1–4**) and possibly three-trait (**Box 1**) matrices, but beyond that, we rely on matrix algebra and trigonometry to distill and describe the geometry of **G** (Lynch and Walsh, 1998; Strang, 1998; Blows, 2007).

One basic idea that is helpful for talking about **G** is that of 'space.' Each individual in a population can be thought of as occupying a position in phenotypic space (**Figure 2(a)**) and the dimensionality of phenotypic space depends on the number of coordinates required to define an individual's position in that space. Phenotypic space can have as many dimensions as the number of traits that are measured, but might have fewer if traits covary. For instance, if the value of an individual for one trait can be exactly predicted from their value for a second trait (i.e., the traits are perfectly correlated), then the dimensionality of this two-trait phenotypic space is reduced to one (**Figure 2(b)**). Individuals also occupy a position in genetic space, described by their breeding values (**Figure 2(c)**). An individual's position in genetic space is translated to their position in phenotypic space through the processes of development, although this developmental mapping between genetic and phenotypic space remains an unresolved challenge for most traits. Just as for phenotypic space, genetic covariance among traits can also reduce the number of dimensions in genetic space.

Symmetrical covariance matrices, such as **G**, can be diagonalized to find a set of orthonormal eigenvectors and eigenvalues (**Figures 1(c)** and **2(d)**). The orientation of eigenvectors and the magnitude of eigenvalues describe the geometry of the (genetic) space, and form the basis of many methods for comparing **G**. Basically, the eigenvectors of **G** are unit-length (**Box 2**) linear combinations of the measured traits that describe directions in genetic space. The corresponding eigenvalues describe the length of the vector, indicating the amount of genetic variation for that specific direction in genetic space. If all our traits of interest were genetically independent of one another, then each eigenvector would correspond exactly to one trait, and the eigenvalues would be equal to the additive genetic variance of that trait. At the opposite extreme, if all traits covary perfectly with one another, there would be a single eigenvector, with the eigenvalue equal to the sum of the individual trait genetic variances. Real **G** are likely to fall between these extremes.

We can describe differences in the geometry of **G** using three terms based on the eigenanalysis: rank, size, and shape. Rank (or dimensionality) is the number of independent

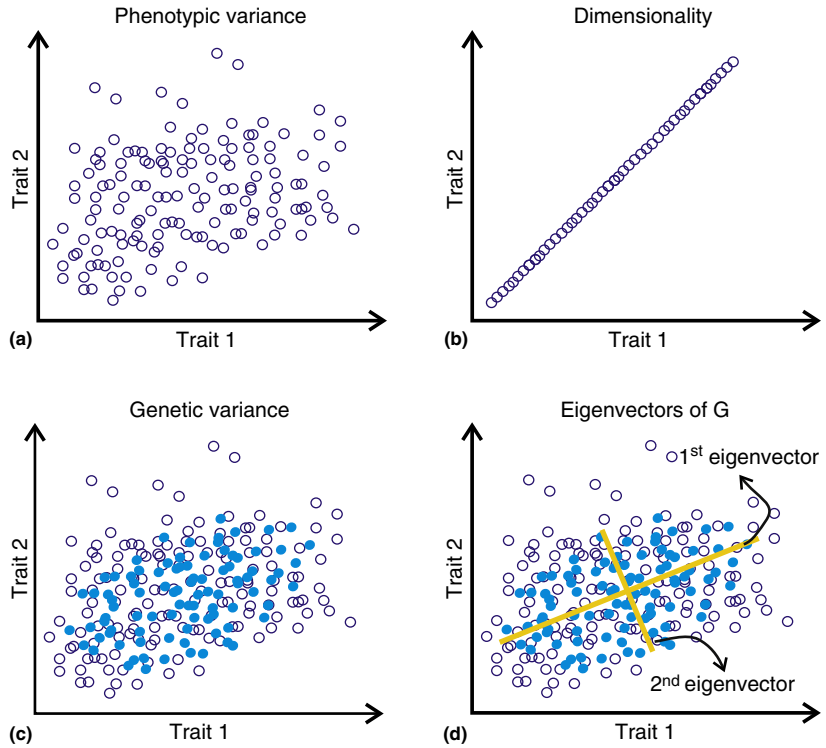


Figure 2 Illustration of some matrix geometry concepts. In any population, we can characterize differences among individuals through their phenotypic values. (a) Shows the values of two traits for individuals in our hypothetical population. This phenotypic space has a maximum number of dimensions equal to the number of traits measured, but if traits covary then the dimensionality of the phenotypic space can be reduced. For example, if two traits perfectly covary, such that the value of one can be predicted from the value of the other, as in (b), the dimensionality of the space is reduced to a single axis. When we measure individuals from a breeding design, whether by planned crosses or the maintenance of a pedigree, we can partition phenotypic variation to determine each individual's breeding values (the average value of the trait in their offspring). This distribution of breeding values (shown in (c)) is the variation that underpins **G**. We can diagonalize this **G** (as in Figure 1(c)) to examine the organization of genetic variance in multidimensional space. (d) For a two-trait matrix, diagonalization returns two unique (orthogonal – at right angles to one another) linear combinations of the measured traits (shown in yellow), each associated with different amounts of variation (indicated here by the lengths of the vector, corresponding to the eigenvalue).

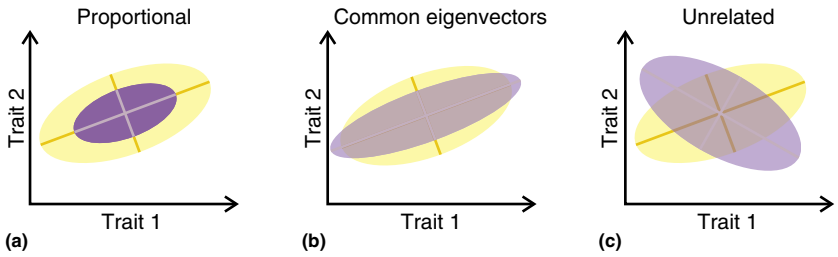


Figure 3 Size and shape variation in **G**, illustrated for two taxa (shown in yellow and purple). Matrices might differ from one another in: (a) size (the total genetic variance); (b) the amount of genetic variation associated with specific, shared, eigenvectors, as apparent in differences in eigenvector length; and (c) in orientation (the directions of eigenvectors in genetic space). In (a), the purple population has a smaller G-matrix than the yellow population, but the matrices are proportional with the same relationship between trait 1 and 2 identified by the eigenvectors. In (b), the purple taxon has more variation associated with eigenvector 1 than does the yellow taxon. However, because the total genetic variation is the same in both populations, this leaves less genetic variation for eigenvector 2 in the purple taxon compared with the yellow taxon. In (c), the purple population has a negative genetic covariation between the two traits, while the yellow population has a positive covariation, but the total variance in the two traits is the same in both populations.

directions (eigenvectors) in genetic space, determined by the number of eigenvalues that are greater than zero (Mezey and Houle, 2005; Hine and Blows, 2006). Typically, most of the genetic variance is found in relatively few dimensions (eigenvectors) of genetic space (Kirkpatrick, 2009), suggesting rank of genetic space might be less than the number of traits. However, whether we can distinguish a given eigenvalue from zero will depend greatly on the power of the experiment, specifically the sample size; with small sample sizes, the power to demonstrate that small variances (such as associated with

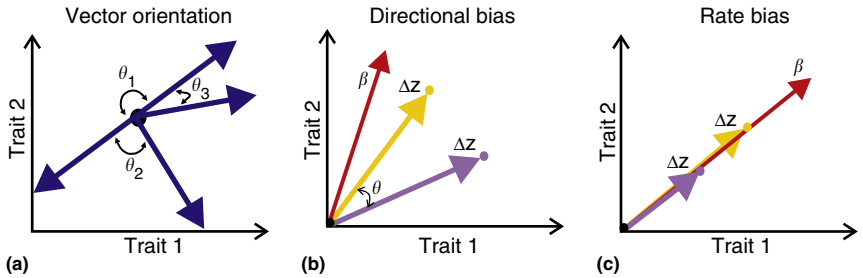


Figure 4 The effect of **G** on the response to selection. (a) Unit-length vectors (**Box 2(A)**) can differ in orientation, from indicating opposite directions in phenotypic (genetic) space ($\theta_1 = 180°$), to being orthogonal to one another ($\theta_2 = 90°$) to being coincident (0°) through any value in between (e.g., $\theta_3 = 25°$). (b) When two taxa have **G** that differ in shape (orientation of variation and the distribution of variation among different directions), the biasing effects of **G** on the response to the same selection gradient (**β**) results in the taxa following different evolutionary trajectories and evolving different mean phenotypes, Δz. (c) When **G** differ only in size, and the geometry of **G** coincides with the selection gradient, populations might diverge in mean phenotype because of greater evolution in a population with a larger **G** (yellow taxon).

lower eigenvalues) are greater than zero will also be commensurately small, and a conclusion of low rank is more likely than in a study with the same distribution of genetic variance, but large sample sizes. Because of the uncertainty (error) associated with estimating eigenvalues, differences in rank are not considered a reliable indicator of differences in **G** (Kirkpatrick, 2009; Blows and McGuigan, 2015). The size of **G** is simply how much genetic variance there is in the trait set, and is determined by the sum of the eigenvalues of **G** (the trace), which must equal the sum of the genetic variances of the original traits (**Figures 1(b)** and **1(c)**). Shape is determined both by the eigenvectors and eigenvalues of **G**; we will consider shape in further detail below as it relates directly to matrix comparison methods (see also **Figure 3**).

How do We Compare G?

Before delving into methods for comparing G-matrices, let us consider the common problem of estimation error. Accurate estimation of **G** requires a large sample size; consequently, empirical estimates of **G** are associated with relatively large error because of logistical constraints limiting the sample size. This sampling variance needs to be taken into account when comparing G-matrices so that we can ask whether any observed differences are greater than we might expect to occur through random sampling error. A widely used approach is randomization or bootstrapping, in which individuals (or the genetic group, e.g., families or sires) are randomly re-assigned among taxa, **G** is reestimated using the same model as for the observed data, and this process is repeated many (~1000) times to generate a distribution, reflecting random sampling, of variation among G-matrices (e.g., Calsbeek and Goodnight, 2009; Roff *et al.*, 2012).

For G-matrices that contain many traits, traits with non-Gaussian (non-normal) distributions, or are estimated from a complex multigenerational pedigree where the genetic unit that should be randomized is not simply family or sire, a Bayesian approach provides a useful way of generating a null distribution for hypothesis testing (Hadfield *et al.*, 2010; Robinson and Beckerman, 2013; Aguirre *et al.*, 2014). Bayesian analyses, in combination with Markov chain Monte Carlo (MCMC) methods (Gelman *et al.*, 2004), can be applied to diverse pedigrees and trait types, to generate the posterior distribution for not only **G**, but also other experimental factors that might contribute to phenotypic variation, greatly facilitating hypothesis testing (Ovaskainen *et al.*, 2008). Under this framework, it is possible to generate a null model **G** by applying the randomization step to the posterior predictive distribution of breeding values (Hadfield *et al.*, 2010; Aguirre *et al.*, 2014), and therefore to generate a null distribution for among-taxa **G** without having to reestimate **G** many times, a great benefit because the computational demands of these analyses can be limiting.

Once we have both our estimates of observed **G**, and their associated errors, as well as an appropriately constructed null **G**, we can compare **G** among groups. Below we outline several methods for comparing **G**. Further detail and background on methods for comparing **G**, including other methods we do not mention, can be found elsewhere (Hansen and Houle, 2008; Calsbeek and Goodnight, 2009; Roff *et al.*, 2012; Aguirre *et al.*, 2014), and we particularly recommend reading Chapter 36 of Walsh and Lynch (2014). As we indicated above, many of the methods for comparing **G** are based on eigenanalyses. However, the focus of the methods as we outline them below is in understanding how G-matrices differ in their distribution of genetic variance. As has been discussed previously (e.g., Cheverud, 2007), eigenvectors themselves do not necessarily reflect underlying genetic causal factors. However, it is not the directions of eigenvectors that are interpreted in the methods described below, but instead it is the genetic variance associated with these different regions of the genetic space.

Flury and Krzanowski Methods

One of the most influential early developments in comparative quantitative genetics was the adaptation by Phillips and Arnold (1999) of Flury's (1988) common principal components (CPC) model. CPC analyses allow a hierarchy of comparisons to determine whether matrices differ in size (proportional matrices: **Figure 3(a)**) or shape, either in the amount of genetic variance associated with specific eigenvectors observed in all matrices under consideration (common eigenvectors: **Figure 3(b)**) or through divergence in orientation of eigenvectors (unrelated structure: eigenvectors are not shared among different **G**: **Figure 3(c)**).

Box 1

Three-dimensional illustrations of the phenotypic and genetic space. The following code takes the G-matrix in **Figure 1(b)** and generates a distribution of phenotypic and genotypic (breeding) values for a population consistent with that G. Code then follows to draw three dimensional versions of **Figures 2(a)** and a modified version of **Figure 2(d)** where only genetic space and the eigenvectors of G are plotted. Last we project the selection and response vectors onto the genetic space as in **Figure 1(d)**. Using your mouse, you can rotate these figures and view them along any of the three axes to consider the shape of phenotypic and genetic space for these three hypothetical traits.

```
#If you don't have the "MASS", "rgl" or "heplots" installed, run the following lines:
install.packages("MASS", dependencies=TRUE)
install.packages("rgl", dependencies=TRUE)
install.packages("heplots", dependencies=TRUE)

#Once the "MASS", "rgl" or "heplots" libraries are installed, load them:
library(MASS)
library(rgl)
library(heplots)

#G-matrix in Figure 1(b)
G<- matrix(c(0.26,0.07,0.16,0.07,0.35,0.08,0.16,0.08,0.39), ncol=3)

#Proportional P with h^2 ~ 0.5
P<- diag(sqrt(diag(G)) * 1.4) %*% cov2cor(G) %*% diag(sqrt(diag(G)) * 1.4)

#100 random points drawn from the multivariate normal distribution with a mean equal to 0 and a variance equal to G
dat_G<- mvrnorm(100, c(0,0,0), G)

#100 random points drawn from the multivariate normal distribution with a mean equal to 0 and a variance equal to P
dat_P<-mvrnorm(100, c(0,0,0), P)

#Selection vector and response vector in Figure 1(c)

beta<- rbind(c(0,0,0), c(0.42,-0.38,-0.82))
delta_z<- rbind(c(0,0,0), c(-0.05,-0.17,-0.28))


#Plots#
#1._____
#Distribution of individuals (blue points) in phenotypic space and 95% confidence ellipsoid (blue wireframe)


open3d(windowRect=c(200,200,1000,700))
par3d(windowRect=c(200,200,1000,700))

plot3d(dat_P, col="blue", size=10, expand=1, box=FALSE, xlab="Trait_x", ylab="Trait_y", zlab="Trait_z")
plot3d(ellipse3d(P, centre=c(0,0,0), level = 0.95), col = "blue", alpha = 0.1, aspect = TRUE, add = TRUE)
wire3d(ellipse3d(P, centre=c(0,0,0), level=0.95), col="grey50", alpha=0.75, aspect=TRUE, add=TRUE)

#2._____
#Distribution of individuals (blue points) in phenotypic space and breeding values (red points) in genetic space as well as the associated and 95% confidence ellipsoids for phenotypic (blue wireframe) and genetic space (red wireframe)

open3d(windowRect=c(200,200,1000,700))
par3d(windowRect=c(200,200,1000,700))

#Phenotypic space
plot3d(dat_P, col="blue", size=10, expand=1, box=FALSE, xlab="Trait_x", ylab="Trait_y", zlab="Trait_z")
plot3d(ellipse3d(P, centre=c(0,0,0), level=0.95), col="blue", alpha=0.1, aspect=TRUE, add=TRUE)
wire3d(ellipse3d(P, centre=c(0,0,0), level=0.95), col="grey50", alpha=0.1, aspect=TRUE, add=TRUE)

#Genetic space
plot3d(dat_G, col="red", size=10, expand=1, add=TRUE)
plot3d(ellipse3d(G, centre=c(0,0,0), level=0.95), color="red", alpha=0.3, aspect=TRUE, add=TRUE)
wire3d(ellipse3d(G, centre=c(0,0,0), level=0.95), col="grey50", alpha=0.5, aspect=TRUE, add=TRUE)

#3._____
#Distribution of breeding values (red points) in genetic space and 95% confidence ellipsoid (red wireframe). Black lines embedded in the genetic space denote the eigenvectors of G

open3d(windowRect=c(200,200,1000,700))
par3d(windowRect=c(200,200,1000,700))

plot3d(dat_G, col="red", size=10, expand=1, alpha=0.2, box=FALSE, xlab="Trait_x", ylab="Trait_y", zlab="Trait_z")
plot3d(ellipse3d(G, centre=c(0,0,0), level=0.95), color="red", alpha=0.3, aspect=TRUE, add=TRUE)
wire3d(ellipse3d(G, centre=c(0,0,0), level=0.95), col="grey50", alpha=0.3, aspect=TRUE, add=TRUE)
plot3d(ellipse3d.axes(G, centre=c(0,0,0), level=0.95, lwd=2), col="black", aspect=TRUE, add=TRUE)

#4._____
#95% confidence ellipsoid (red wireframe) for the genetic space. Black lines embedded in the genetic space denote the magnitude and orientation of the selection vector (beta) and the selection response (detla z)

open3d(windowRect=c(200,200,1000,700))
par3d(windowRect=c(200,200,1000,700))

plot3d(ellipse3d(G, centre=c(0,0,0), level=0.95), color="red", alpha=0.3, box=FALSE, xlab="Trait_x", ylab="Trait_y", zlab="-Trait_z")
wire3d(ellipse3d(G, centre=c(0,0,0), level=0.95), col="grey50", alpha=0.3, aspect=TRUE, add=TRUE)
lines3d(beta, lwd=2)
```

```
text3d(beta[2,], text="beta", adj=c(1,1))
lines3d(delta_z, lwd=2)
text3d(delta_z[2,], text="delta_z", adj=c(1,1))

###END###
```

Box 2

A. General Formulae

There are several operations that are widely used in comparative quantitative genetics, irrespective of the specific method being used to compare matrices. First, we need to be able to describe the effects of size and orientation separately and to do this we work with vectors that are of unit length (so-called normalized vector) such that:

$$\boldsymbol{b}^{\mathrm{T}}\boldsymbol{b} = 1 \qquad [A1]$$

where $\boldsymbol{b}$ is any vector, and $^{\mathrm{T}}$ indicates the transpose of the vector. Vectors are normalized by dividing each element by the square root of the sums of squares of all elements.

If we have more than one normalized vector, $\boldsymbol{b}$ and $\boldsymbol{a}$, for example, we can determine how different their orientation is by calculating the angle between them. We could report this as the dot product (ranging from -1 to 1), which is the sum of the products of the corresponding entries:

$$r = \boldsymbol{b}^{\mathrm{T}}\boldsymbol{a} \qquad [A2]$$

Alternatively, we can report the vector differences as an angle in radians:

$$\theta = \cos^{-1} r \qquad [A3]$$

which can be converted to degrees (ranging from 0° to 180°) by multiplying θ by $(180/\pi)$.

We can also determine how much genetic variation is available for any given vector (i.e., trait combination) by projecting it through the G-matrix of interest (Lin and Allaire, 1977). So, for any taxon, $1 \ldots t$, we can determine the genetic variance in any vector $\boldsymbol{b}$ by projecting it through the relevant population $\mathbf{G}$:

$$V_{G_t} = \boldsymbol{b}^{\mathrm{T}}\mathbf{G}_t\boldsymbol{b} \qquad [A4]$$

B. Krzanowski Common Subspace

Krzanowski's method is aimed at determining whether matrices share a common subspace. If we include all of the eigenvectors of our $\mathbf{G}$ of interest we are constrained to recover a common subspace (Blows *et al.*, 2004). Instead, we consider a subspace of each $\mathbf{G}_t$, $\mathbf{A}_t$, which is the first j eigenvectors of $\mathbf{G}_t$. The number of eigenvectors to be considered can be defined in two ways (Aguirre *et al.*, 2014). First, we can consider the first $n/2$ eigenvectors of each $\mathbf{G}$, where n is the number of traits. Alternatively, we could define the subspace based on the proportion of additive genetic variance accounted for. In either case, the number of dimensions (eigenvectors) of the subspace matrix ($\mathbf{H}$) that can be interpreted, without constraining the method to recover a common subspace, is equal to the rank of $\mathbf{A}_t\,\mathbf{A}_t^{\mathrm{T}}$ for the $\mathbf{A}_t$ with the fewest number of eigenvectors included. Aguirre *et al.* (2014) compared these two approaches for deciding how many eigenvectors to include in $\mathbf{A}_t$ and found that the second, defining $\mathbf{A}_t$ based on the proportion of the variance captured, uncovered greater similarity among $\mathbf{G}$.

Having defined our $\mathbf{A}_t$, we can determine the common subspace shared among $\mathbf{G}$ using (Krzanowski, 1979):

$$\mathbf{H} = \Sigma_{t=1}^{p}\mathbf{A}_t\mathbf{A}_t^{\mathrm{T}} \qquad [B1]$$

For each eigenvector of $\mathbf{H}$, $\boldsymbol{b}_i$, we can determine the angle (θ) between that eigenvector and the subspace of the G-matrix of the tth population, $\mathbf{A}_t$:

$$\theta_t = \cos^{-1}\left\{\left(\boldsymbol{b}_i^{\mathrm{T}}\mathbf{A}_t\mathbf{A}_t^{\mathrm{T}}\boldsymbol{b}_i\right)^{0.5}\right\} \qquad [B2]$$

These angles (θ_t) then tell us which $\mathbf{G}$ has diverged the most from the common subspace shared by all $\mathbf{G}$ in the analysis. Finally, we can project the eigenvectors of the common subspace ($\boldsymbol{b}_i$) through each $\mathbf{G}$ (using A4) and determine whether $\mathbf{G}$ differ in the magnitude of genetic variance associated with the common subspace.

C. Vector-Based Approaches

For a given selection gradient, $\boldsymbol{\beta}$, represented as a column vector for traits 1 to n, and a given $\mathbf{G}$ we can determine the response to selection (Δz) using the multivariate breeder's equation (Lande, 1979), represented here in matrix form (see also **Figure 1**):

$$\begin{pmatrix}\Delta z_1\\ \vdots\\ \Delta z_n\end{pmatrix} = \begin{pmatrix}G_{11} & \cdots & G_{1n}\\ \vdots & \ddots & \vdots\\ G_{n1} & \cdots & G_{nn}\end{pmatrix}\begin{pmatrix}\beta_1\\ \vdots\\ \beta_n\end{pmatrix} \qquad [C1]$$

By comparing among taxa how this response is biased away from $\boldsymbol{\beta}$ by $\mathbf{G}$ we can determine whether the taxa differ in their evolutionary trajectory because of differences in $\mathbf{G}$. Comparison of the direction of evolution can be made by calculating the angle (A3) between Δz vectors. Alternatively, we could consider the magnitude of the evolutionary response in the different taxa by projecting $\boldsymbol{\beta}$ through $\mathbf{G}$ (A4). Differences in both direction and magnitude are captured by the difference in response (e.g., the distance between the two endpoint dots in **Figure 4(b)**), given by Hansen and Houle's (2008) equation:

$$\begin{aligned} d(\boldsymbol{\beta}) &= |\Delta\overline{z_1} - \Delta\overline{z_2}|\\ &= |(\mathbf{G}_1 - \mathbf{G}_2)\boldsymbol{\beta}| = \sqrt{\boldsymbol{\beta}^{\mathrm{T}}(\mathbf{G}_1 - \mathbf{G}_2)^2\boldsymbol{\beta}} \end{aligned} \qquad [C2]$$

Instead of considering the response to an hypothesized direction of evolution, we can probe $\mathbf{G}$ using any other unit-length vectors, including a large set of random vectors spanning the phenotypic space (Cheverud and Marroig, 2007). We can assess similarity among matrices based on whether they differ (more than among the null model $\mathbf{G}$ representing sampling error) in the direction or magnitude of predicted responses.

D. Tensors

Two approaches exist for determining the difference between just two covariance matrices. First, we can determine the relative difference between the matrices by first finding the ratio (Flury, 1983; Mitteroecker and Bookstein, 2009; Houle and Fierst, 2013; Bookstein and Mitteroecker, 2014):

$$\mathbf{C} = \mathbf{A}^{-1}\mathbf{B} \qquad [D1]$$

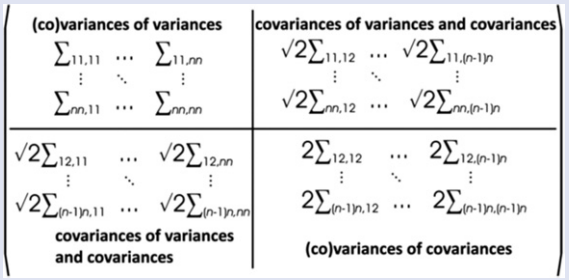
where '$^{-1}$' indicates the inverse of the matrix.

Alternatively, we could find the difference between the two matrices on the absolute scale by first taking the difference (Schott, 2010):

$$\mathbf{C} = \mathbf{A} - \mathbf{B} \qquad [D2]$$

By diagonalising **C** it is then possible to summarize the way in which the two matrices differ the most. This idea of scaling matrices by one another can be generalized to many matrices by the genetic tensor analysis. The method is undeniably complex; here, we simply provide an overview, and refer readers to Hine *et al.* (2009) for details.

To interpret divergence in **G** among taxa we must first calculate a 2nd-order representation, **S**, of the 4th-order tensor, $\sum_{\mathbf{G}}$. The mapping of $\sum_{\mathbf{G}}$ to **S** results in four quadrants, as described in detail in Hine *et al.* (2009) and illustrated below (see also Aguirre *et al.*, 2014).



The eigentensor approach depends on two steps of diagonalization. First, eigenanalysis of **S** uncovers the eigentensors, which are analogous to the eigenvectors of **G** in that they describe the subspaces where independent aspects of variation among **G** exist, and their associated eigenvalues describing the variance in that subspace. When dealing with multiple **G**, this allows that different **G** might have evolved through independent changes, driven by different evolutionary processes.

We can then rearrange these eigentensors into their own 2nd-order representations, allowing us to calculate the eigenvalues and eigenvectors of the eigentensors. This second analysis identifies the trait combinations contributing to the among-matrix subspaces captured by each eigentensor. Note, unlike the diagonalization of a standard variance–covariance matrix, diagonalization of eigentensors can result in negative eigenvalues. This occurs when increases in genetic variance associated with one combination of traits is opposed by a decrease in genetic variance associated with a second, independent, combination of the measured traits.

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The Flury method was developed based on product-moment covariance matrices, and while it is readily applicable to comparisons of such matrices, for the more derived variance-component matrices, typical of estimates of **G**, the degrees of freedom are unknown, and the CPC model cannot be applied with certainty. Krzanowski (1979) proposed a related approach, also focused on common subspaces, which has been adapted to the comparison of **G** (Blows *et al.*, 2004; Aguirre *et al.*, 2014). Like CPC, Krzanowski's approach is focused on determining whether G-matrices share a common subspace, that is, whether the different G-matrices have eigenvectors in common. Krzanowski's common subspace differs from CPC both by being more broadly applicable to **G** from different designs, and more limited in that it is focused only on the subspace associated with the most variance, not the entire genetic space (**Box 2(B)**).

The Krzanowski method is really a three-step procedure (**Box 2(B)**). First, through some matrix algebra we calculate a matrix that contains the subspace common to the G-matrices we are interested in comparing. Second, we apply eigenanalysis to this new matrix to determine whether all of our G-matrices share eigenvectors; if they do not, we can then determine which **G** differ from the common subspace the most by calculating the angle (**Box 2(A)**) between the subspaces of each **G** and the common subspace. First and second steps of Krzanowski's approach focus solely on the orientation of the eigenvectors of **G**, however, as made clear by the CPC hierarchy, even if matrices share a common subspace, they might differ in the magnitude of the genetic variance associated with this subspace. Hence, in the third step, we use vector projection (**Box 2(A)**) to calculate the length of each eigenvector of the common subspace matrix in the space of each **G**. Thus, the Krzanowski method can be used to identify linear trait combinations (covariances) that are unique to different taxa, and to characterize differences in the genetic variation associated with the common subspace.

Vector Approaches

There are several broadly similar approaches that assess the similarity (or dissimilarity) of G-matrices through the use of vectors (**Box 2(C)**). For multivariate phenotypes, the evolutionary response ($\Delta\bar{z}$) will be biased away from the direction of selection (β) by the genetic variance to an extent that will depend on the geometry of **G** (Lande, 1979). The multivariate breeder's equation quantifies this biasing effect of **G** (**Figure 1**; **Box 2(C)**) and analytical comparisons of G-matrices from different populations can distinguish differences in orientation of the corresponding response vectors (**Figures 4(a)** and **4(b)**)

and in the magnitude of the evolutionary response (**Figure 4(c)**). This type of informed comparative approach, based on the breeder's equation, can be applied using an estimated selection gradient (Hansen and Houle, 2008; Chenoweth *et al.*, 2010; Aguirre *et al.*, 2014), or a hypothetical selection gradient which reflects a researcher's prior observations of selection in their study system (Calsbeek and Goodnight, 2009; Stinchcombe *et al.*, 2010). Alternatively, if there is no prior information on selection, we can probe the entire phenotypic space using a large set of unit-length random vectors (Cheverud and Marroig, 2007). In the absence of knowledge of the selection regime, the biological interpretation of the differences in G-matrices depends on the aims of the study. For example, Marroig *et al.* (2011) focused at the trait level, comparing **G** in terms of the predicted direct and indirect evolution of each trait contained within **G**, allowing them to draw conclusions about the evolutionary potential of individual traits. In contrast, Aguirre *et al.* (2014) focused on the total phenotypic space described by the measured traits, identifying the specific directions in phenotypic space that differed significantly among taxa in the associated genetic variation, and thereby evolutionary potential.

Tensors

The final method that we will consider is the genetic tensor approach (Hine *et al.*, 2009, 2011; Robinson and Beckerman, 2013; Aguirre *et al.*, 2014), which is possibly the most versatile, but also the most intuitively challenging, approach for determining if matrices differ. The power of the genetic tensor approach lies in its generality – it considers the entire genetic space of each **G** and it can be used to compare any number of G-matrices simultaneously. A useful first step is recognizing that **G** is a 2nd-order tensor – each element in **G** is denoted by two indices, for example, the covariance between traits 1 and 2 can be identified as G_{12} (**Figure 1**). To compare the elements of different G-matrices we need four indices – the covariance of traits x and y in population 1 with traits w and z in population 2, $G_{xy,wz}$. Because of this dependence on four indices, we refer to the matrices describing the variation among G-matrices as 4th-order tensors.

Although the idea of working with 4th-order tensors sounds intimidating, for the simple case where we want to compare only two G-matrices, all this complexity boils down to simple scaling of one matrix by the other (**Box 2(D)**). As recently highlighted by Bookstein and Mitteroecker (2014), a two-matrix comparison is closely related to multivariate analysis of variance (MANOVA) followed by a principal components analysis (PCA): first multiplying one **G** by the inverse of the other (the matrix equivalent of division); and then subjecting the resultant matrix to an eigenanalysis. This eigenanalysis identifies linear combinations of traits that differ between the two G-matrices, in decreasing order of the amount of variance they differ by. Although algebraically complex (**Box 2(D)**), the eigentensor approach is conceptually an extension of this simple approach – first we generate a matrix capturing the differences among G-matrices and then we decompose that matrix to determine the divergence in genetic covariance among our G-matrices, and the characteristics of those independent axes (eigenvectors) of divergence (**Box 2(D)**).

Admittedly this sounds complicated; however, the mathematical complexity is justified by the ease and accuracy of the biological interpretation that is permitted. For example, Hine *et al.* (2009) applied a tensor approach to characterize the divergence in the genetic variance associated with a set of eight contact pheromone traits among nine natural populations of *Drosophila serrata*. Most (68%) of the divergence among G-matrices was due to a change in genetic variance for one linear combination of pheromones – increasing or decreasing all pheromones simultaneously. This trait combination was associated with greater genetic variance, and thus more potential to evolve, in the northern most populations (Hine *et al.*, 2009).

Evolutionary Processes and G

How does **G** evolve? As this matrix depends on allele frequencies, it will evolve through the same processes as allele frequencies do – mutation, migration, selection, and drift. Teasing apart the relative effects of different processes relies on experimental populations in which the contribution of different processes is manipulated, with findings from such studies potentially contributing to the interpretation of divergences in **G** of wild populations (Phillips and McGuigan, 2006). Nonetheless, relatively few manipulative experimental evolution studies have considered the changes in **G** when one evolutionary process is known to dominate. Below, we consider what is known from these manipulative experiments.

Under random genetic drift, theory predicts additive genetic variance will decrease as alleles are lost through sampling error, and **G** of populations diverging through drift should be proportionally smaller (e.g., **Figure 3(a)**) than the ancestral **G**. This prediction of proportionality suggests proportionality itself might be a criterion for determining whether **G** has diverged through drift rather than selection (Roff, 2000). In several taxa, inbreeding has been experimentally imposed to test the effect of drift on genetic variance. Most of these studies have considered few traits, and reported on individual trait variances (the diagonal of **G**) only. Moreover, contrary to the prediction that additive genetic variance will decrease under drift, inbreeding has been observed to often increase additive genetic variance (e.g., Bryant and Meffert, 1993; van Heerwaarden *et al.*, 2008). A possible mechanism driving this counterintuitive result is the conversion of nonadditive genetic variance to additive genetic variance (Barton and Turelli, 2004). This effect might therefore be greatest for life history traits, which are expected to harbor the most nonadditive genetic variance.

What happens to the covariances under drift? Phillips *et al.* (2001) bred 52 pairs of brothers and sisters of *Drosophila melanogaster*, and compared **G** for wing shape among these and the ancestral population. The average **G** of the 52 inbred populations, as predicted, was proportionally smaller than the ancestral **G**. However, when Phillips and colleagues inspected **G** from each of the 52 populations individually, they found that they differed not just in total size, but in shape as well, with changes in orientation and in the distribution of variance

among directions. Because these changes in shape differed among populations, they canceled one another out, resulting in no net change in shape across the large sample of populations. Similarly, idiosyncratic effects on covariances have been observed in other taxa (e.g., Calsbeek and Goodnight, 2009), suggesting that for the unreplicated experiments represented by many natural populations, the expectation that proportionality of **G** reflects drift, and conversely that nonproportional change indicates the action of selection, is not likely to be met.

In contrast to the expectation of proportional decrease in **G** under drift, the unknown genetic targets of selection make theoretical predictions of changes in **G** due to selection more challenging. Further, the small size of most experimental populations make it more difficult to impose selection without allowing drift to also operate, and changes in the **G** of nonselected control populations (e.g., Wilkinson *et al.*, 1990) challenge interpretations of the effects of selection on **G**. One factor that is likely to impact on the evolution of **G** is variation in the selection regime; are populations evolving under stabilizing selection in a constant environment, or are they experiencing novel environments and directional selection? Computer simulations suggest that stabilizing selection will promote the stability of **G** (Jones *et al.*, 2003; Revell, 2007), while directional selection might alter **G**. Perhaps most simplistically, under novel directional selection, the size of **G** is predicted to increase if the phenotypic response to selection depends on increased frequency of rare alleles (Barton and Turelli, 1987; Reeve, 2000). Increased genetic variance under artificial selection in the laboratory has been observed (Blows and Higgie, 2003; Hine *et al.*, 2011), but whether rare alleles typically contribute to continued evolutionary change remains an open question. Changes to **G** might be particularly dramatic if the alleles underlying the selection response have large effects on phenotype (Agrawal *et al.*, 2001). However, because the contribution of an allele to **G** depends on its frequency, variation among G-matrices from different populations, or the same population at different times, might be transitory, only appearing while adapting populations are segregating alleles at intermediate frequencies.

We know very little about how novel alleles, arising through mutation, might change **G**. One theory is that selection drives the evolution of the mutational machinery to cause the mutational variance and covariance matrix, **M**, to align with selection (Jones *et al.*, 2014). Under this scenario, we would not expect mutation to have much impact on **G**. However, Camara and Pigliucci (2000) induced mutations in *Arabidopsis thaliana* and applied Flurry's CPC analysis to show that the geometries of **G** pre- and post-mutagenesis were unrelated, or shared only some common components. Estimates of **M** and **G** from outbred populations evolving under different types of selection are needed for us to understand whether mutation is likely to alter **G** over time frames of interest.

From studies in naturally evolving populations, there are three general themes apparent (Doroszuk *et al.*, 2008; Walsh and Lynch, 2014), which are consistent with several of the theoretical expectations. First, the size of **G** might be more labile than its shape. Second, when the environment changes, presumably causing a population that was under stabilizing selection to come under directional selection toward a new adaptive optimum, **G** is likely to change. Third, while the environment (and selection) remains similar, **G** might not evolve. Nonetheless, there remains considerable variation in observations from natural populations, which makes it difficult to predict the evolution of **G** for any specific case. One aspect of variation in natural populations that is likely to be contributing to this lack of consensus is the effect of the environment on the expression of genetic variance. Phenotypes, which are measured to estimate **G**, can respond plastically to environmental variation; if that environmental variation also influences the way that genetic information contributes to phenotypes, through context-dependent allelic contributions, then **G** is also likely to respond to environmental variation (McGuigan *et al.*, 2011; Berger *et al.*, 2013; Bjorklund *et al.*, 2013; Li *et al.*, 2014). How much this non-evolutionary component of variance contributes to among-population variation in G-matrices is unclear, as is the evolutionary consequences of environment-specific **G** (McGuigan and Sgro, 2009).

As we pointed out above, estimates of **G** will typically be associated with large sampling errors. Furthermore, because we need to know the population pedigree, **G** can be difficult to estimate for many organisms and impossible for some (e.g., extinct taxa). These difficulties led Cheverud (1988) to suggest substituting the phenotypic variance–covariance matrix, **P**, for **G** when making evolutionary predictions. If the environmental effects on the traits are similar to the genetic effects, which might occur if environmental and genetic factors act through the same developmental processes, then **P** is expected to be very similar to **G** (Cheverud, 1984). In contrast to the complex breeding designs and analyses required to estimate **G**, **P** can be estimated as a simple product-moment covariance matrix and is estimated more accurately than **G** from a given dataset. Furthermore, P-matrices have statistical properties that are more amenable to certain types of analyses, such as CPC. Comparisons of phenotypic and genetic correlations have revealed some similarity (Cheverud, 1988; Roff, 1996; Waitt and Levin, 1998), although the extent of the similarity might depend on the trait type. Different types of traits are affected to a different extent by environmental variation, reducing the similarity of **P** and **G** for some trait types (Willis *et al.*, 1991; Roff, 1996; Hadfield *et al.*, 2007). Importantly, we still do not know how different **G** and **P** need to be to affect conclusions about the response of populations to future selection, or the processes that might have contributed to the divergence of covariance matrices. Comparative analyses of **P** provide information on variation in what selection can act on in each population, but whether these analyses provide insight into the transmission of that selection response across generations is still not clear.

See also: Multivariate Quantitative Genetics. Quantitative Genetic Variation

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Quantitative Trait Variation, Molecular Basis of

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Glossary

Additive genetic effect The effect of substituting one allele at a locus with another allele. For a given bi-allelic locus, this value is typically measured as half of the difference between the mean phenotypic values of the two homozygotes.

Cloning The process of refining a quantitative trait locus to specific genes and genetic variants that have phenotypic effects. Cloning typically requires a combination of genetic mapping and genetic engineering techniques.

Co-immunoprecipitation A biochemical approach for studying physical interactions among proteins and other molecules.

Cryptic genetic variation Polymorphisms that only show phenotypic effects when a genetic or environmental perturbation occurs.

Dominance When a heterozygote exhibits a phenotype that is associated with one allele of a quantitative trait locus.

Dominance genetic effect For a given bi-allelic locus, the difference between the phenotype of heterozygotes and the average phenotype of the two homozygotes.

Dominant negative When one allele of a gene also prevents another allele from functioning when they co-occur in a heterozygote.

Fluorescence resonance energy transfer A microscopy approach for studying physical interactions between proteins.

Genetic background effect When the effect of an allele is influenced by the genome in which the allele occurs.

Genetic buffering When inherited mechanisms prevent mutations and genetic variants from having phenotypic effects.

Genetic interactions (or epistatic interactions) When sets of two or more genetic variants show a combined phenotypic effect that is nonadditive.

Genome An organism's complete set of genetic material.

Genotype–environment interaction When the effect of one or more genetic variants change across environmental conditions. Alternatively, when the effect of the environment changes across genotypes.

Haploinsufficiency When a diploid organism possesses a single functional copy of a gene, resulting in insufficient gene product and an abnormal phenotypic state.

Heritability The proportion of variance in a trait that is explained by genetic factors, as opposed to the environment.

Linkage The tendency of genes or alleles that are located on the same chromosome to be inherited together.

Linkage disequilibrium The nonrandom association of genetic polymorphisms at different loci. Linkage disequilibrium is expected among sites that are closely linked, but may also arise for other reasons, such as selection on loci that both influence a beneficial phenotype.

Orthologs Genes that occur in different species, but have the same evolutionary origin and molecular function.

Pleiotropy When a genetic variant influences multiple traits.

Quantitative trait gene A gene that harbors a genetic variant that has a phenotypic effect.

Quantitative trait locus A region of the genome that harbors one or more genetic variants with phenotypic effects.

Quantitative trait nucleotide A genetic variant that contributes to heritable variation in a phenotype.

Statistical genetic architecture A statistical characterization of a phenotype's genetic basis. This may include information on the number of loci that contribute to a phenotype, as well as their effects and interactions.

Yeast two-hybrid A molecular approach for studying physical interactions between proteins.

Introduction

Genetic mapping is commonly used to dissect quantitative traits that are important to agriculture, evolutionary biology, and medicine (Mackay *et al.*, 2009; Georges, 2007). Most mapping efforts detect quantitative trait loci (QTLs) that span tens to hundreds of genes (Remington *et al.*, 2001; Flint and Mott, 2001). These QTLs are useful for studying the statistical genetic architecture of a phenotype. However, they are of limited value in determining how a trait is specified at the molecular level (Flint *et al.*, 2005; Drinkwater and Gould, 2012).

Knowing how genetic variants alter cellular and developmental processes to produce phenotypic changes is important for many reasons. Such knowledge can be used to improve our ability to predict individuals' phenotypes based on their genotypes and to treat heritable diseases with targeted therapies (Albert and Kruglyak, 2015). Moreover, this information can reveal the types of functional genetic variants that segregate in populations and provide a potential substrate for evolution (Seidel *et al.*, 2011).

To determine the functional mechanisms that give rise to heritable phenotypic variation, specific quantitative trait genes (QTGs) and quantitative trait nucleotides (QTNs) for a trait must be identified (Mackay *et al.*, 2009; Georges, 2007; Remington *et al.*, 2001; Flint *et al.*, 2005; Drinkwater and Gould, 2012; Aitman *et al.*, 2010; Liti and Louis, 2012; Weigel and Nordborg, 2005; **Figure 1**). After these QTGs and QTNs have been determined, bioinformatic and experimental approaches can be used to assess how alleles of these factors

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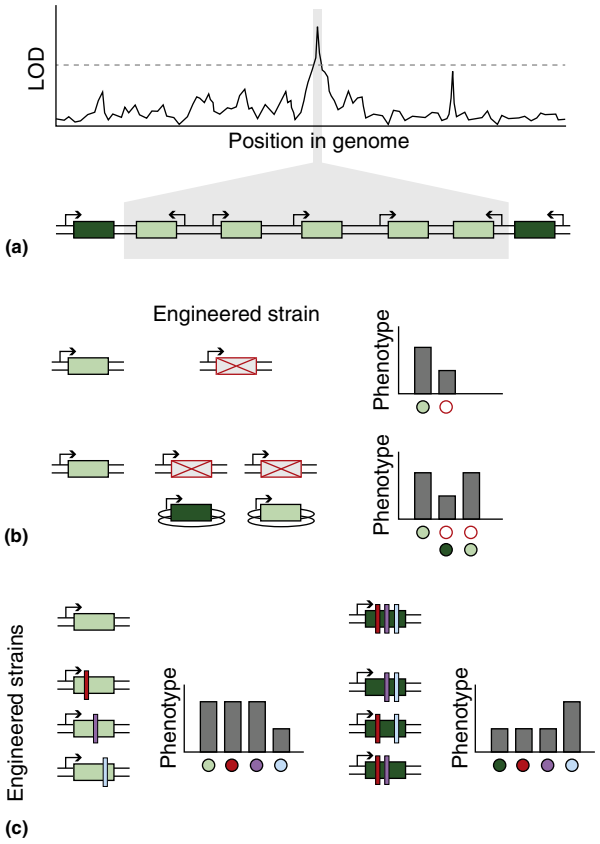

Figure 1 Determining the molecular factors underlying a quantitative trait locus (QTL). An abstract example of how quantitative trait genes (QTGs) and quantitative trait nucleotides (QTNs) are typically identified is shown. In (a), a QTL is detected at the resolution of five genes. To identify the QTG, different strategies may be employed, such as gene deletion and plasmid complementation (b). Gene replacement, in which genetic engineering is used to switch the allele of a gene that an individual carries, and complementation by crossing are other approaches that might be used to go from QTL to QTG. Further resolving a QTG to a specific QTN may be achieved by replacing individual nucleotides (c). We use haploid examples for simplicity. Furthermore, we note that these approaches may be more difficult for genes that are involved in genetic interactions (Mackay, 2014; Taylor and Ehrenreich, 2015) or show different effects across genetic backgrounds (Chandler *et al.*, 2013). In such cases, it might be necessary to conduct the experiments shown in this figure in multiple genetic backgrounds.

functionally differ (e.g., Deutschbauer and Davis, 2005; Grisart *et al.*, 2004; Smith and Kruglyak, 2008; Rosas *et al.*, 2014; Yvert *et al.*, 2003; Filiault *et al.*, 2008; Smemo *et al.*, 2014; Balasubramanian *et al.*, 2006; Fridman *et al.*, 2004; Johanson *et al.*, 2000). Furthermore, combinations of QTNs can be examined at the molecular and systems levels to assess how these variants collectively influence a trait (e.g., Sudarsanam and Cohen, 2014; Zhu *et al.*, 2008).

In this article, we describe how research on the molecular basis of heritable phenotypes enhances our understanding of quantitative genetics. We discuss the distinct insights into phenotypic variation that arise from moving beyond QTLs, and identifying QTGs and QTNs. We then highlight how work on the molecular basis of quantitative trait variation has begun to shed light on functional mechanisms that give rise to commonly observed quantitative genetic effects.

Characterizing the Molecular Basis of a Quantitative Trait

Determining the molecular basis of a quantitative trait requires pinpointing QTGs and QTNs, and assessing how changes in molecular function caused by these variants result in phenotypic effects. Here, we describe specific insights gained at each of these steps.

Identifying QTGs

Relative to mapping QTLs at the resolution of many genes, determining specific QTGs represents a major step forward in our understanding of a trait's molecular basis (Flint *et al.*, 2005; Drinkwater and Gould, 2012; Weigel and Nordborg, 2005). This is because a QTG connects diversity in a phenotype to variation in specific biochemical, metabolic, and regulatory processes. Making these links to molecular mechanisms is the easiest when the genes that contribute to trait variation were previously identified through mutagenesis screens, and characterized using biochemical and molecular approaches (e.g., Rosas *et al.*, 2014; Filiault *et al.*, 2008; Balasubramanian *et al.*, 2006). In such instances, this prior knowledge can be used to formulate hypotheses about the mechanisms that enable alleles of a QTG to differ in their phenotypic effects. This is true even in non-model species, where valuable information about a QTG's function can be obtained from its orthologs in model organisms. However, efforts to identify QTGs can also discover new genes and novel functions for known genes (e.g., Seidel *et al.*, 2011), thereby advancing our core knowledge of the genetics and development of the species and trait of interest.

Characterizing QTNs

While QTGs reveal specific genes and pathways involved in heritable trait variation, QTNs provide valuable insights into how these QTGs contribute to phenotypic diversity. Once a QTN has been identified, primary sequence analysis can be used to predict the polymorphism's effect on its cognate gene and protein. QTNs can affect transcription and translation, alter protein structure and activity, or have other effects at the molecular level (see **Figure 2** for some examples). After QTNs have been found, molecular, cellular, developmental, and physiological assays can be conducted to study how these polymorphisms result in phenotypic changes (e.g., Deutschbauer and Davis, 2005; Grisart *et al.*, 2004; Smith and Kruglyak, 2008; Rosas *et al.*, 2014; Yvert *et al.*, 2003; Filiault *et al.*, 2008; Smemo *et al.*, 2014; Balasubramanian *et al.*, 2006; Fridman *et al.*, 2004; Johanson *et al.*, 2000). For a polymorphism located in a *cis*-regulatory element, one might examine whether the SNP causes a change in transcription factor binding or gene expression. Likewise, for a QTN that changes the amino acid sequence of a protein, one might look at its effects on protein activity, stability, or physical

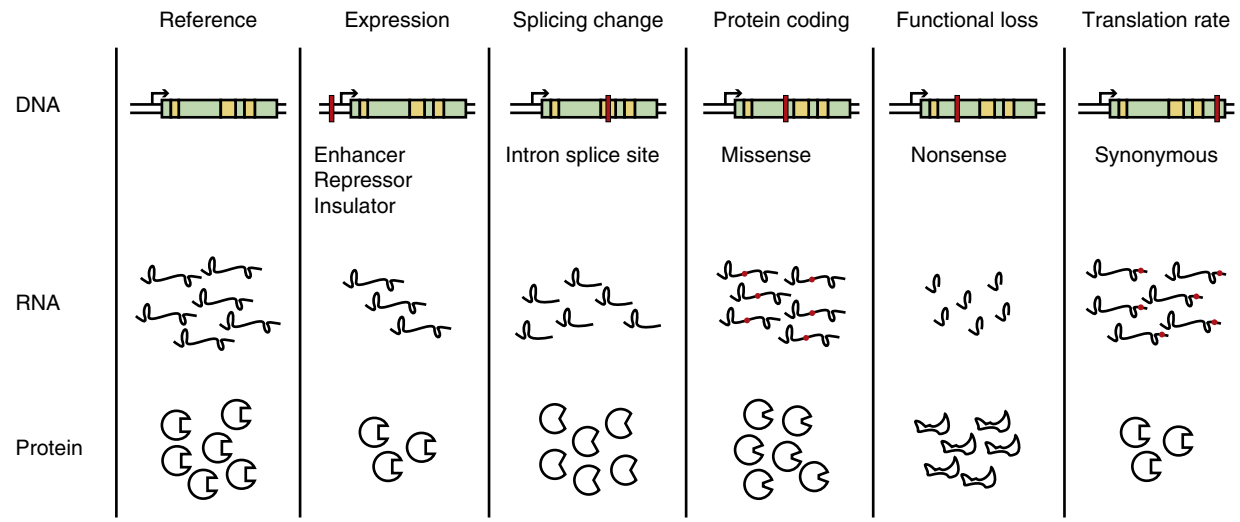

Figure 2 Molecular effects of quantitative trait nucleotides (QTNs). Here, we show a number of ways that polymorphisms can lead to functional changes in quantitative trait genes (QTGs) at the levels of gene regulation and protein function. Effects are separated roughly into the levels of DNA, RNA, and protein.

interactions with other proteins. After examining how a QTN alters the activity of a gene, it is important to assess how this functional perturbation impacts downstream cellular, physiological, and developmental processes. In some organisms, these studies can be performed in individuals that are genetically identical other than at a QTL. Such individuals can be produced using either crossing (e.g., Kooke *et al.*, 2012) or genetic engineering (e.g., Storici *et al.*, 2001) and serve as a powerful resource for determining how a QTN's effect is transduced from the molecular level to the phenotypic level.

Studying the Mechanisms by which Multiple QTNs Influence a Trait

Given that quantitative traits arise due to the effects of multiple QTNs, determining how these factors collectively influence a phenotype is important. This is especially true when sets of QTNs involved in genetic–environment and genotype–environment interactions influence a phenotype (e.g., Gerke *et al.*, 2010, 2009; Taylor and Ehrenreich, 2014). A variety of techniques, such as yeast two-hybrid (Fields and Song, 1989), fluorescence resonance energy transfer (Ma *et al.*, 2014), and co-immunoprecipitation (Sambrook and Russell, 2006), can be used to study how pairs of QTNs affect direct functional interactions between genes. However, a large fraction of nonadditive genetic variation may arise due to statistical interactions that occur between QTNs that are involved in different pathways and cellular processes (Omholt *et al.*, 2000; Gjuvsland *et al.*, 2007; Taylor and Ehrenreich, 2015). This suggests that examining the effects of multiple QTNs at the systems level is important. An increasingly common strategy for such work is to measure global gene expression, as well as the levels of metabolites, proteins, and other molecular intermediates, across genetically distinct individuals (Ayroles *et al.*, 2009; Civelek and Lusis, 2014). By integrating these data with information on the identities and functions of QTNs, researchers can develop network models that explain how gene regulation, metabolism, and physiology are influenced by multiple polymorphisms (Zhu *et al.*, 2008; Rockman, 2008). These models can then be linked to phenotypic outcomes, thereby providing insights into the systems level mechanisms that specify variation in a trait (Zhu *et al.*, 2008; Rockman, 2008).

The Molecular Underpinnings of Quantitative Genetic Effects

Heritable variation in quantitative traits arises due to QTNs that influence the activities and functions of genes, proteins, pathways, and networks. Given that all organisms are comprised of these same building blocks, shared functional mechanisms may underlie commonly observed quantitative genetic effects, such as additivity, dominance, pleiotropy, genetic interaction, and genotype–environment interaction (Omholt *et al.*, 2000). Here, we summarize how these different classes of statistical effects may arise at the molecular level.

Additivity

A large fraction of quantitative trait variation is caused by additive QTNs that act independently of the genetic background in which they occur (Hill *et al.*, 2008). Although many QTNs are likely additive because they have subtle loss- or gain-of-function effects on their cognate genes, even seemingly large molecular perturbations, such as deletions of entire genes, can have additive effects (e.g., DeLuna *et al.*, 2008; Baryshnikova *et al.*, 2010). This suggests that part of the reason that some QTNs appear to be additive is that their phenotypic effects are constrained by genetic buffering. A number of mechanisms are known to buffer biological systems against the effects of genetic variants and the environment, including redundancy among genes and pathways, genetic network architecture, and specific proteins and protein complexes (e.g., heat shock proteins) (as discussed in Hartman *et al.*, 2001; Gu *et al.*, 2003;

Rutherford, 2000; Jeong *et al.*, 2001; Jarosz *et al.*, 2010; Boone *et al.*, 2007; Kitano, 2004 and elsewhere).

Dominance

The molecular basis of dominance has long been a subject of interest among both empiricists and theorists (e.g., Keightley, 1996; Kacser and Burns, 1981; Orr, 1991). Extensive research on mutations and QTNs has identified a number of general molecular mechanisms that result in dominance (Wilkie, 1994). One of the most straightforward causes of dominance is when a QTN has a complete or partial loss-of-function effect on its cognate gene, which is complemented in a heterozygote (**Figure 3(a)**). However, loss-of-function alleles can also be dominant if they result in 'haploinsufficiency,' which occurs when heterozygotes possess a level of an important transcript or protein that falls below a critical threshold (**Figure 3(b)**). 'Dominant negatives' are an additional class of dominant QTN; such variants prevent the other allele of a gene from functioning properly (**Figure 3(c)**). Gain-of-function polymorphisms can exhibit dominance if they increase the abundance of a transcript or protein above a tolerable threshold, or lead to a new spatiotemporal expression pattern (**Figure 3(d)**). Gain-of-function dominant alleles can also arise at the level of protein structure–function relationships: a dominant polymorphism may change a protein's activity or structure, or may even render a protein toxic to cells (Wilkie, 1994). Overdominance may occur if being heterozygous at a locus confers higher fitness than being homozygous. A classic example of such overdominance is the genetic polymorphism that causes sickle cell anemia. This same variant provides protection against malaria and is therefore beneficial in parts of the world where malaria is prominent. Thus, individuals that are heterozygous for the sickle cell allele receive some protection against both malaria and sickle cell disease (Serjeant, 2013).

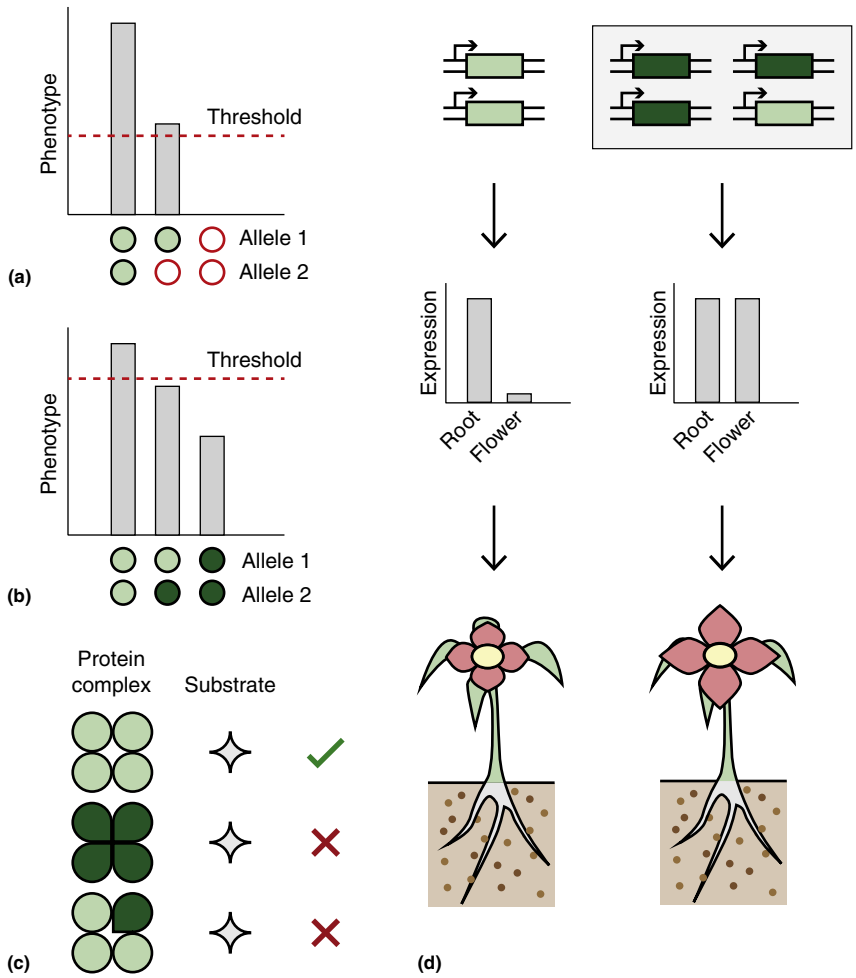

Figure 3 Mechanisms of allelic dominance. As described in the text, dominance can arise due to a number of reasons at the molecular level. (a) Shows an example where a single copy of a gene is sufficient to maintain gene dosage above a required threshold. In contrast, (b) shows an example where a single loss-of-function allele at the level of gene expression is sufficient to reduce gene dosage below a required threshold. (c) Illustrates an example of a dominant negative, where the dominant allele prevents the other allele from functioning. This component of **Figure 3** is based on a figure shown in Alberts *et al.* (2002). In (d), a quantitative trait nucleotide (QTN) causes a gene to be expressed in a new tissue (commonly referred to as 'ectopic expression'), resulting in an increase in flower size that segregates in a dominant manner.

Pleiotropy

When a QTN either directly or indirectly has an effect on multiple traits, it is described as 'pleiotropic' (Paaby and Rockman, 2013; **Figure 4(a)**). As shown in **Figure 4(b)**, a pleiotropic QTN may affect a trait that plays a role in determining an individual's outcome for other phenotypes. Alternatively, a QTN may directly impact multiple downstream pathways that each influences a distinct phenotype (**Figure 4(c)**). Distinguishing between direct and indirect pleiotropy requires knowing the pathways and networks that underlie a trait, as these forms of pleiotropy may be indistinguishable at the phenotypic level. Research attempting to find common features of pleiotropic genes suggests that genes with higher levels of pleiotropy tend to encode proteins that are distributed throughout more cellular compartments or tissues, and are involved in more protein–protein interactions (He and Zhang, 2006; Dudley *et al.*, 2005). Genetic mapping studies focused on quantitative traits have also provided an additional important insight into pleiotropy: pleiotropic QTLs often harbor multiple QTNs that influence different traits, rather than a single QTN that affects multiple traits (Chen and Lubberstedt, 2010). Because closely linked QTNs that underlie distinct phenotypes will segregate together in a population if they are in linkage disequilibrium, the traits they affect may be correlated. Thus, cloning the QTGs and QTNs underlying pleiotropic QTLs is essential for determining the molecular basis of correlations among traits.

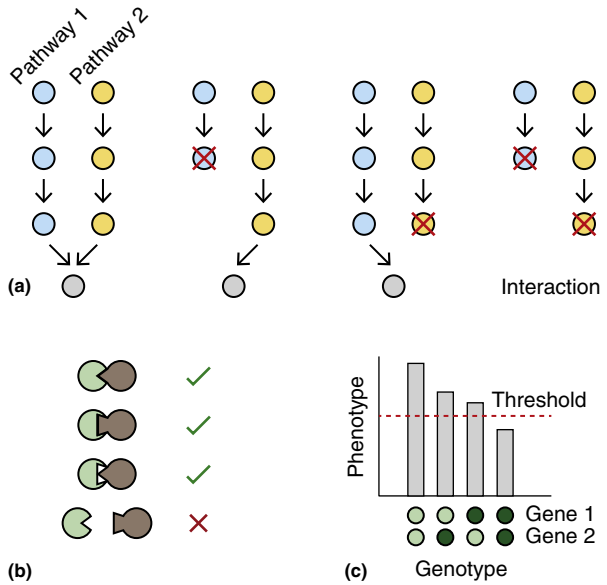

Figure 4 Mechanisms of genetic interactions. We show some mechanisms that can cause genetic interactions, using haploid examples for simplicity. In (a), loss-of-function quantitative trait nucleotides (QTNs) in parallel pathways that converge on the same metabolite or downstream gene result in a genetic interaction when they occur in a particular combination. In (b), two QTNs that disrupt protein structure show an interaction by preventing protein complex formation. Lastly, in (c), we illustrate how combinations of QTNs can impact the level of an important metabolite, protein, or transcript, resulting in a phenotypic effect when the level falls below a threshold.

Genetic Interactions

Genetic interactions occur when the effects of QTNs depend on an individual's genotype at other positions in the genome. These interactions can involve two QTNs (referred to as 'gene–gene interactions') or more than two QTNs (referred to as 'higher-order genetic interactions'). The mechanisms underlying gene–gene interactions have largely been determined through work on large collections of mutants in model organisms (Boone *et al.*, 2007; Lehner, 2011). Typically these interactions arise when genetic variants compromise two pathways or protein complexes that act in parallel, or perturb proteins that function in the same complex (Boone *et al.*, 2007; **Figures 5(a)** and **5(b)**). However, other sources of gene–gene interactions have been proposed; these include the disruption of robustness and the uncovering of otherwise cryptic genetic variants, combinations of QTNs collectively altering the dosage of critical biomolecules either above or below a threshold level (**Figure 5(c)**), and variation in gene regulatory networks (Omholt *et al.*, 2000; Gjuvsland *et al.*, 2007; Lehner, 2011). Less is known about the mechanisms underlying higher-order genetic interactions because these interactions have proven to be more difficult to detect and dissect than gene–gene interactions (Taylor and Ehrenreich, 2014; Pettersson *et al.*, 2011). The most likely source of higher-order genetic interactions is thought to be combinations of three or more genetic variants in gene regulatory networks (Gjuvsland *et al.*, 2007; Taylor and Ehrenreich, 2015). However, there has yet to be empirical proof that this or any other mechanism underlies higher-order genetic interactions.

Gene–Environment and Genotype–Environment Interactions

Many QTNs show effects that depend on the environment. Interactions with the environment can involve single genetic

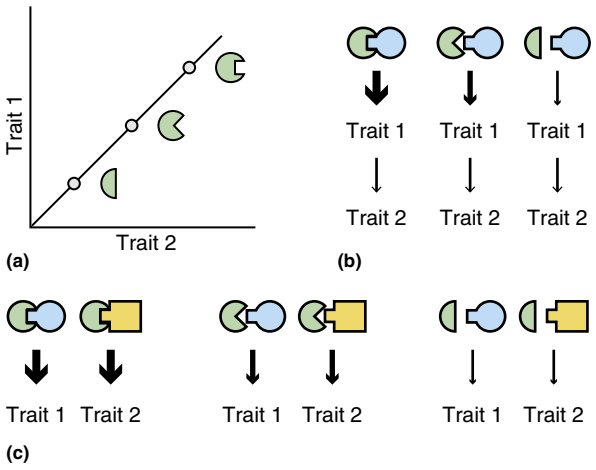

Figure 5 Mechanisms of pleiotropy. Quantitative trait nucleotides (QTNs) that occur in genes that regulate multiple phenotypes can cause correlated trait changes (a). In this figure, we show how this can arise due to polymorphisms that alter the function of a single protein. In (b), QTNs directly affect Trait 1 and only influence Trait 2 through Trait 1. In (c), QTNs directly affect Traits 1 and 2 by influencing how the protein interacts with different partners involved in the respective phenotypes.

variants (referred to as 'gene–environment interaction') or multiple genetic variants (referred to as 'genotype–environment interaction') (Des Marais *et al.*, 2013). The molecular basis of gene–environment and genotype–environment interactions may depend on the conditions being considered and the mechanisms an organism uses to perceive these conditions. Gene–environment and genotype–environment interactions can arise from QTNs in sensors that respond to specific environmental cues (e.g., Filiault *et al.*, 2008; Balasubramanian *et al.*, 2006). Furthermore, transcription factors, signaling cascades, or other components of the cell that act downstream of these sensors and mediate perception of cues may harbor QTNs that interact with the environment (e.g., Smith and Kruglyak, 2008). Biological systems can also show more general responses to the environment (Des Marais *et al.*, 2013). For example, temperature can generally change the kinetic rates and patterns of physical interactions among proteins. Environmental change may also disrupt an organism's ability to buffer itself against genetic variants, thereby uncovering genetic variants that typically do not have phenotypic effects (Gibson and Dworkin, 2004; Paaby and Rockman, 2014).

Conclusion

Quantitative genetics has historically been a field rooted in statistics. However, statistical patterns of heritable phenotypic variation result from the individual and collective effects of functional genetic variants that segregate in populations. Thus, work aimed at identifying these factors and examining how they exert their effects is crucial for bridging the gap between our statistical and molecular understanding of heritable trait variation. Such information will be crucial in improving our ability to predict and treat heritable diseases, and will also likely improve our understanding of how traits evolve in natural populations. Moving forward, technological advances in genetic mapping and genome editing (Ryan *et al.*, 2014; Cong *et al.*, 2013) are likely to accelerate progress in this area. Such research will undoubtedly continue to improve our understanding of the molecular mechanisms that determine the relationship between genotype and phenotype.

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See also: Genetic Architecture. Genotype-by-Environment Interaction. Quantitative Genetic Variation

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Quaternary Biogeography and Climate Change

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Glossary

Albedo The reflecting power of a surface.
Ancient DNA (aDNA) DNA that has survived in the remains of ancient organisms.
Meridional overturning circulation A global oceanic circulation pattern wherein surface waters in the high latitudes are cooled, thereby becoming denser; this dense water sinks and flows toward the equatorial regions. In tropical and subtropical regions around the world these waters eventually mix with other waters, becoming less dense, and they return to the surface to ultimately flow toward the higher latitudes and complete the circuit.
No-analog assemblages Combinations of species observed at particular places and times in the past that are not seen anywhere on the landscape today.

Introduction

The Quaternary, which started 2.588 million years ago (Ma) and encompasses both the Pleistocene (2.588 Ma to 11.7 thousand years ago (ka)) and the Holocene (11.7 ka to the present), is characterized by substantial climatic and biogeographic change. Climatically, this time period is known as the 'Ice Ages' because the earth has cycled between a series of cold glacial and warmer interglacial periods (Hays *et al.*, 1976). A significant amount of biogeographic change also occurred, including the evolution of *Homo sapiens* in Africa and subsequent dispersal to the rest of the world, and the evolution of modern biotic communities (Blois and Hadly, 2009; Henn *et al.*, 2012). Finally, this time period also encompasses the Anthropocene, a proposed new epoch within the Quaternary characterized by significant human impacts on natural processes (Zalasiewicz *et al.*, 2011).

Here, we discuss Quaternary climate change and biogeography, primarily using examples from North America over the past 21 000 years. This well-studied time period encompasses the most recent transition from a glacial to an interglacial period (Denton *et al.*, 2010). It also saw the spread of humans from Eurasia into North and South America (Achilli *et al.*, 2013; Hamilton and Buchanan, 2010; Henn *et al.*, 2012), the near-global extinction of large megafauna (e.g., Barnosky *et al.*, 2004; Koch and Barnosky, 2006), and significant population-level changes in vegetation and the surviving smaller animals (e.g., Blois *et al.*, 2010; Gill *et al.*, 2009; Williams *et al.*, 2004). Many of these biogeographic changes happened, at least in part, due to climate change (e.g., Barnosky *et al.*, 2004; Lorenzen *et al.*, 2011; Williams and Jackson, 2007). Thus, the Quaternary and the last 21 000 years in particular provide baselines for expected rates and magnitudes of climate change in normal conditions, as well as for how populations, species, communities, and ecosystems have responded to those changes.

Quaternary Climate Change

Orbital Variation and Quaternary Glacial–Interglacial Cycles

The Quaternary occurred at the end of a long-term cooling trend that followed the warm early Eocene climatic optimum roughly 50 Ma. Thus, while the glacial–interglacial cycles that characterize the Quaternary encompass both cold and warm times, even the warm periods are colder than most times in the Cenozoic (the past 65 million years) (**Figure 1**; Zachos *et al.*, 2001). This long-term cooling trend facilitated the development of extensive ice sheets in both the southern and northern hemispheres. While permanent ice sheets have been in place in Antarctica for tens of millions of years (Kennett, 1977; Zachos *et al.*, 2001), the start of the Quaternary, 2.588 Ma, roughly coincided with the development of permanent ice sheets in the northern Hemisphere (Ruddiman and Raymo, 1988).

One of the most striking physical features of the Quaternary is the periodic growth and decline of the northern hemisphere ice sheets, which provide visible evidence of the accompanying climatic changes that occurred throughout the Quaternary. These glacial–interglacial cycles are driven primarily by changes in the position of the earth relative to the sun, which fluctuates due to orbital variations (Raymo *et al.*, 2006). Together, orbital variations influence the amount and distribution of solar radiation across the earth and the seasonal contrasts of solar radiation, pacing the build up and dissipation of large ice sheets at the poles (Lisiecki and Raymo, 2005; Raymo *et al.*, 2006; Ruddiman, 2008; Zachos *et al.*, 2001).

Orbital variations have a number of consequences for both marine and terrestrial environments and physical landscapes, including interrelated changes to ice sheets, sea levels, CO_2 and other atmospheric gases, temperature, and aridity. Ice sheet buildup is spurred initially by orbitally forced decreases in summer insolation which causes summer cooling, amplified (at least in North America) by changes in vegetation from forest to tundra (Kageyama *et al.*, 2004). Subsequent feedbacks between land and ice through changes to albedo, changes in the altitude of ice sheets, and restriction of freshwater flows into the adjacent oceans helps amplify the buildup of the ice sheets (Kageyama *et al.*, 2004). Increases in the extent and volume of ice on land correspond to decreases in sea levels, which were dramatically lower during glacials, for example, reaching around 120 m below present-day sea level (Denton *et al.*, 2010).

Similar to the role of insolation in glacial inception, deglaciation is initiated when summer insolation increases. However, increases in summer insolation do not always cause glacial terminations; other factors are also important (Denton

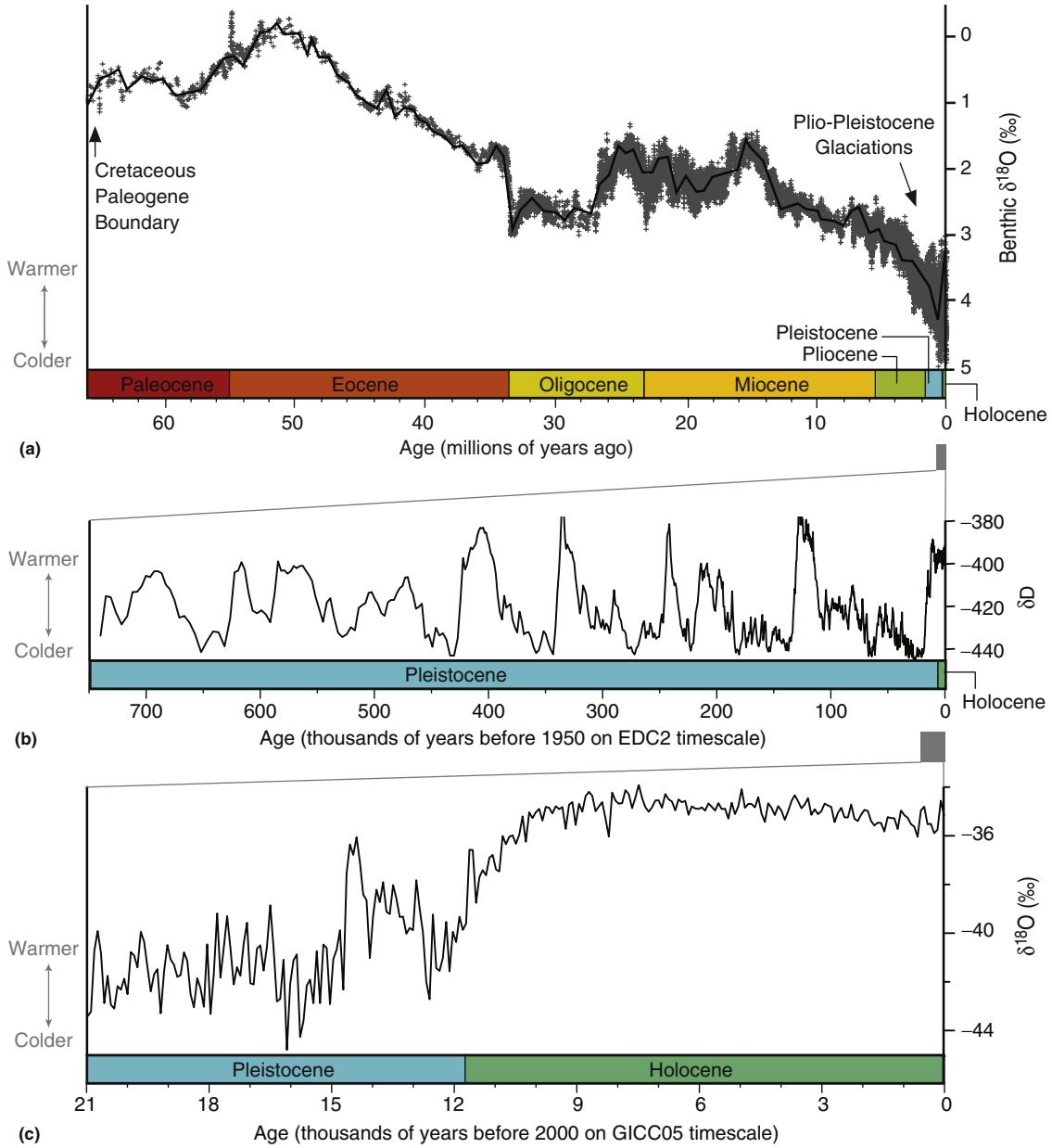

Figure 1 Three views of climate change across the Cenozoic. (a) Global cooling from the early Cenozoic to the present inferred from changes in oxygen isotopes ($\delta^{18}O$), modified from Blois, J.L., Hadly, E.A., 2009. Mammalian response to Cenozoic climatic change. Annual Review of Earth and Planetary Sciences 37, 181–208 and adapted from Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693, with permission from American Association for the Advancement of Science. (b) Glacial–interglacial variation over the past 750 000 years, inferred from changes in deuterium (δD) throughout the EPICA Dome C ice core in Antarctica (Augustin *et al.*, 2004). (c) Transition from the Last Glacial Maximum to the present, inferred from $\delta^{18}O$ from the NGRIP ice core in Greenland (Andersen *et al.*, 2004; Rasmussen *et al.*, 2006).

et al., 2010). One factor appears to be continental ice volume: when continental ice volume nears its maximum, enough fresh meltwater flows from the continental ice sheets into the North Atlantic to decrease North Atlantic meridional overturning circulation. This initiates a cascade of global effects to both oceans and atmospheres that causes atmospheric CO_2 levels to rise high enough to sustain an interglacial (Denton *et al.*, 2010). Sea levels also rise during transitions from glacial to interglacial states as the large amount of water formerly locked up as ice on land drains into the ocean (Lambeck *et al.*, 2002). Changes to the ice sheets and sea levels during deglaciation affect the physical space available to terrestrial and marine species; in some places (e.g., northern North America), new physical space becomes available to terrestrial species as ice sheets melt and retreat northward, whereas in other locations physical environments are lost as sea levels rise (e.g., coastlines). The converse is true of the physical changes that occur during glacial inceptions.

Each glacial and interglacial period was unique – in magnitude of warming and cooling, in duration, and in the variation in short-term climate events overlain on the glacial–interglacial cycles (Augustin *et al.*, 2004; Petit *et al.*, 1999). However, several general patterns emerge. Glacial–interglacial cycles in the early Pleistocene were characterized by high-frequency cycles of relatively low magnitude, and cycles were symmetric; that is, duration of the glacial–interglacial transitions (entry into interglacial) was relatively similar to that of the interglacial–glacial transitions (termination of glacial) (Ruddiman and Raymo, 1988; Tziperman and Gildor, 2003). However, the periodicity and magnitude of glacial–interglacial cycles changed between 1 Ma and 600 ka (**Figure 1**): cycles became longer (less frequent, on the order of 100 000 years duration), and the amplitude of each cycle increased (Lisiecki and Raymo, 2005; Ruddiman and Raymo, 1988). Since then, the duration of the glacial portion of the cycle has been between 80 000 and 120 000 years in length (Denton *et al.*, 2010). In addition, the rates of change at the initiation and termination of glacials became asymmetric: initiation of glacial periods was slow, characterized by an oscillating buildup, but termination of the glacial period was rapid (Denton *et al.*, 2010; Tziperman and Gildor, 2003).

Rapid Climate Change and the Last Glacial–Interglacial Transition

A majority of the biogeographic data available for the Quaternary are from the most recent glacial–interglacial transition, so we focus on this transition to illustrate the decadal to millennial-scale climatic variability that accompanies the more general glacial to interglacial transition (**Figure 1**). We also illuminate specific patterns and mechanisms of shorter-term variation that occurred at earlier glacial–interglacial transitions (e.g., Raymo *et al.*, 1998).

The global climate transition from the Last Glacial Maximum (LGM) *ca.* 21 ka to the start of the Holocene interglacial period 11.7 ka was marked by collapse and retreat of the ice sheets, substantial rises in sea levels, and relatively rapid global warming (Denton and Hughes, 2002). However, these changes did not occur smoothly and monotonically but were instead marked by starts and reversals, and periods of rapid change followed by periods of little change. For example, ice sheet retreat and corresponding changes in sea levels were discontinuous (Carlson and Winsor, 2012). In North America, the Cordilleran and Laurentide ice sheets covered northern North America, and an additional ice sheet still occurs on Greenland. Portions of these ice sheets reached their maximum extent as early as 30 ka and persisted at maximal extent until roughly 19 ka (Carlson and Winsor, 2012; Clark *et al.*, 2009), at which point they extended south of the 49th parallel into the United States. The most rapid rates of ice sheet retreat occurred around 19 ka and during the Bølling–Allerod warm period (14.7–12.9 ka), though final collapse of the Laurentide did not occur until 8.2 ka (Carlson and Winsor, 2012). Sea level rose in spurts corresponding to changes in the ice sheets, and finally leveled off around 6 ka (Carlson and Winsor, 2012). Altogether, sea levels rose roughly 120 m across the last glacial–interglacial transition (Denton and Hughes, 2002).

Global temperatures rose 3–5 °C on average from the LGM to the present (Jansen *et al.*, 2007), but the transition was marked by substantial spatial and temporal variability. Among the most significant climatic changes during deglaciation were two periods of rapid warming with an intervening cold interval, all of which occurred within roughly 3000 years (**Figure 1**; Steffensen *et al.*, 2008). Following initial deglaciation, rapid warming occurred around 14.7 ka during the entry into the Bølling–Allerod period. For example, a Greenland ice core showed that around 9 °C degrees of warming occurred within 70 years (Severinghaus and Brook, 1999). The causes of this event are still being investigated, but warming was likely related to reduced amounts of freshwater runoff into the North Atlantic (Liu *et al.*, 2009; Denton *et al.*, 2010). Following this warm period, the climate system entered into the Younger Dryas (12.9–11.7 ka) when large pulses of freshwater into the North Atlantic reduced meridional overturning circulation and thus global heat transport (McManus *et al.*, 2004). The end of the Younger Dryas was marked by rapid warming into the Holocene interglacial, on the order of 5–10 °C in Greenland in as little as a few decades (Dansgaard *et al.*, 1989; Severinghaus and Brook, 1999; Taylor *et al.*, 1997). Both of these rapid climate changes have been detected in diverse proxy records around the world (Clark *et al.*, 2009) and were truly global events, though the details differ between the northern and southern hemispheres and the magnitude of climate change was variable across space and time. Changes were also seen in many other aspects of the climate system, such as temperature, aridity, and the seasonality of both (e.g., Bartlein *et al.*, 1998).

Holocene Variability

Climates also varied during the Holocene interglacial period, but the magnitude of change was generally less than during deglaciation (**Figure 1**; Mayewski *et al.*, 2004; Shuman, 2012; Viau *et al.*, 2006). Mayewski *et al.* (2004) identified at least six periods of rapid climate change occurring throughout the Holocene of varying durations. Each event experienced significant enough change that it was detected in global arrays of paleoclimate proxy records (though not every site showed every change). The first period (9–8 ka) was a cool period in the northern Hemisphere. It represented a lagged response to orbital forcings at the end of the Pleistocene and corresponded to the final collapse of the Laurentide ice sheet into the North Atlantic Ocean at 8.2 ka (Alley *et al.*, 1997). Low latitude regions generally saw increased aridity during this time. Subsequent Holocene periods of rapid climate change were also characterized generally by polar cooling and tropical aridity (Mayewski *et al.*, 2004). The primary exception is the most recent period of climate change, 600–150 years BP, when the polar regions were cool but low latitudes wet. The most significant Holocene climate changes involved changes in aridity rather than temperature.

Quaternary Biogeography

Overall, the environmental variation that characterized the Quaternary – both in climates and in physical environments – significantly affected the biological diversity of the globe (e.g., Blois and Hadly, 2009). Understanding these impacts and how

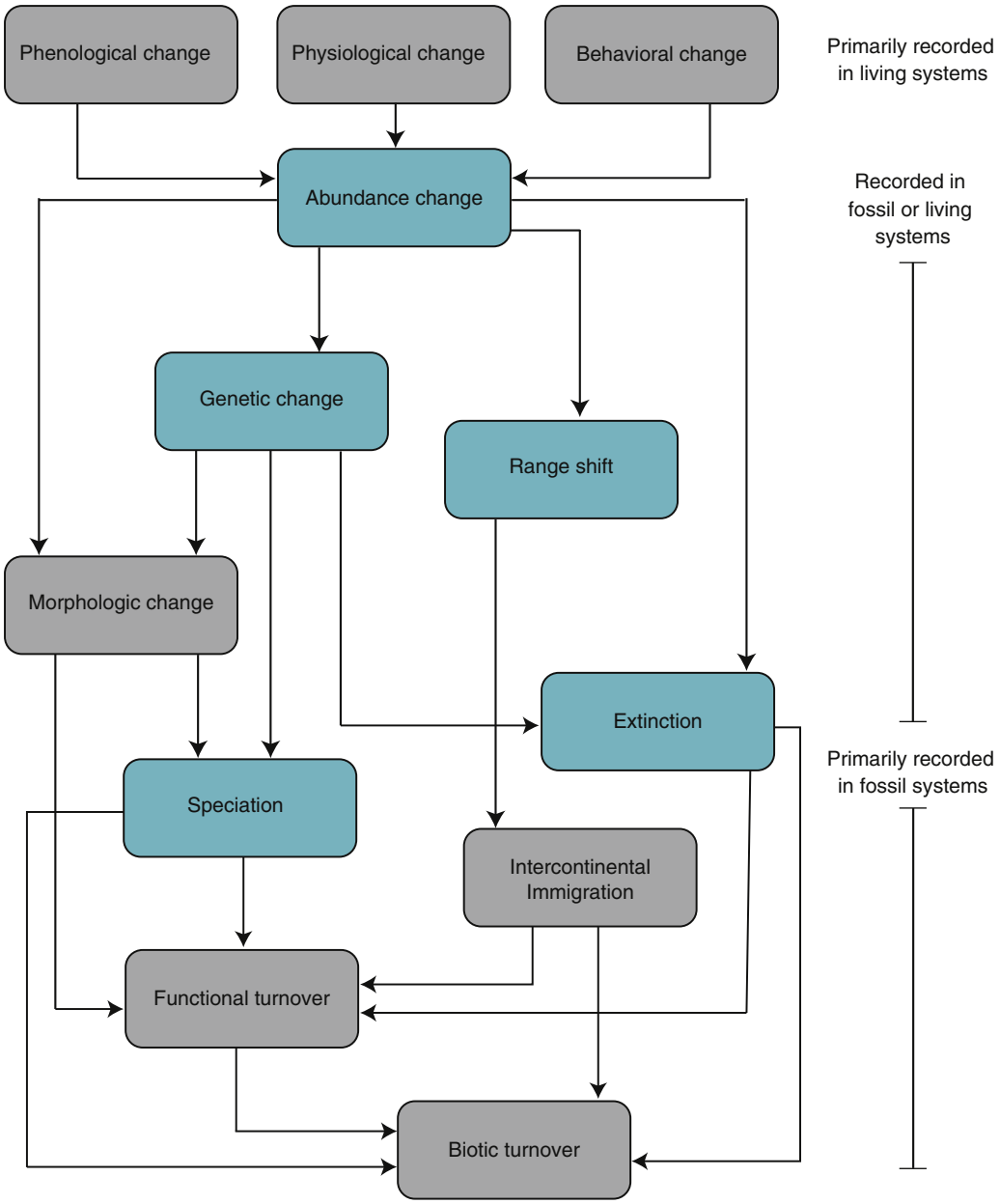

Figure 2 Different responses that species may exhibit to climate change, and the systems (modern and/or fossil) in which they are typically studied. For simplicity, only the main pathways between responses are indicated. Responses discussed in the text are highlighted in teal color. Modified with permission from Blois, J.L., Hadly, E.A., 2009. Mammalian response to Cenozoic climatic change. Annual Review of Earth and Planetary Sciences 37, 181–208.

organisms have responded to past climate changes is important for designing effective conservation strategies that optimize the ability of extant species to persist in the face of significant future climate changes. Below, we review some of the biogeographic changes that occurred in response to Quaternary climate change (Figure 2; Blois and Hadly, 2009), among them abundance changes, genetic structure and diversity changes, range shifts, speciation, and extinction. These changes are not independent of one another, nor are they the only responses that can be exhibited by species (e.g., physiological or morphological responses are also seen in response to climate change; Millien *et al.*, 2006; Pörtner and Farrell, 2008); however, they are the responses most often detected in fossil systems. We then examine how changes within single populations and species scale up to biogeographic responses detected at the assemblage and ecosystem levels.

Population and Species Responses to Climate Change

Abundance change

One of the first responses that a species shows to climate change is changes in abundance (Lundberg *et al.*, 2000). Because abundance changes determine the size and/or location of populations within a species distribution, they can drive other observed responses to climate change such as genetic

change, morphological change, and range shifts (**Figure 2**; Blois and Hadly, 2009; Hewitt, 2000). As climate changes and the abundance of a species decreases or increases, the species' ability to withstand perturbations will similarly decrease or increase (Johnson, 1998; Kiessling and Aberhan, 2007; Nogués-Bravo *et al.*, 2008; Purvis *et al.*, 2000).

Abundance is a function of the amount and availability of suitable habitat present (Andrewartha and Birch, 1954; del Monte-Luna *et al.*, 2004). How abundance changes as climate changes is a complex function of the interaction between climate and/or habitat change, life history strategies, habitat preferences of a species, and interactions with other species (e.g., Forchhammer *et al.*, 2002). Although a single species has a general range of conditions within which it can survive (e.g., its environmental niche *sensu*; Grinnell, 1917; Soberón, 2007), the specific shape of environmental fluctuations (e.g., magnitude, duration, and rate of climate change), combined with intraspecific variation in tolerances within populations (such as between adults and juveniles), can result in many possible outcomes: sustained populations, periodic extirpation-recolonization events, or permanent extirpation (Jackson *et al.*, 2009).

In the fossil record, abundance changes appear to be largely individualistic responses that vary according to the environmental niche of each species (Blois *et al.*, 2010; Terry *et al.*, 2011; Williams *et al.*, 2004). Paleo-records have also shown that rapid climate change can affect population abundances almost immediately, at times leading to rapid ecosystem restructuring. For example, Yu (2007) determined that forested vegetation in New Jersey responded to the Bølling–Allerod warming event with a lag of at most 200 years (**Figure 1**), with the cold-tolerant *Picea* declining and the warm-tolerant *Pinus* increasing. Responses to subsequent rapid cooling during the Younger Dryas (**Figure 1**) again occurred almost immediately (e.g., decreased abundances of warm-tolerant oak). Similarly, rapid vegetation response to climate changes led to the restructuring of communities elsewhere in northeastern North America, where pine, hemlock, and spruce abundances declined only to be replaced by another dominant species (e.g., Shuman *et al.*, 2009). Changes in abundance have also been seen in mammals in response to late Quaternary climate changes (e.g., Blois *et al.*, 2010; Grayson, 2006), though limits of dating precision and the taphonomy of many mammalian fossil deposits do not typically allow accurate assessment of mammalian responses to rapid climate change.

Genetic change

The advent of ancient DNA (aDNA) has identified significant genetic changes that occurred within species across the past several millennia. Under optimal preservation conditions such as in permafrost settings, aDNA as old as 50 000–65 000 years old can be recovered (Willerslev *et al.*, 2003), though in some exceptional cases recovery of much older aDNA is possible (e.g., Orlando *et al.*, 2013). Ancient DNA provides the opportunity to study the impact of climate change on the structure and genetic diversity within past populations and species (e.g., Hadly *et al.*, 2004; Hofreiter *et al.*, 2004b; Hofreiter and Stewart, 2009). Genetic change is a result of population-level processes such as recombination, mutation, selection, random genetic drift, and gene flow (Charlesworth *et al.*, 2003), all of which take place in species over tens to thousands of years. Most of these processes may be influenced by climate, either directly such as when climate selects for or against key traits or indirectly through abundance or habitat change, which influences gene flow and genetic drift. For example, aDNA has been used to determine changes in the relative abundance of ancient populations (Chan *et al.*, 2006; Hadly *et al.*, 2004; Lorenzen *et al.*, 2011), whether populations became connected or remained isolated during periods of climate change (Hadly *et al.*, 2004, 1998; Hofreiter *et al.*, 2004a; Teacher *et al.*, 2011), and whether and which clades survived across periods of climate change (Brace *et al.*, 2012; Foote *et al.*, 2013; Fulton *et al.*, 2013). Species reservoirs of genetic diversity and their potential for genetic change may become increasingly important for species to successfully adapt to anthropogenic climate change (e.g., Jump and Penuelas, 2005).

Range shifts

One of the most prominent and well-supported responses that species have to climate change is range shifts, both today and in the past (Lyons, 2003; Ordonez and Williams, 2013; Sexton *et al.*, 2009; Tingley *et al.*, 2009; Waltari *et al.*, 2007). Species ranges are limited at broad scales by environmental factors such as temperature, precipitation, and relative humidity (Brown *et al.*, 1996; Eronen and Rook, 2004). As climate changes and environmental gradients shift, species may track their preferred environments at varying directions and rates according to each species physiological limitations (Graham *et al.*, 1996; Tingley *et al.*, 2009; Walther *et al.*, 2002). At one end of the scale, changes in climate may result in only minimal adjustments to species ranges, particularly for species that are not tightly controlled by climate or are located in regions with a large amount of topographic and thus microclimatic complexity (Ackerly *et al.*, 2010; Loarie *et al.*, 2009). On the other hand, changes in climate may result in large range shifts, particularly in areas where climate velocity is large and for species that have good dispersal capabilities (Loarie *et al.*, 2009; Ordonez and Williams, 2013; Schloss *et al.*, 2012). Shifts in species ranges are a direct result of changes in abundance (**Figure 2**), and occur when existing populations are extirpated and/or new populations are formed just beyond the edges of ranges (**Figure 3**; Walther *et al.*, 2002). Range expansion is due to episodes of colonization into favorable habit (Walther *et al.*, 2002), and the rate at which a species colonizes new habitat is dependent upon many factors such as climate change, dispersal capability, and microclimate (Lesser and Jackson, 2013). At the very broadest scale, species can move into entirely different continents, such as when humans crossed the Bering land bridge into North America (Achilli *et al.*, 2013; Goebel *et al.*, 2008). These events are more rare than within-continent range shifts and are often accompanied by speciation or biotic turnover.

Studies on extant species have illuminated the processes that may underlie present and past range shifts. Not all species alter their ranges in response to climate change, but the species that do are typically ones that live at higher latitudes or elevations and are often found near the edge of their physiological limits (Hickling *et al.*, 2006; McCain and King, 2014; Walther *et al.*, 2002). The magnitude and direction of range shift depends on the species life history characteristics

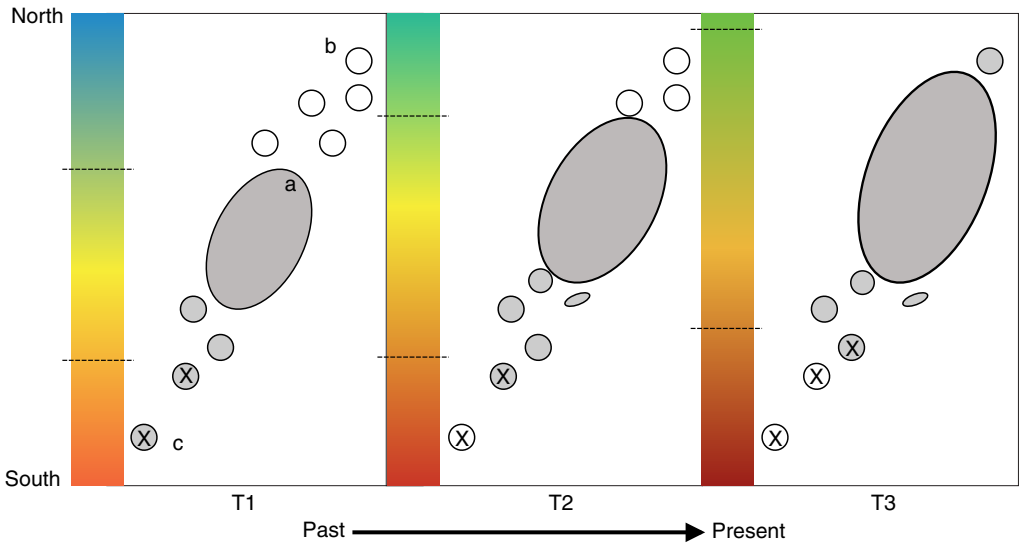

Figure 3 Potential range shifts of a species as a response to climate change across three time periods. In each panel from time 1 (T1) to time 3 (T3), the geographic extent remains the same but the climatic gradient across space shifts from colder (blue) to warmer (red). At T1, the species is able to persist in a range of environments (gray filled circles), though some of them are unsampled (X) so the range limits (both geographic and climatic, indicated by the dashed line) appear to be narrower than they are. They are not able to persist in other environments (unfilled circles). With increasing global temperatures (e.g., from T1 to T3) individuals from the northern leading edge of the distribution will colonize new localities in search of habitats with suitable climates. As temperatures continue to increase from T2 to T3, previously unsuitable habitats become suitable as local climates warm, while previously suitable habitats become unsuitable. Finally, when temperatures reach a maximum (T3), populations at the southern trailing edge of the distribution become extirpated, due to the loss of suitable habitat. Also, the entire species range has shifted in geographic space to the north. Shifts have occurred in climate space as well (i.e., temperatures at the southern localities are warmer at T3 than anything observed in T1). Shifts in climate space arise from, potentially, adaptation to warmer climate regimes in the south, but adaptation was not as strong as empirically observed because some localities from warm places were unsampled in T1, creating the perception of a narrower climatic niche.

such as dispersal ability, their sensitivity to the environment, and the amount of climate change that occurs at a local scale (McCain and King, 2014). Species that have narrow tolerance ranges are affected more by climate change than species that can tolerate varied climates and habitats (Liow *et al.*, 2009; McCain and King, 2014).

The location and distribution of species are also a function of biotic interactions. Direct evidence shows that biotic interactions such as competition can regulate the extent of a species range (Sexton *et al.*, 2009). Additionally, species ranges are smaller at lower latitudes, suggesting that increased biotic interactions and competition in areas with higher species diversity, i.e., the tropics, can dictate the size of a species distribution (Brown *et al.*, 1996; Rapoport, 1982). However, the extent to which biotic interactions are detected in the fossil record is limited (Blois *et al.*, 2014).

Information about past species localities has been fairly well preserved in the fossil record and these data have been aggregated in public databases (i.e., FAUNMAP) and museum repositories (Brewer *et al.*, 2012; Uhen *et al.*, 2013), facilitating reconstruction of species ranges at various times in the past (Graham *et al.*, 1996; Lyons, 2003). For example, between the Pre-Glacial to Glacial and Glacial to Holocene time periods, North American mammals shifted their ranges more northward than they did southward (Graham *et al.*, 1996; Lyons, 2003). Plant populations also generally expanded northward across the late Quaternary. Davis and Shaw (2001) provided evidence of this northward shift by examining spruce pollen percentages in lake sediments from eastern North America and determined that spruce had a more southerly range approximately 21.5 thousand years ago compared to 500 years ago. Similar changes occurred in many other plant genera (Davis, 1983; Huntley and Webb, 1989). The advent of paleo-species distribution modeling (SDM) and paleoclimate simulations has facilitated correlation of past changes in species distributions with climate change (Nógues-Bravo, 2009; Svenning *et al.*, 2011; Varela *et al.*, 2011). Paleo-SDMs have provided insight into, for example, the potential location of glacial refugia (Gavin *et al.*, 2014; Svenning *et al.*, 2008), how past distributions changed as climate changed (e.g., Nogués-Bravo *et al.*, 2008; Ordonez and Williams, 2013; Rodriguez-Sanchez *et al.*, 2010), and the role of climate in past extinction events (Lorenzen *et al.*, 2011; Nogués-Bravo *et al.*, 2008).

Speciation

The many glacial–interglacial cycles of the Quaternary may have driven speciation within many plants and animals by shifting climate zones, forming and/or removing environmental barriers, and fragmenting populations (Barnosky, 2005; Hewitt, 1996). Habitat fragmentation can result in populations that are isolated from one another; if isolation persists for an extended period of time, substantial genetic divergence could occur between populations and result in speciation (Barnosky, 2005; Hewitt, 2000; Kadereit *et al.*,

2004; Stewart *et al.*, 2010). However, whether and for how long a population is isolated depends on the interaction between the nature of the barrier and the species environmental tolerances (Hewitt, 2000, 1996). For example, if a species is adapted to cooler climates it will occupy refugia during warmer climate cycles (which are typically short in duration during the Quaternary), whereas species that are adapted to warmer climates will occupy refugia during the longer glacial periods; these differences in isolation time can also affect the timing of divergence between species (Gavin *et al.*, 2014; Kadereit *et al.*, 2004; Stewart *et al.*, 2010).

Because so many climate fluctuations occurred, the rate of speciation during the Quaternary should be elevated compared to similar time periods without glacial–interglacial cycles (Avise and Walker, 1998). However, evidence has been provided both for (Jakob *et al.*, 2007) and against (Barber and Jensen, 2012) climate-induced speciation. The glacial and interglacial cycles led to the isolation of populations for relatively short periods of time (at most, approximately 100 thousand years), which resulted in intraspecific genetic differences. In some cases, these genetic differences led to speciation events (Brochmann and Brysting, 2008; Galbreath and Cook, 2004; Jakob *et al.*, 2007). However, the rates of speciation during the Quaternary period are not higher than previous time periods, at least for mammals (Barnosky, 2005; Bobe and Behrensmeyer, 2004), perhaps because the duration of isolation was not long enough to facilitate speciation in these groups. The reconnection of fragmented populations during favorable conditions may have allowed for increased gene flow, which resulted in lower than expected speciation rates for the Quaternary period (Barnosky, 2005; Klicka and Zink, 1997; Zink *et al.*, 2004).

Extinction

A major extinction event occurred during the Quaternary between approximately 50 ka and 10 ka (Barnosky *et al.*, 2004; Koch and Barnosky, 2006). During this time, 97 of the 150 largest mammalian genera went extinct across all continents (Barnosky *et al.*, 2004; Lyons *et al.*, 2004; Roberts *et al.*, 2001), though extinction severity was spatially heterogeneous. Africa was not affected as heavily as the other continents and only lost 18% of its genera, whereas continents such as North America and South America lost 76% and 86% of their genera, respectively (Nógues-Bravo *et al.*, 2010). Megafaunal extinction was also heterogeneous through time (Barnosky *et al.*, 2004; Hofreiter and Stewart, 2009; Stuart and Lister, 2012).

Many hypotheses about the cause of the megafaunal extinction have been postulated, but the two main hypotheses are climate change and human interactions (Barnosky *et al.*, 2004; Koch and Barnosky, 2006; Lyons *et al.*, 2004). Humans clearly impacted megafaunal populations and caused some species to go extinct, but the exact mechanisms of human impact are still unclear. Potential human drivers of extinction include habitat degradation and fragmentation by practices such as deforestation and fire (Burney *et al.*, 2004; Robinson *et al.*, 2005), overhunting (Brook and Johnson, 2006; Lyons *et al.*, 2004; Martin, 1967), and pandemic disease introduced by humans (Alroy, 2001; Rothschild and Laub, 2006). Of these drivers, the leading hypothesis is overhunting (Barnosky *et al.*, 2004; Haynes, 2013; Koch and Barnosky, 2006; Lyons *et al.*, 2004; Martin, 1967). Several studies have examined the timing of the megafaunal extinctions on each continent and found a strong correlation between human arrival and megafaunal extinction (e.g., North America and Australia; Barnosky *et al.*, 2004; Johnson *et al.*, 2013). Human arrival has also been correlated with the extinction of other groups such as birds (Steadman, 1995). The ability of humans to severely impact megafauna through overhunting is thought to result from the naivety or lack of coevolution of animals with humans on most continents and the slow reproductive rates of large mammals (Haynes, 2013; Lyons *et al.*, 2004; Zuo *et al.*, 2013). However, many of the extinct megafauna show no signs of being hunted by humans (Grayson, 1984; Koch and Barnosky, 2006) and some species may have coexisted with humans for thousands of years before going extinct (Lima-Ribeiro and Diniz-Filho, 2013; Wroe *et al.*, 2004). Another alternative is that extinction could have occurred due to indirect effects of hunting (Lyons *et al.*, 2004), such as habitat change or ecological cascades resulting from extinction of just a few key megafaunal species (Gill *et al.*, 2009). All of these factors contribute uncertainty about the total impact of overhunting on megafaunal extinction (Sandom *et al.*, 2014).

Climate change at the end of the last glacial period has also been hypothesized as a cause of megafaunal extinction, perhaps by reducing the types and quality of habitat available to the animals and thus reducing the resources necessary to support species (Koch and Barnosky, 2006). For example, the megafaunal extinction rate was positively correlated with the magnitude of climate change on all continents except for South America, which only had a moderate amount of climate change during the late Quaternary period (Nógues-Bravo *et al.*, 2010). Some species went extinct before the local arrival of humans and the timing of extinction was correlated with significant climate changes in some systems, ruling out humans as the cause of extinction for those taxa (Barnosky, 1986; Stuart and Lister, 2012; Wroe *et al.*, 2013). Despite the apparently clear link between extinction and climate, the climate change hypothesis has had a difficult time explaining why only large mammals were affected and not other groups such as marine organisms or plants (Lyons *et al.*, 2004; Martin, 1984), though size-dependent differences in reproductive rates may have been a factor (Johnson, 2002; Zuo *et al.*, 2013). Also, large mammals that were nocturnal, arboreal, and lived in inaccessible forests (e.g., mammals that were not as exposed to human hunting) were not as drastically affected by the megafaunal extinction (Koch and Barnosky, 2006). Finally, climate change hypotheses have a difficult time explaining why there was an increased extinction rate for large mammals during the LGM and not in previous glacial periods (Lyons *et al.*, 2004).

Although many studies have proposed either humans or climate change as the culprit for the late Quaternary megafaunal extinctions, recent studies show that the mechanism of extinction for many species is likely a combination of both human impacts and climate change (Haynes, 2014; Lorenzen *et al.*, 2011; Nogués-Bravo *et al.*, 2008; Prescott *et al.*, 2012). These studies postulate that climate change altered species abundances and distributions at the end of the last glacial period, such that the smaller populations were more susceptible to overhunting from humans (e.g., Nogués-Bravo *et al.*, 2008). Since some continents had high extinction rates with low climate change (Nógues-Bravo *et al.*, 2010) and other species went extinct with no evidence of human hunting or interference, extinction mechanisms are likely location- and/or species-specific.

Community Responses to Climate Change

The combined effect of changes within individual species across the late Quaternary led to significant changes in community attributes such as diversity, evenness, and composition. For example, the small mammal assemblage in northern California showed significant losses in diversity across the last glacial–interglacial transition, even though no small mammal species went globally extinct (Blois *et al.*, 2010). These changes were similar to those seen in other small mammal assemblages across the west (e.g., Grayson, 2006; Lyman, 2014) and were postulated to be due to climate change favoring more generalist species (Blois *et al.*, 2010).

Another significant biogeographic change was the appearance and disappearance of 'no-analog' assemblages. Small mammals occurred in no-analog assemblages, where species that today live in very different and spatially separated localities coexisted in the past (e.g., Graham *et al.*, 1996). No-analog assemblages have also been seen in plant communities and were linked to climate changes. For example, peak dissimilarity in plant assemblages occurred at the same time as peak dissimilarity in climates, in both Alaska and eastern North America (Williams and Jackson, 2007) and dissimilarity in northeast pollen assemblages spiked at times of significant climatic transitions (Shuman *et al.*, 2009). Similarly, spatial dissimilarity among plant assemblages in eastern North America peaked from 14–11 ka. These relationships were primarily due to climatic factors (Blois *et al.*, 2013) and not to biotic interactions (Blois *et al.*, 2014). Despite the clear evidence for the impact of climate change on assemblage structure and diversity, biotic interactions (at least cross-trophic interactions) clearly also were a factor. For example, no-analog conditions were closely correlated with the functional loss of megafauna from the system, indicating that herbivory by megafauna was a key driver maintaining plant assemblages (Gill *et al.*, 2009, 2012).

Conclusion

Overall, the Quaternary encompassed a time of significant climatic and biogeographic change. While species and communities responded to a multitude of drivers, the physical and climatic changes that affected the earth system over the past several million years were primary. However, much of the data for both climatic and biogeographic processes in the Quaternary is drawn from the past few millennia – from the LGM to the present. Although this perspective has provided a detailed understanding of biogeographic responses to deglaciation and global warming, comparatively less is known about biogeographic responses to environmental change during the thousands and millions of years prior to the LGM, and across other periods of rapid climate changes. Additionally, much of the focus has been on biogeographic responses of a few groups, such as pollen or mammals, due to the different nature of the fossil record between groups. Today, however, biogeographic patterns and processes are being altered by extensive human impacts, so much so that a new geological epoch has been proposed, the Anthropocene (Zalasiewicz *et al.*, 2011). The extent to which ecological processes today are altered compared to the past is unknown, and a question that can only be answered by combining paleo- and present-day data. Integrating paleobiogeographic data from multiple components of our biotic systems, understanding responses of all components across periods of rapid warming events, and establishing relevant biogeographic baselines for past processes all represent key frontiers in paleobiogeography and avenues that are particularly relevant for conservation for present-day species.

See also: Biogeography, Evolutionary Theories in. Responses to Climate Change, Evolution and

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R

r- and *K*-Selection in Fluctuating Environments, Theory of

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Glossary

Density dependence Change in population size depends on current and/or previous population sizes.
Density-dependent selection Relative fitnesses of genotypes depend on population size.
Environmental stochasticity Random environmental variation that affects vital rates of all individuals, or groups of individuals, similarly.
***r*- and *K*-selection** Different phenotypes are selected for small and large population sizes.
Trade-offs Population parameters, for example, $\overline{r}$ and $\overline{\gamma}$ depend on the same phenotype and therefore cannot vary independently.

An Introduction to *r*- and *K*-Selection

Classical population genetics is often based on simplifying assumptions including constant population sizes and stable environments. The theory of *r*- and *K*-selection is conceptually important because it demonstrates that conclusions arising from basic population genetic models can be dramatically altered when more biologically unrealistic assumptions are relaxed from models. First, in populations subject to density dependence in environments without environmental stochasticity, evolution will maximize the carrying capacity *K* if the relative fitness of genotypes depends on population size. Second, including environmental stochasticity in density-dependent models result in an evolutionary process maximizing a more complex function determined by the ecological parameters driving the dynamics of the population. Third, empirical analyses of the theory of *r*- and *K*-selection requires estimation of trade-offs between phenotypic characteristics affecting the growth rate *r* at small population sizes and the response of the growth rate γ to changes in population size.

Deterministic Models

As is the case for many important concepts in modern evolutionary ecology, the concept of *r*- and *K*-selection was first introduced by the American Robert H. MacArthur. In an attempt to include more ecological realism in population genetic models, he showed in a classical paper in 1962 that under density-dependent selection in a stable environment evolution maximizes the equilibrium population size or the carrying capacity *K*. This result is based on the simple assumption that the relative fitness of two genotypes is dependent on population size (Figure 1). By simple stability analyses he was able to derive conditions for two alleles differently affected by changes in population size to remain in the population. An important implication of this result was that evolution no longer tends to maximize the relative growth rate *r* of a genotype in the population, as in the classic theory of Fisher (1930) and Wright

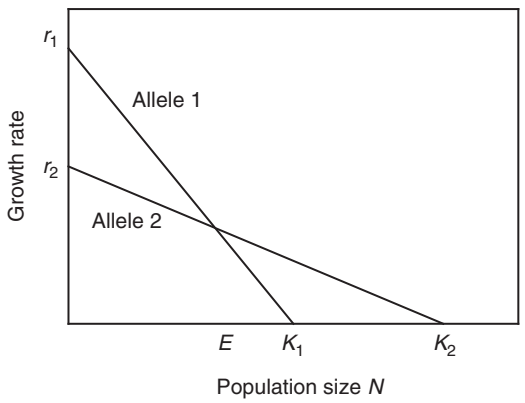

Figure 1 The rate of increase of two alleles as a function of population size assuming a logistic model of density regulation. At population sizes below the intersection point of the lines *E* allele 1 will increase and we get *r*-selection. In contrast, at larger population sizes (above *E*) allele 2 has the higher growth rate, resulting in *K*-selection. r_1 and r_2 are the intrinsic growth rate, and K_1 and K_2 are the carrying capacity of allele 1 and allele 2, respectively (modified from MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography. Princeton, NJ: Princeton University Press). *E* is the population size where there is a switch from *r*- to *K*-selection.

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(1931) for populations that are not density regulated. This is illustrated in Figure 1, where allele 2, which has a lower intrinsic growth rate at small population sizes than allele 1, is favored when population size exceeds a certain level E.

Another important advance was provided by MacArthur and Wilson (1967), who suggested a trade-off between traits affecting selection at small population sizes, when the population is not density regulated, and those favored at larger population sizes, when density dependence is operating. Based on the theory of MacArthur (1962), they argued that high population growth rates should be favored when the number of individuals was small (r-selection). In contrast, at large population sizes, selection should result in increased carrying capacity (K-selection) (Figure 1). As an example of how the trade-off between r- and K-selection could arise, they suggested (MacArthur and Wilson, 1967, p. 151) that in an environment with no crowding, genotypes which harvested the most food to achieve higher productivity in terms of production of offspring should be favored. In contrast, at high population sizes where there is much crowding and intense competition, individuals that can replace themselves with a minimal amount of food should outcompete more wasteful individuals.

This early theory of r- and K-selection was later extended to include more explicit genetic models and deterministic seasonal fluctuations in the environment (summarized in Roughgarden, 1979). An especially important contribution was provided in a series of papers by Brian Charlesworth, that he summarized in his 1994 book *Evolution in Age-Structured Populations*. A central concept in this theory is the critical age-class, which is the age-class with the largest negative effect on the growth of the population. Using the concept of evolutionary stable strategy (ESS) as proposed by Maynard Smith and Price (1973), Charlesworth was able to show, using a haploid model, that if the density regulation is only affected by the number of individuals in the critical age-class, evolution tends to maximize the size of this age-class. More generally, if density regulation is determined by the total number of individuals or biomass in the population, total population size or biomass is maximized.

r- and *K*-Selection in Fluctuating Environments

Much of the prevailing theory summarized above is based on the assumption of a constant, or periodic, environment. When environmental stochasticity caused by random variation in the environment is included, one may assume that selection can be expressed simply by replacing the parameters by their average values. Unfortunately, this is not correct. Tuljapurkar and Orzack (1980) showed that the appropriate measure of fitness in stochastic environments in the absence of density regulation is the long-run growth rate $s = \bar{r} - \frac{1}{2}\sigma_e^2$, where $\bar{r}$ is the growth rate in the average environment and σ_e^2 is the environmental variance defined as the temporal variance in $\bar{r}$. Thus, evolution maximizes s as long as there is no density dependence.

Lande (2007) developed a model in which the dynamics were density-dependent, but with no density-dependent selection. This means that the relative fitnesses of the genotypes were independent of population size. However, the mean relative fitness of a phenotype determining average selection is still not s, but the Malthusian parameter in the average environment minus the environmental covariance between its growth rate and that of the population.

To include density-dependent selection, i.e., that fitnesses of the genotypes respond differently to changes in population size, Lande *et al.* (2009) analyzed a haploid or asexual model of inheritance with theta-logistic density regulation, meaning that population size N is regulated through N^θ. This model represents a general class of density regulation expressed only by a single parameter θ (Gilpin *et al.*, 1976; Lande *et al.*, 2003). For small values of θ density regulation starts to act at small population sizes. In contrast, if θ is large there is almost no density regulation at smaller population sizes, whereas strong density regulation occurs above the carrying capacity K. Thus, the theta-logistic model allows us to vary the form of density regulation and the corresponding stationary distribution of population sizes, assuming large enough population sizes to ignore the effects of demographic stochasticity. For this kind of model with a constant nongenetic θ, Lande *et al.* (2009) showed that evolution maximizes the expectation

$$E(N^\theta) = \frac{s}{r}K^\theta = \left[1 - \frac{\sigma_e^2}{2r}\right]K^\theta$$

where r is the growth rate at small population sizes in the average environment and σ_e^2 is the environmental variance in the population growth rate. Thus, evolution maximizes the average of N^θ over the stationary distribution of population sizes, which is a function of the basic ecological parameters r, K, and σ_e^2 specific for each genotype.

This evolutionary maximization principle for density-dependent population can be used to explore how density-dependent selection acts in fluctuating environments. When σ_e^2 is small, $\left(1 - \frac{\sigma_e^2}{2r}\right)$ is close to 1 and hence evolution maximizes K^θ (and thus K as K and K^θ are obviously ranked identically), as in MacArthur's (1962) model for a constant environment. We also see that for large environmental variances σ_e^2, large s and r are favored, producing r-selection. Lande *et al.* (2009) also showed that there exists, for given values of the other parameters, one optimal value of θ, often around $\theta = 2$.

The results from analyses of density-dependent selection in a haploid model was extended by Engen *et al.* (2013) to include a quantitative character z in a sexual model which enables analysis of the joint process for the mean of the character $\bar{z}$ and log population size in a fluctuating environment. Again, it was possible to derive an evolutionary maximization function. It was shown that the expected evolution of the mean phenotype $\bar{z}$ subject to density-dependent selection was governed by the function

$$Q(\bar{z}) = \frac{\bar{s}(\bar{z})}{\bar{\gamma}(\bar{z})} = \left[1 - \frac{\sigma_e^2(\bar{z})}{2\bar{r}(\bar{z})}\right]g(K(\bar{z}))$$

where $\bar{s}(\bar{z})$is the long-run growth rate of the population in the absence of density regulation so that $\bar{s}(\bar{z}) = \bar{r}(\bar{z}) - \frac{1}{2}\sigma_e^2(\bar{z})$, $\bar{\gamma}(\bar{z})$ is the average strength of density dependence in the population growth rate, and $g(N)$ is an increasing function of N describing the density regulation (for theta-logistic density regulation $g(N) = N^\theta$ and for the logistic model $g(N) = N$). The function is similar to the function maximized by evolution in the simple haploid model of Lande *et al.* (2009),

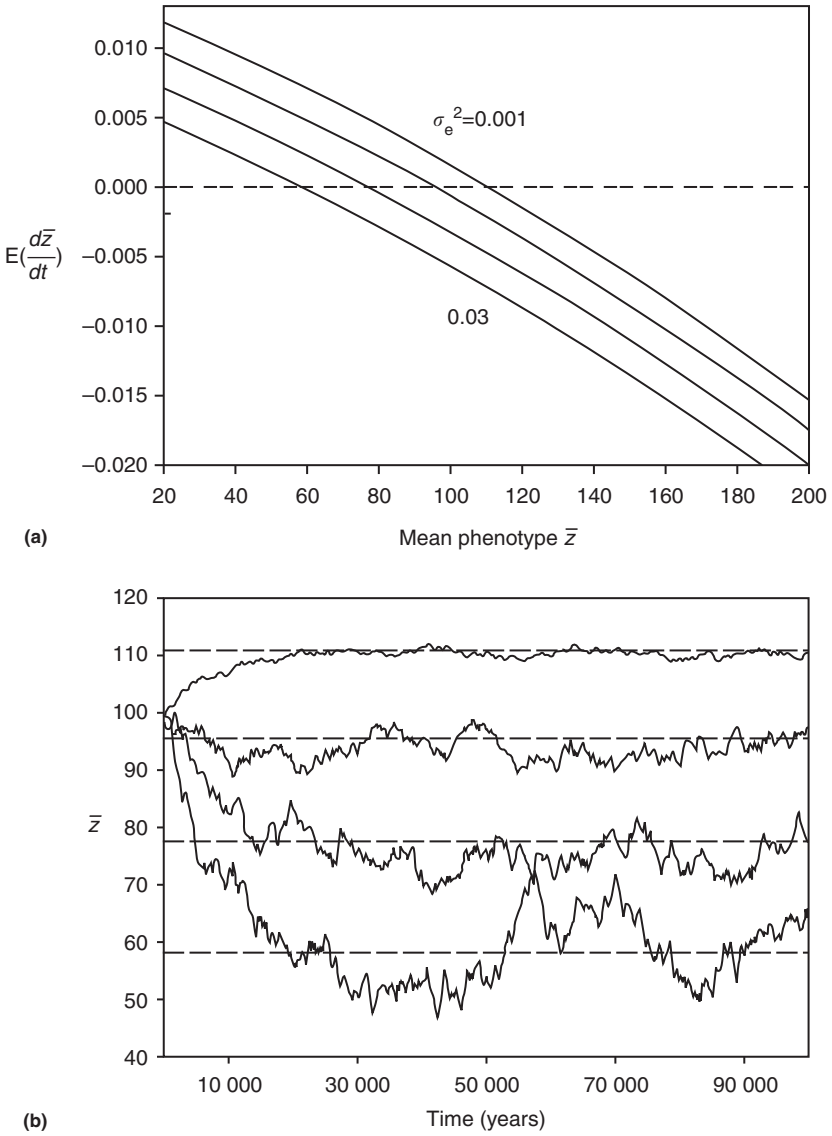

Figure 2 Expected change in the mean phenotype $E\left(\frac{d\bar{z}}{dt}\right)$ as a function of the mean phenotype $\bar{z}$ (a) and simulations of temporal variation in mean body size $\bar{z}$ (b) for different values of the environmental variance σ_e^2. Larger environmental noise yields smaller average body size. The model assumes a trade-off between fitness and susceptibility to increased population density so that both Malthusian fitness and strength of density dependence decrease with increasing z. Thus, a decrease in $\bar{z}$ means increased *r*-selection. The dashed lines in (b) represent the mean values.

but with parameters averaged over all individuals in the population. The expected evolution of the mean phenotype $\bar{z}$ under density-dependent selection will be toward the value z_{opt} maximizing $Q(\bar{z})$. The expected evolution of $\bar{z}$ thus corresponds to an increase of $Q(\bar{z})$, but environmental stochasticity always perturbs it back to values slightly below the maximum (Engen *et al.*, 2013) so that $\bar{z}$ has temporarily stationary fluctuations around the optimal phenotypic value z_{opt}.

We can use the function $Q(\bar{z})$ to analyze the balance between *r*- and *K*-selection. In a constant environment ($\sigma_e^2 = 0$), the mean phenotype evolves to z_{opt} and hence evolution maximizes $N = K$, as first shown by MacArthur (1962). When environmental stochasticity is present, both population size and the mean phenotype will fluctuate. A stationary distribution of $\bar{z}$ is achieved, which produces more *r*-selection for more environmental noise, resulting in evolution toward larger values of $\bar{s}(\bar{z})$ and $\bar{r}(\bar{z})$.

It is necessary that there exists a trade-off between r and γ (or between r and K) because otherwise the evolution of the mean phenotype would always increase $\bar{r}$ and decrease $\bar{\gamma}$, resulting in correlated selection for large $\bar{r}$ and K. To illustrate the effect of such a trade-off, Engen *et al.* (2013) used a simple example, in which Malthusian fitness and the strength of density dependence are both decreasing with increasing body size z. For instance, this may occur in practice if large individuals have lower fecundity but are less sensitive to population density than small individuals. Increased environmental stochasticity then results in *r*-selection toward smaller individuals with higher reproductive rates (**Figure 2**).

Discussion

We have seen that density dependence will produce selection toward large values of r at small densities and larger K when competition for a limiting resource is operating. This assumes a trade-off between the density-independent component of the Malthusian fitness r of a genotype in the absence of density regulation and the sensitivity of its fitness to changes in population size, measured as the strength of density dependence γ. In addition, low stochasticity in the environment will favor a small γ or large K, whereas large environmental stochasticity will cause r-selection (**Figure 2**) and smaller K. Thus, selection due to fluctuations in population size may be an important process to include when estimating expected phenotypic evolution in a fluctuating environment (Boyce, 1984). Some empirical evidence does suggest that such density-dependent selection may have a significant effect on the evolutionary response to fluctuating selection in natural populations (Reed *et al.*, 2013).

Empirical analyses of r- and K-selection are difficult. A major reason for this is that it requires estimates of genetic trade-offs between major life history characters. In general, obtaining such estimates are difficult in natural populations (Kruuk *et al.*, 2008). In analyses of r- and K-selection this represents a particular challenge because estimates of the genetic component of the phenotypic response to variation in population size are required for estimating the additive genetic variance in γ. Many early studies of r- and K-selection were based on comparison of populations or even species in different environments (Pianka, 1970, 1972). This approach is only valid if the environments only differ with respect to the number of individuals present so that all genetically based differences are due to variation in population density. In most cases it is almost impossible to remove environmental effects (Boyce, 1984; Mueller, 1997). Furthermore, Charlesworth (1994, pp. 265–266) pointed out that some of these patterns interpreted as support for r- and K-selection are indistinguishable from those expected from the impact of age-specific variation in selection on vital rates.

Evidence accumulated from laboratory systems provide some support for r- and K-selection strongly affecting evolution of phenotypes in density-dependent populations (Mueller *et al.*, 1991). In fruit flies *Drosophila* spp., for example, r- and K-selection operated through an effect of crowding, resulting in a trade-off in population growth rates at low and high densities. Larval competitive ability increased in populations kept at high densities, whereas early-developing genotypes favored at low densities had low tolerance to ammonia that increases under crowded conditions (Borash *et al.*, 1998).

An illustration of the importance of considering density-dependent selection was provided by Bassar *et al.* (2013), who manipulated population sizes of Trinidadian guppies *Poecilia reticulate*. Strong density dependence was recorded in populations with small risk of predation. Artificial alteration of population size showed that the clear advantage of the high-predation phenotypes exhibited at low densities disappeared at high densities. However, the low-predation phenotypes did not have a consistent advantage at high densities. This shows that the effects of population regulation and density-dependent selection were important to include for explaining adaptive life history variation in this fish species.

An issue that recently has been raised is whether evolutionary phenotypic changes can be produced so rapidly that they can reduce the long-term impact on the biodiversity of rapid human-induced alterations of the environment. In such analyses of, for example, the effects of climate change, it is important to include density dependence in models. For instance, Reed *et al.* (2013) showed that strong selection for earlier breeding in great tits *Parus major* due to an increase in spring temperatures was counteracted by a density-dependent increase in juvenile survival in years with poor breeding success. Similarly, evidence now strongly indicates that harvest alters the dynamics of harvested fish stocks (Anderson *et al.*, 2008). These altered dynamics may produce phenotypic evolution caused by r- and K-selection that can affect the long-term yield of the harvest.

Acknowledgments

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See also: Life Histories, Axes of Variation in. Life History Trade-offs

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Recombination and Molecular Evolution

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Glossary

Adaptation The process of increasing in frequency a trait that is beneficial under natural selection.
Allele A gene type that is present at a locus.
Anisogamy The situation where male and female gametes are highly dimorphic; usually the male produces smaller gametes. Isogamous species are where males and females have similar-sized gametes.
Background selection The loss of variation in the genome through purging deleterious mutation.
Effective population size, N_e A quantity used to describe the genetic diversity present in a population; the principle being that an idealized population of size N_e would harbor the same level of diversity, if all mutations acted independently.
Epistasis The effect where a collection of loci exhibit different fitness, compared to cases where each mutation acts independently.
Fitness Broadly, the ability of an individual to reproduce and leave descendants.
Hitchhiking Where linked alleles (neutral or deleterious) fix with an adaptive allele.
Interference When selection acts on mutations in a non-independent manner (e.g., a deleterious allele hitchhikes to fixation with an adaptive mutant).
Linkage disequilibrium Nonrandom association of mutations in the genome.
Mutation A change made to the molecular sequence of a genome.
Recombination Reciprocal exchange of genetic material.
Segregation When two gene copies are separated and are passed at random during sexual reproduction.

Introduction

Many organisms reproduce sexually – 99.9% of animal species, for instance (Vrijenhoek, 1998) – but the evolutionary reason for the widespread prevalence of sex is not immediately obvious. Sexual reproduction comes at a heavy price: mates must be found, fought for, and won; sexually transmitted diseases braved; and, after all these trials, sexually-produced offspring only carry half the genes of the parent. In fact, as John Maynard Smith pointed out (Maynard Smith, 1978), asexual reproduction should have a twofold advantage, all else being equal. That is, asexual females will reproduce at twice the rate of sexual ones, quickly outcompeting them, so that a mutation causing clonal reproduction in an anisogamous species is expected to quickly take over (**Figure 1**).

Nevertheless, sex is pervasive in many taxa. In fact, phylogenetic analyses (Bell, 1982) show that sexual species tend to occur deeper in phylogenetic trees than asexual ones,

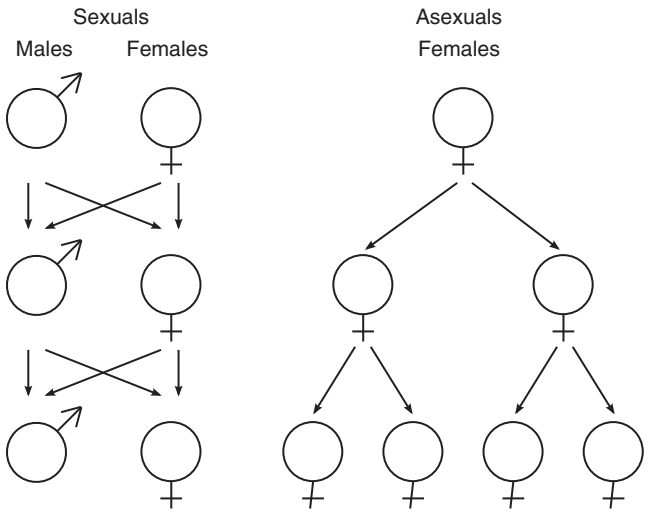

Figure 1 A cartoon describing Maynard Smith's 'twofold cos of sex' argument. The sexual population, on the left, requires a male and a female pairing to reproduce; if one son and daughter are born every generation, the population is maintained at the same size. Asexual females, on the right, can produce two clonal offspring without needing a male partner. Adapted from Hartfield, M., Keightley, P.D., 2012. Current hypotheses for the evolution of sex and recombination. Integrative Zoology 7, 192–209.

suggesting that only sexual species persist through time. For example, within the species *Daphnia pulex*, which has both sexual and asexual forms, the asexual lineages appear to be short-lived (Tucker *et al.*, 2013). What, then, might account for the apparent advantage of sexual lineages? One possibility is that there are immediate advantages to sexual reproduction; for example, receiving care from two parents might improve the survival of offspring. That is, while the twofold cost of sex is useful as a thought experiment, it might rarely be realized in nature, as reproductive rates alone are not always the principal determinant of evolutionary fitness. However, this rationale depends on details of the life history of each species, and is therefore unsatisfying as a general explanation for the prevalence of sex.

Instead, it is generally thought that sexual lineages have a long-term fitness advantage over asexual lineages. Sexual species are expected to adapt faster to changing environments, and to better maintain fitness in constant environments, compared to asexual organisms. The reason is that sex shuffles genomes, making new combinations of genotypes available for selection to act on. Mechanistically, this shuffling comes in two flavors: segregation and recombination between loci (**Figure 2**). Both are expected to promote the spread of beneficial mutations and the purging of deleterious ones. Without segregation, for example, the descendants of a heterozygous individual will remain heterozygous *ad infinitum*, or at least until a second mutation occurs at the same locus. A single beneficial mutation in such a population can increase from low frequency to 50%, but then faces a barrier to fixation. With segregation, there is no such barrier; homozygous individuals are easily generated from heterozygous ones, and the beneficial mutation can fully spread (Kirkpatrick and Jenkins, 1989).

The overwhelming body of research, though, is directed toward the evolutionary benefits of genetic recombination. As we will discuss below, the implications of recombination have been spelled out in a large body of theory, some of which has been tested with molecular data.

Recombination and Adaptation

Theoretical Background

Any selected allele, whether deleterious or beneficial, must begin as a mutation, either as a single or multiple copies. Each copy of the allele will arise on some genetic background, usually one randomly drawn from the population. Without recombination, the fate of the selected allele is strongly affected by whichever genetic background it arises on. For example, a beneficial allele unlucky enough to arise on an unfit genetic background may be lost, driven out of the population along with the deleterious alleles it is linked to. When the fates of selected alleles are non-independent, they are said to 'interfere' with one another. Felsenstein (1974), in an engaging review of the topic, termed this phenomenon 'Hill–Robertson interference,' after the seminal investigation into selection, linkage, and drift interactions by Hill and Robertson (1966). There are several flavors of Hill–Robertson interference, but all ultimately attribute the evolutionary advantage of recombination to its ability to uncouple selected alleles from their genetic backgrounds, so selection can act on them independently.

1. Fisher–Muller interference. Fisher (1930) and Muller (1932) pointed out how asexual populations suffer from interference between beneficial mutations. That is, in a non-recombining population, in order for two (or more) beneficial mutations to fix at the same time, they must occur in the same genetic background. This composition is unlikely, unless the first beneficial mutation to occur has already reached a high frequency. With recombination, two beneficial mutations can occur on different backgrounds, recombine onto the same genome, and sweep to fixation together (**Figure 3**). Thus, fixing two mutations takes roughly half the time that it does in an asexual population (Christiansen *et al.*, 1998), unless mutation rates are high enough to create double mutants without

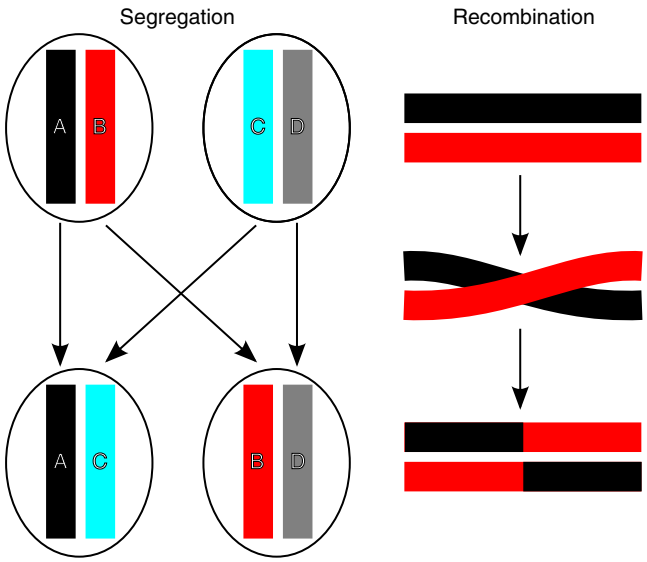

Figure 2 Segregation and recombination during sex. Segregation shuffles the manner in which gene copies (denoted here A–D and labeled with different colors) are organized within genomes. Recombination exchanges material between genomes.

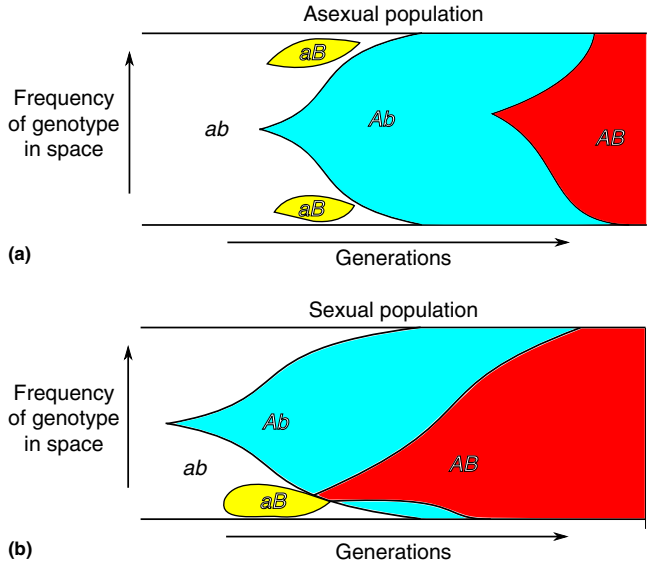

Figure 3 Fisher–Muller argument for the evolution of sex. In asexuals (top), beneficial mutations have to arise and fix in sequence due to competition between them. In sexuals (bottom), however, recombination can form the optimal AB genotype much more rapidly. Adapted from Muller, H.J., 1932. Some genetic aspects of sex. American Naturalist 66, 118–138.

recombination (Kim and Orr, 2005). When applied to microbes, Fisher–Muller interference is sometimes called 'clonal interference' (Gerrish and Lenski, 1998), which can be tested experimentally (e.g., Lang *et al.* (2013)).

2. Muller's ratchet. Muller's (Muller, 1964) ratchet does not describe a failure to adapt to a changing environment, but instead a failure to maintain fitness even in a constant environment. In any population, particularly a small one, it is always possible to lose the fittest current genotype by chance. One way to model this phenomena is to consider deleterious mutations to be Poisson distributed over chromosomes – essentially, distributed in the same way beans dropped onto a chessboard would be randomly spread over the squares. As Haigh (1978) showed, the mean number of mutations would be the ratio of the genomic mutation rate (U) to the average selection coefficient acting against these mutations (s_d). The fraction of deleterious-mutation-free genomes is then just the size of the zero class for the Poisson distribution, $\exp(-U/s_d)$. Thus, if the fraction of individuals carrying no deleterious mutations is expected to be one in a thousand, a population with a census size in the hundreds may not contain even one individual without deleterious mutations. In an asexual population, with no back mutation, there is also no way to regenerate a mutation-free genome – the name 'Muller's ratchet' comes from this irreversibility, as ratchets are wrenches that turn only one direction. Recombination, however, can reverse the process: as long as no particular deleterious mutation fixes, two genomes can recombine to form a reconstituted mutation-free genome.

   As the arguments above suggest, Muller's ratchet is most important in small populations, with high genomic mutation rates (due to either high overall mutation rates, and/or long genomes), or with weak selection (Felsenstein, 1974). Without these factors, the ratchet acts so slowly as to be unimportant; otherwise it can be a powerful force, causing genomic degradation (Charlesworth and Charlesworth, 2000).

3. Ruby-in-the-rubbish interference. Ruby-in-the-rubbish interference (Peck, 1994) describes the loss of beneficial mutations that happen to arise on unfit backgrounds. The original ruby-in-the-rubbish model considers adaptation in an asexual population, where the beneficial mutations are not strong enough to overcome the effect of linked deleterious mutations. In this case, the only beneficial mutations with any chance of not going extinct must arise on deleterious-mutation-free genomes. In sexual populations, recombination can free the beneficial mutation from its loaded genetic background, improving its prospects for success. Cases in which the beneficial mutations are strongly selected for, and therefore override linked deleterious mutations, are more complex. If there are many deleterious mutations, adaptive alleles can still be lost in asexual populations if mutation rates are high (Johnson and Barton, 2002). With pairs of advantageous–deleterious mutations, sex can prevent the fixation of deleterious alleles. The benefit to recombination is greatest if deleterious mutations have slightly lower selection coefficients than their beneficial drivers (Hartfield and Otto, 2011). However, the case with many deleterious mutations affecting adaptive alleles in sexual populations has not yet been fully solved.

In each of these cases, recombination counteracts interference by offering new allele combinations for selection to act on. This effect is only useful, though, when the fittest alleles are not already found together; otherwise, recombination breaks apart optimal genotypes. In technical terms, recombination only provides a benefit if there is negative linkage between beneficial mutations – when genomes more often have a mixture of beneficial and deleterious alleles at different loci, rather than possess only beneficial or only deleterious

alleles. What, then, should give rise to these kinds of nonrandom associations? One answer is epistasis, where individuals carrying multiple mutations have better or worse fitness than expected based on the selective coefficients of the single mutations. However, only specific kinds of epistasis promote increased recombination (see Kondrashov (1988) and Kouyos *et al.* (2007)). A general explanation may simply be that population sizes are limited. As Fisher (1930) pointed out, in any finite population, all possible combinations of alleles never occur, so there will always be nonrandom associations between alleles. In cases where beneficial mutations are coupled, selection will quickly fix these genotypes (and, conversely, rapidly purge genotypes where deleterious mutations are coupled). The upshot is that the genotypes containing a mixture of beneficial and deleterious mutations would, on average, remain segregating for the longest amount of time. Over time, then, most linkage disequilibrium between beneficial mutations is negative, of just the sort to promote an advantage of recombination. Barton and Otto (2005) solidified this logic and showed how associations created in this manner select for increased recombination rates.

Empirical Evidence for an Advantage of Recombination

If there is an advantage of recombination in aiding adaptation, can we see it in molecular population genetic data? Some of the earliest answers to this question come from sex chromosomes, which have evolved independently many times from autosomes. X and Y chromosomes proceed to diverge when recombination between them stops; as the Y only occurs while paired with the X, it can no longer recombine at all. What happens to this non-recombining chromosome? The long-term outcome can be seen on the *Drosophila* Y, for example. In terms of DNA content, this chromosome is huge – roughly twice the size of the X – but it harbors only a handful of genes, compared to thousands on the X. Initially, these chromosomes were identical, but the Y has lost most of its genes, and gained a large amount of 'junk DNA.' By studying young Y chromosomes, we can watch this process in action. For example, there is a very young Y chromosome in *Drosophila miranda*, which still retains many of its genes. Compared to their homologous on the X, however, these copies show less evidence of adaptation (Bachtrog and Charlesworth, 2000) as predicted by the theories outlined above. Similar surveys have been performed in humans (Wilson Sayres *et al.*, 2014), mice (Soh *et al.*, 2014), and plants (Hough *et al.*, 2014); see Bachtrog (2013) for a recent review.

Other studies looking for an evolutionary advantage of recombination have been done within chromosomes, comparing low to high recombination regions. In *Drosophila melanogaster*, for example, there are long regions exhibiting very low recombination rates. Molecular evidence suggests that these regions show fewer substitutions of adaptive alleles and more substitutions of deleterious alleles, compared to regions of average recombination (Betancourt and Presgraves, 2002; Haddrill *et al.*, 2007; Mackay *et al.*, 2012). Gossmann *et al.* (2014) also found such a result in birds, by comparing chromosomes with different average recombination rates. Humans, in contrast, do not show a strong pattern of this type (Bullaughey *et al.*, 2008), possibly due a low rate of adaptive protein evolution in our own species (Eyre-Walker and Keightley, 2009).

Recombination and Neutral Variation

Even in sexual organisms, the amount of recombination can vary, for example, across sexes and individuals, and even across the genome. Studies of how evolutionary rates differ due to this variation have yielded fascinating insights into the effects of selection interference. *D. melanogaster* is an organism that shows a large amount of variation in recombination rates across its genome. In the early 1990s, Begun and Aquadro (1992) used this fact to show that, compared to highly recombination regions, low recombination regions had very little genetic diversity – in these regions, individual sequences of the same gene, which in *D. melanogaster* differ from each other at 1–2% of silent sites, showed only a small fraction of the normal level of variation. They invoked an explanation for which the theory had been worked out 20 years before: hitchhiking of neutral mutations (Maynard Smith and Haigh, 1974), based on Hill–Robertson interference caused by beneficial mutations. Briefly, hitchhiking argues that if selection fixes a single copy of a beneficial allele, it necessarily also fixes any neutral variants that arise on the same background (**Figure 4**). As a result, the nearby regions will be stripped of neutral variation. Recombination, however, allows the selected allele to move to other genetic backgrounds, and be linked to

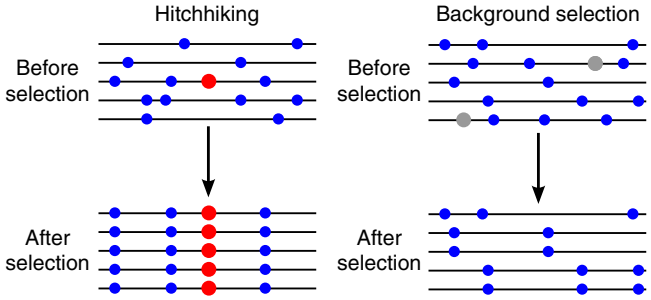

Figure 4 How selective effects reduce genetic variance. Hitchhiking (left) occurs when an adaptive allele, shown in red, spreads to all individuals in the population. Linked neutral mutations (blue dots) are driven with it to fixation as well, unifying the genetic signal around the adaptation. Background selection (right) occurs when deleterious mutations, the gray dots, are lost by selection, and are replaced by other neutral genotyped (after selection, the second genotype is replaced by the third, and the fifth by the fourth).

other neutral alleles; thus, the extent of the region affected by hitchhiking depends on the recombination rate.

More formally, we can see why recombination should affect neutral sequence diversity if we first understand what affects it, namely, mutation rate (u), and the number of ancestors contributing genetic material to a population, quantified as effective population size (N_e). Diversity increases as mutation rate increases, because, as more mutations are introduced into a population, more sites can contain differences. Diversity also increases as N_e increases – if the number of genetic ancestors is small, individuals will be closely related, and therefore genetically similar to one another. Begun and Aquadro were able to eliminate an effect of recombination on mutation rate, and therefore concluded that recombination affects N_e. As diversity is measured across the genome of a single species of fly, it is unlikely that there are different numbers of actual ancestors contributing to different regions of the genome. Instead, it may be that the fixation of beneficial mutations reduces the number of genetic ancestors for long regions in low recombination regions *via* the hitchhiking effect (**Figure 4**).

Hitchhiking, then, appears to explain the relationship between diversity and recombination, which also implies that adaptive evolution is frequent enough to change levels of neutral diversity. However, Charlesworth *et al.* (1993) almost immediately challenged this explanation, pointing out that hitchhiking is not the only possible cause for a relationship between neutral diversity and recombination, recurrent deleterious mutations is another. The reason is that deleterious mutations also reduce the effective population size of linked regions: as they are removed from the population, so is any neutral linked variation (**Figure 4**). Without recombination, the only genomes that ultimately contribute ancestry to the population are those deleterious-mutation-free genomes; that is, the zero class of the Muller's ratchet model. With recombination, some of the genetic material that would have otherwise been lost can recombine onto mutation-free backgrounds. As a result, the effect of background selection, like that of hitchhiking, varies with the recombination rate. Trying to disentangle the two effects still motivates current research, since the two phenomena can be difficult to tease apart (Cutter and Payseur, 2013).

Begun and Aquadro's observations were based on a limited amount of data (appropriate for the time), so it is worth asking how their findings have held up. For *Drosophila*, the answer is quite well, as shown using whole-genome polymorphism datasets (Mackay *et al.*, 2012). Besides *Drosophila*, these effects have been examined in a broad range of taxa (Cutter and Payseur, 2013); though the results are mixed, most other taxa show similar patterns to those in *Drosophila*, with recombination rate positively correlated with neutral genetic diversity. The effect of recombination on N_e is also reflected in patterns seen for weakly selected alleles: alleles whose selection coefficients hover near the value of $1/N_e$, rendering them borderline neutral. A local reduction in N_e can push them under this threshold, so that they act effectively neutrally. In *Drosophila*, sites evolving under weak selection do, in fact, show reduced adaptation in regions of very low recombination. However, this effect appears to be restricted to these regions, rather than scaling with increased recombination rate (Marais *et al.*, 2001; Haddrill *et al.*, 2007; Campos *et al.*, 2014). The reason is likely historical: weakly selected sites will take a long time to reach their equilibrium level of adaptation, while most reduced recombination regions in *D. melanogaster* seem to have only recently suffered from a reduction in recombination rate (Campos *et al.*, 2014). Further, with whole-genome polymorphism data, we have been able to refine our picture of the local effect of selection on linked neutral sites. In the immediate vicinity of putatively adaptive substitutions, there is a dip in average neutral diversity, just as expected under hitchhiking (Sattath *et al.*, 2011), though not in humans (Hernandez *et al.*, 2011). In our own species, the role of background selection is well-established; in fact, the effect of linkage to selected sites has been quantified in a 'background selection map' (McVicker *et al.*, 2009). The contrast between *Drosophila*, which shows evidence consistent with adaptive evolution, and humans, where deleterious mutations appear to play a bigger role, may be due to their contrasting effective population sizes. All else being equal, larger populations may experience higher overall rates of adaptive substitutions: in support of this, mice, which are mammals like humans but have larger population sizes (Phifer-Rixey *et al.*, 2012), show a *Drosophila*-like pattern near substitutions (Halligan *et al.*, 2013).

Conclusion

Here, we have seen how recombination frees organisms to adapt at multiple sites, allows them to preserve the integrity of their genomes, and conserves genetic variation. With reduced sequencing costs, these questions can now be addressed in new species and in new ways. Future results will refine our picture on how recombination shapes genomes, and hopefully throw up unexpected outcomes, generating new avenues of research.

See also: Effective Population Size. Recombination and Selection. Selective Sweeps. Sex, Evolution and Maintenance of

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Recombination and Selection

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Glossary

Clonal interference Competition between beneficial mutations in the absence of recombination such that the fate of the lineages carrying the beneficial mutations is not independent of one another.

Genetic load The difference in fitness between the optimum population fitness and the actual mean fitness observed in a population.

The Interaction between Recombination and Selection

The early theory of population genetics focused on individual loci and considered the dynamics of allele and genotype frequency change at these loci one at a time. But the nature of evolving genomes is modular – multiple sites within loci, and multiple loci within genomes. To describe and understand the evolutionary dynamics of these systems requires an understanding of the interplay between evolutionary forces such as selection, and the genetic forces of Mendelian segregation and chromosomal recombination that cause sites and loci to share evolutionary histories, or that free them to follow separate trajectories over time.

The interaction between selection and recombination plays a key role in our understanding of the evolution of sex. At the heart of this discussion has been an attempt to understand when and how the breaking up by recombination of genotypes that have already survived to reproductive age can be beneficial. The evolutionary theory that has focused on nonmechanistic discussions of the origins of recombination shares a common theme – that what recombination breaks apart, it also must necessarily bring together. It is the bringing together of either multiple beneficial or multiple deleterious mutations (in the language of population genetics, the dissolution of *negative linkage disequilibrium*, here defined as the statistical excess of haplotypes combining negative and positive alleles) that lies at the core of modern models of recombination and selection.

Two contrasting approaches have been applied to the evolutionary theory of recombination and selection – *optimality theory* and *modifier theory*. These two approaches differ both in their theoretical details and in their focus on how natural selection acts – optimality theory focuses on equilibrium models that often implicitly employ group selection types of arguments, while modifier theory explicitly focuses on individual selection and the dynamics of evolutionary change.

Optimality Arguments

In optimality theory, the optimization of a specific criterion under various values of key parameters is considered. Optimality criteria for the theory of recombination have included individual or population mean fitness, the time to fixation of a beneficial mutation, the rate of evolution, or the amount of genetic load, among others. The assumption for these types of models is that evolution proceeds in a direction that optimizes the criterion with respect to the evolutionary parameter or parameters of interest. It should be stressed that there is no assumption as to whether real organisms can or will be optimal, nor do the theories explicitly consider whether or not natural selection can act to optimize the parameter. These will depend on details of the natural system, such as the genetic and phenotypic nature of the characteristic under selection, the amount of additive genetic variance that is available for natural selection to act upon, the strength and direction of natural selection, and random effects such as genetic drift (a review of optimality theory in evolutionary biology is given by Parker and Maynard Smith, 1990). Optimality arguments (also called equilibrium models, see Otto and Lenormand, 2002) consider how increased or decreased rates of recombination affect the value of the optimization criterion, which is often a characteristic of a group, such as the population mean fitness. The assumption that follows is that groups with a more optimal value of the criterion would outcompete and replace groups with suboptimal values, and thus the direction that evolution would take recombination is ascertained. The models most often focus on how recombination redistributes mutations that are under selection (beneficial mutations under *Fisher–Muller models*, and deleterious mutations under *Muller's ratchet*, see below), and then in turn how this redistribution by recombination sets the value of the optimality criterion.

Modifier Theory

The second large family of models that consider selection and recombination focus on nonequilibrium situations for the evolution and spread of recombination. They are based on the concept of a modifier locus that alters the level of genetic segregation or recombination and that acts epistatically with the loci that determine organismal fitness (Feldman *et al.*, 1997). In these models, individual selection is explicitly considered, since the focus is on the conditions under which alleles acting to change the rate of recombination will increase in frequency within a population. Work that considered a single large population under a constant selective environment found a very limited set of conditions where modifiers of recombination can spread (Otto and Lenormand, 2002). Less

restrictive conditions are possible with the addition of some type of structure (spatial structure, temporal structure, or the structure imposed by the genetic architecture of the organism).

Important Optimality Models of Recombination and Selection

The Fisher–Muller Model and the Role of Negative Linkage Disequilibrium

It might seem that a clear benefit of recombination is the creation of novel, beneficial genotypes upon which selection can act. However, when alleles are randomly associated across loci (*linkage equilibrium*), the creation of new beneficial combinations of alleles by recombination is exactly balanced by the loss of these beneficial combinations (Felsenstein, 1988). It is only when there is a statistical excess of haplotypes combining negative and positive alleles (*negative linkage disequilibrium*) that recombination gives a benefit (**Figure 1**). A key model for the interaction of selection and recombination is the Fisher–Muller model (Fisher, 1930; Muller, 1932) – here, recombination is beneficial to a population because it brings together new favorable alleles that first arose on different haplotypes. The new beneficial alleles thus are initially in negative linkage disequilibrium, to use modern terminology.

The Fisher–Muller model assumes a population that is finite in size, but large enough that multiple beneficial mutations may segregate at the same time (in a small population, the assumption is that beneficial mutations will either be lost due to drift or fix quickly, so that only one is likely to exist at any given time). In an asexual lineage lacking recombination, new beneficial mutations at different loci that first arise on different genetic backgrounds cannot go to fixation at the same time – they will necessarily compete against one another. These beneficial mutations are in negative linkage disequilibrium. To see this, we might imagine a population fixed for the *a* allele at locus A and the *b* allele at locus B (only the *ab* haplotype exists). If a new, favorable allele (*A* allele) arises in one asexual lineage (so that the combination *Ab* exists), and another new, favorable allele (*B* allele) arises on another asexual lineage (so that the combination *aB* also exists), we have negative linkage disequilibrium. In this case, the combination *AB* is missing, and cannot occur without recombination ($D = p_{AB}p_{ab} - p_{Ab}p_{aB} = 0 - p_{Ab}p_{aB}$ and is necessarily negative).

The advantage of recombination under the Fisher–Muller model is given by a *rate of evolution* optimality argument; recombination allows the population to have a higher rate of fixation of beneficial mutations than a population lacking recombination. Felsenstein (1974, 1988) pointed out that the assumption of finite size is an important aspect, since in an infinite population a proportion μ^2 of either new individuals (for haploids) or new gametes (for diploids) would be double mutants (haplotype *AB*) and the population would then be in linkage equilibrium, and there would be no advantage to recombination.

Hill–Robertson Effect

The Hill–Robertson effect (or *Hill–Robertson interference*, Hill and Robertson, 1966; Felsenstein, 1974) is closely related to the basic idea presented in the Fisher–Muller model, and extends it to describe how genetic linkage, even in the presence of recombination, causes an interaction between selection and genetic drift. The Fisher–Muller model above focused on the interference between beneficial mutations arising in the case of complete linkage (for asexual lineages). But in a finite population, even partial linkage (any deviation from free recombination, or a recombination frequency of $r = 0.5$) causes linked beneficial genes to interfere with each other's ability to reach fixation.

With free recombination, a new beneficial mutation is exposed to selection against all available genetic backgrounds; good and bad backgrounds (with regard to fitness) tend to cancel each other out on average, and thus the average fitness of organisms carrying the new beneficial mutation will only reflect the particular selective advantage of the mutation itself. However, in the case of linkage, the initial association between the beneficial mutation and the background where it arose will persist for some time (until recombination 'frees' the mutation). Thus, under linkage, the average fitness of organisms carrying new beneficial mutations will now also depend on the background within which the mutations arose. This acts to increase the variance in the frequency of the beneficial mutation above what it would be if its spread depended only on its own fitness. In effect, linkage increases the amount of genetic drift (here genetic drift is being measured by the variance in frequency from generation to generation) that accompanies selection, and thus the Hill–Robertson effect can be viewed as a decrease in the effective population size (N_e) for the locus where the beneficial mutation arose. This decreased N_e means that the probability that the beneficial mutation will fix will be less, on average, under linkage than under free recombination. For two linked loci of similar beneficial effects, the probability of fixation reaches a limit of $2N_e r/(2N_e r + 1)$, where r gives the recombination frequency between the two loci.

Muller's Ratchet

In contrast to the models discussed above, the focus of the model proposed by Muller (1964) is on linked deleterious mutations. We consider an asexual lineage with some number of loci at which deleterious mutations may arise; if the population is of finite size, genetic drift may occasionally fix a deleterious mutation. Once this mutation is fixed, the most fit haplotype (the haplotype with the lowest number of deleterious mutations) now carries an additional deleterious mutation – the number of deleterious mutations on the most fix haplotype goes from n to $n+1$. The mean fitness of the population decreases, and the 'ratchet' clicks forward. In the absence of back-mutations and recombination, there is no way to regain the more fit, n deleterious mutation haplotype, and so, as is true of an actual physical ratchet, there is no moving backwards to higher fitness. As pointed out by Felsenstein (1988), Muller's ratchet is a variant of the Fisher–Muller model, which we can see if we focus on the unmutated, favorable variant at each locus. We imagine deleterious alleles arising at different locations (in different loci). Once all of the asexual lineages or haplotypes have at least one deleterious

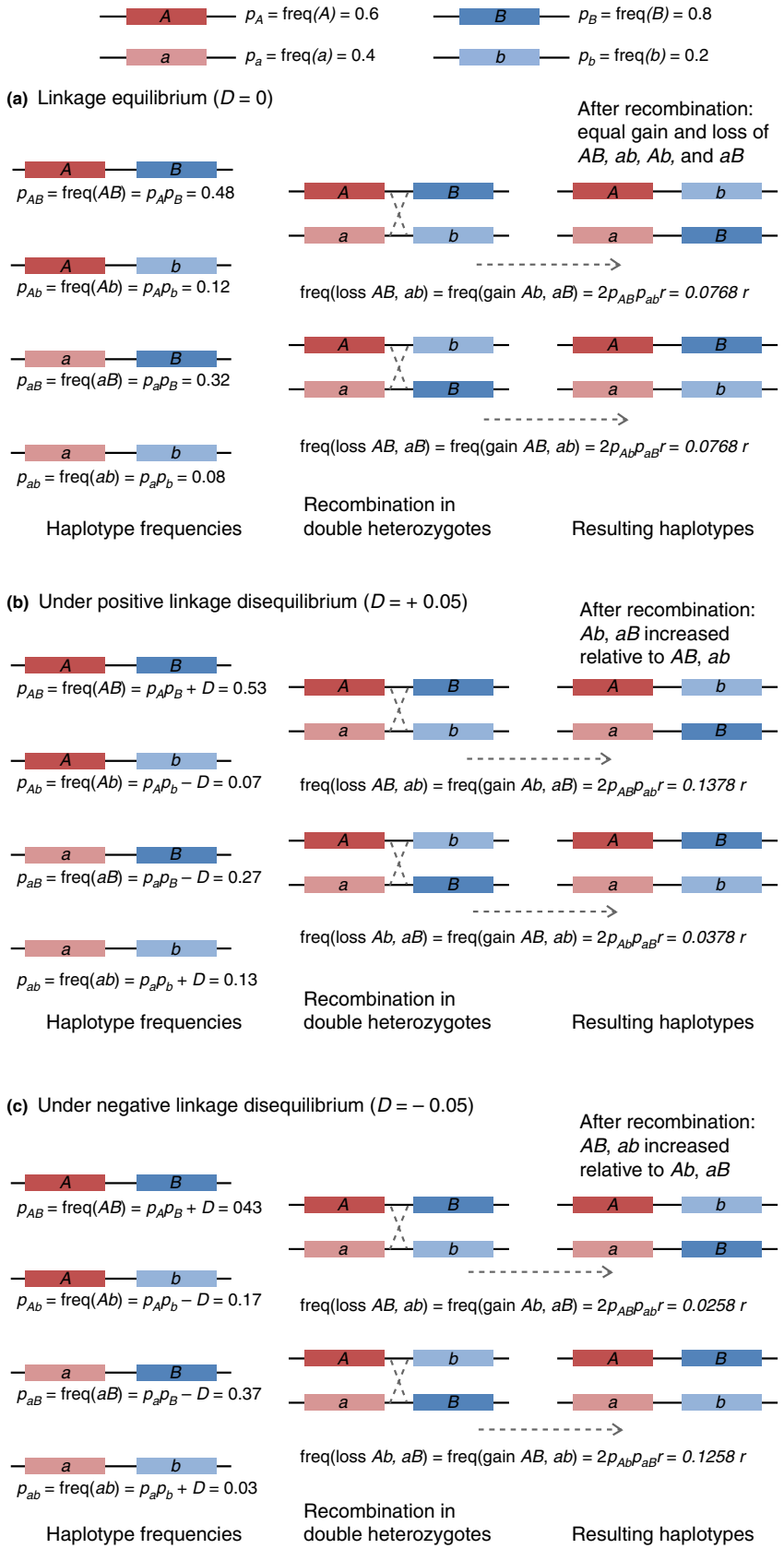

Figure 1 Effects of recombination under (a) linkage equilibrium, (b) positive linkage disequilibrium, and (c) negative linkage disequilibrium. In this example, we assume that the *A* and *B* alleles are favorable and the *a* and *b* alleles are deleterious. Only recombination under negative linkage disequilibrium (where there is a statistical excess of haplotypes combining negative and positive alleles) leads to a relative increase in the proportion of positive + positive haplotypes (*AB*) favored by selection, and of negative + negative haplotypes (*ab*) cleared by selection (panel c).

mutation, the favorable alleles at all of the loci where a deleterious mutation has occurred will be in negative disequilibrium with one another, since there is no haplotype that exists with all of the favorable alleles (and none of the deleterious mutations).

Haigh (1978) gave a quantitative description of Muller's ratchet, where the optimality criterion is the expected number of individuals in the optimal, zero mutation class. We assume that the relative fitness of an individual with k deleterious mutations is $(1-s)^k$, where s is the selective disadvantage per mutation. In a finite population of size N that is undergoing deleterious mutations at a rate of U per genome per generation, the expected number of individuals in the zero class is given by

$$E(n_0) = Ne^{-U/s} \qquad [1]$$

Muller's ratchet moves slowly if at all if $E(n_0)$ is large, and in this case deleterious mutations accumulate independently of one another (this will happen in a population of large size, for example). On the other hand, if $E(n_0)$ is small, the ratchet quickly moves the population distribution along k, so that the average individual in the population has a larger and larger number of deleterious mutations, and the mean fitness of the population becomes smaller and smaller. The expectation given in [1] is smallest for small populations (where drift is strong) and for weakly deleterious mutations (small s) that are easily increased in frequency by drift. This formulation also emphasizes the role that genome size may play in how vulnerable a population may be to Muller's ratchet. If U increases as genome size increases, then $E(n_0)$ necessarily decreases as genome size increases, implying that the ratchet may be more important for larger genomes (Maynard Smith, 1988), and that, all other things being equal, there may be an upper limit on the functional genome size of strictly asexual lineages. This has been an argument for why asexual eukaryotes (with larger genome sizes than asexual prokaryotes) may not persist over long evolutionary timescales.

Selection Fluctuating Over Time and Space – the Red Queen and Other Models

In addition to the above models, where the negative linkage disequilibrium favoring recombination is generated stochastically, there are a very large number of models that consider linkage disequilibrium that is generated deterministically. In these models, *epistatic interactions* (where the fitness effects of alleles at different loci interact such that their total effect is not determined by adding or multiplying their individual effects) are generated by selection that fluctuates over time or space. These include the *Red Queen model* (Van Valen, 1973) where environmental fluctuations (either abiotic, or biotic, such as due to parasites or pathogens) cause the optimal genotype to change from generation to generation, and thus lead to selection favoring changes in the sign of linkage disequilibrium (Maynard Smith, 1988). As an example, imagine that the haplotype combination A_1B_1 is favored in one generation – linkage disequilibrium will build up, due to an overrepresentation of this particular allelic combination. In another generation, when A_1B_1 is no longer favored, recombination breaking up this linkage disequilibrium will be favored – thus this type of selection leads to cyclical changes to both genotype frequencies and linkage disequilibrium. Other families of models depend on environmental variation that is spatial rather than temporal – so-called *Tangled Bank models* and *Lottery models* that focus on the ability of a lineage to occupy ecologically diverse environments (Ghiselin, 1974; Williams, 1975; Maynard Smith, 1976; Bell, 1982; Koella, 1988). For all of these models, linkage disequilibrium is formed via deterministic, nonrandom processes involving epistasis and population structure, and they are thus infinite-population size models.

Modifier Theories of Recombination

Modifier Theory in Large Populations

Theories considering the fate of modifiers of recombination must consider the balance between two different types of selection – short-term selection on individuals to have the highest mean offspring fitness, and long-term selection to increase the genetic variation in population fitness (Barton, 1995; Otto and Lenormand, 2002). To consider the long-term selection effects, we follow the fate of a modifier allele (M) that alters the recombination rate (r) between a set of loci and consider both the association between those loci (linkage disequilibrium, D) and the amount and type of fitness interactions between the loci (epistasis, ε, **Figure 2**) (for a review, see Feldman *et al.*, 1997). Generally, epistasis generates linkage disequilibrium of the same sign – positive epistasis (where allelic effects on fitness are greater in combination than expected from their individual effects) leads to an overrepresentation of these allelic combinations relative to what we would expect from their marginal frequencies ($D > 0$), whereas negative epistasis (where allelic effects are smaller in combination than individually) generates $D < 0$. As discussed above for optimality models, negative D always favors recombination via its long-term effect on population fitness. When there is recombination within allelic combinations of intermediate fitness that combine both beneficial and deleterious alleles (and have $D < 0$), the haplotypes arising after recombination have either high fitness (beneficial alleles brought together) or low fitness (deleterious alleles brought together). This increases the genetic variation in population fitness, and allows long-term selection to act (**Figure 3**).

The short-term effects of recombination on individual fitness are more complex – these depend on the signs of both D and ε, and also on the form of selection acting on the loci. To try to understand this complexity, we can consider the change in frequency of the modifier allele, Δp_M. We follow the general model of Barton (1995), and consider weak selection, where alleles at two different loci (A, B) change fitness by some small amount individually (s_A,s_B), and interact to give a deviation ε from multiplicative fitness. If the modifier allele changes recombination by a small amount ∂r, the change in modifier allele frequency is then

$$\Delta p_M = -\frac{\partial r p_M q_M D}{r_{MAB}}\left[\varepsilon + s_A s_B\left(\frac{1}{r_{MA}} + \frac{1}{r_{MB}} - 1\right)\right] \qquad [2]$$

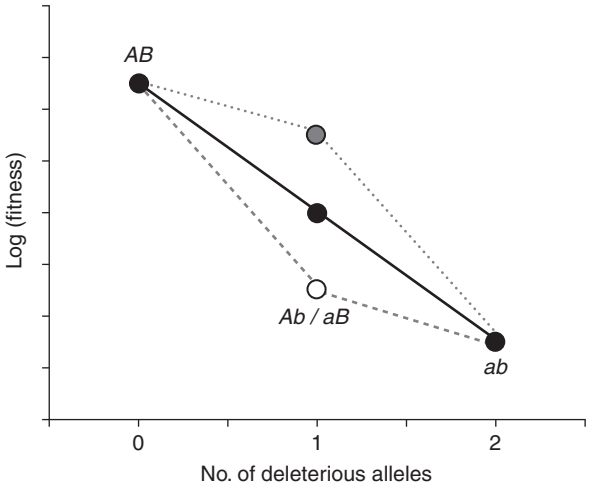

Figure 2 Fitness effects of positive and negative epistasis. In this example, we let *AB* and *ab* have the most extreme differences in fitness, with *AB* having the greatest fitness, and *ab* the lowest fitness. The relative intermediate fitnesses of the *Ab* and *aB* combinations depend on the form of epistasis (we assume for simplicity that *Ab* and *aB* have equal fitness). With no epistasis ($\varepsilon = 0$, black dot, solid black line), the *Ab* and *aB* haplotypes have exactly intermediate fitness. With positive epistasis ($\varepsilon > 0$, white dot, dashed gray line), the *Ab* and *aB* haplotypes are less fit than expected, while with negative epistasis ($\varepsilon < 0$, gray dot, dotted gray line), the *Ab* and *aB* haplotypes are more fit than expected. Adapted from Figure 1, Kouyos, R.D., Silander, O.K., Bonhoeffer, S., 2007. Epistasis between deleterious mutations and the evolution of recombination. TREE 22 (6), 308–315.

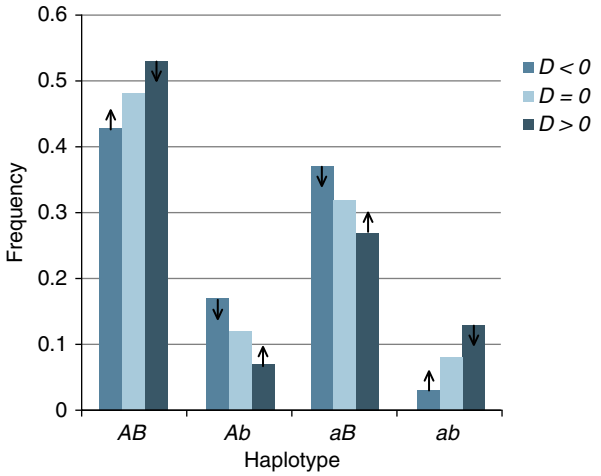

Figure 3 Change in haplotype frequencies under recombination. We imagine a simple case with two loci and two alleles at each locus, leading to four possible haplotypes (*AB*, *Ab*, *aB*, and *ab*). The graphs give the frequencies of the four haplotypes under negative linkage disequilibrium ($D < 0$, medium blue bars), under linkage equilibrium ($D = 0$, pale blue bars), and under positive linkage disequilibrium ($D > 0$, dark blue bars). The values for the haplotype frequencies and for D are as given in **Figure 1**. The black arrows indicate the direction of change for the haplotype frequencies under recombination: for $D > 0$, *AB* and *ab* decrease in frequency, while *Ab* and *aB* increase in frequency, and the opposite is true for $D < 0$. Note that recombination always takes the haplotype frequencies toward the linkage equilibrium values (pale blue bars in center). If we assume that *AB* and *ab* have the most extreme phenotypes (highest fitness and lowest fitness, respectively, as in **Figure 2**), we see that recombination under negative linkage disequilibrium increases the frequency of extreme phenotypes and decreases the frequency of less extreme phenotypes, increasing the ability of natural selection to act. Adapted from Figure 1, Kouyos, R.D., Silander, O.K., Bonhoeffer, S., 2007. Epistasis between deleterious mutations and the evolution of recombination. TREE 22 (6), 308–315.

where r_{MAB} gives the rate of recombination for M, A, and B, r_{MA} the rate for M and A, and r_{MB} the rate for M and B (adapted from Eq. A1.5*e*, Barton, 1995). This equation summarizes both the short- and long-term effects of selection. We can see from [2] that negative D always lead to an increase in the modifier (positive Δp_M), as discussed above. Since the modifier allele needs to stay associated with the beneficial allelic combination it creates long enough for their increase in frequency to also lead to an increase in frequency for the modifier, there is also a dependence on the recombination rate between the modifier and fitness loci (r_{MAB}). These are the results of the long-term effects of selection.

To understand the effects of short-term selection, we note that the effects of changing the average fitness of offspring is greatest when D and ε have opposite signs (more exactly, when $(\varepsilon + s_A s_B)D < 0$; Otto and Lenormand, 2002). As stated above, epistasis generates disequilibrium of the same sign; factors other than epistasis can generate either positive or negative disequilibrium (such as spatial correlations in selection coefficients, generating positive D, or random genetic drift, generating negative D), and thus influence the relative effect of epistasis on the short-term results of recombination (Otto and Lenormand, 2002).

What can cause the conditions that favor a recombination modifier under this type of selection? One situation is that of fluctuating epistasis, where the epistatic effects of allelic combination change over time. This could be caused by biotic coevolution, as described above in Red Queen-type models, if the epistasis imposed by one species is in direct response to linkage disequilibrium arising in the other species (Nee, 1989). A second situation is as a response to directional selection, where there is a need for epistasis to be both weak and negative (Barton, 1995). Many biologically reasonable scenarios would give rise to weak epistasis, but it is not clear if epistasis is generally negative (so that allelic effects are smaller in combination than expected from their individual effects) (see discussion of epistasis, below). Some support for the idea of widespread negative epistasis comes from consideration of stabilizing selection on additive quantitative traits (Maynard Smith, 1988; Charlesworth, 1993), from consideration of mutational load arguments under deleterious mutations (Kondrashov, 1988, but see MacCarthy and Bergman, 2007 for a counter-argument), and from work considering artificial gene networks (Azevedo *et al.*, 2006). Studies seeking to empirically measure epistasis have found mixed results – some have found negative epistasis some positive, and some have found either no epistasis or variable epistasis (some of these are summarized in de Visser and Elena, 2007 and in Kouyos *et al.*, 2007; see references therein).

Modifier Theory in Small Populations – The Role of Genetic Drift

In small populations, genetic drift acts as a stochastic force in generating nonrandom associations. Directional selection acting on beneficial mutations in such populations generates, on average, negative linkage disequilibrium. Allelic associations in positive linkage disequilibrium (either beneficial with beneficial, or deleterious with deleterious) are rapidly either fixed or lost when they arise due to chance – it is combinations that include both beneficial and deleterious mutations that, on average, persist the longest (Barton and Otto, 2005). This leads to increases in frequency for recombination modifier alleles, with the strongest effects occurring in small populations (Otto and Barton, 2001), in large populations where spatial structure increases the effect of genetic drift (Martin *et al.*, 2006), and in population where directional selection is acting simultaneously on multiple loci (Iles *et al.*, 2003). However, this combined effect of genetic drift and directional selection on recombination requires a relatively high rate of beneficial selective sweeps to fix or remove allelic combinations in positive disequilibrium.

Modifiers of Recombination and Genetic Drift in Large Populations

Genetic drift acts in all populations, and so the stochastic effects of finite population size can play a role in large populations as well. Under Hill–Robertson interference (discussed above), genetic linkage is seen to increase the amount of genetic drift near a selected locus, thus reducing the effective population size for the locus when either a beneficial mutation arises or in the presence of purifying selection against a deleterious allele. Keightley and Otto (2006) contrasted the probability of fixation for an allele modifying recombination with a neutral allele, and showed that purifying selection against repeated deleterious mutations provided an advantage to modifier alleles, causing them to fix with a higher probability. Surprisingly, this effect increased with increasing population size.

To understand this somewhat counter-intuitive result, we note that recombination frees the focal locus from Hill–Robertson interference, allowing deleterious mutations to be purged by selection. A larger number of polymorphic loci increases the opportunity for Hill–Robertson interference, which increases the advantage seen for recombination. Larger populations (where genetic drift is overall weaker) will maintain greater polymorphism, and thus see on average a greater amount of Hill–Robertson interference, and a larger advantage to recombination. The Keightley–Otto model gives a truly synthetic treatment of the role of negative disequilibrium where both selection and drift determine how selection on a new mutation affects the fate of other loci, and recombination frees loci from these shared fates.

Epistasis, Genomic Architecture, and Fitness Landscapes

As mentioned above, empirical studies seeking to measure the amount and type of epistasis have found mixed results. Kouyos *et al.* (2006) considered a multilocus model for a recombination modifier under a broad range of epistatic effects, and found that epistatic interactions of a given strength could generate very different types of linkage disequilibrium and that epistatic interactions had the greatest effect under weak selection. Thus it may be that the evolution of recombination under mutation–selection balance is driven by a small number of interactions under weak selection, instead of the average epistasis of all interactions, which is the quantity that is usually measured in empirical studies.

There are several biological scenarios under which the negative epistasis predicted by modifier theory to favor the evolution of recombination might be generated. Among these are density-dependent regulation of population size under limiting resources via truncation selection (Crow and Kimura, 1979; Kondrashov, 1988; de Visser and Elena, 2007) and selection for optimum (rather than maximum flux) along an enzymatic pathway under high competition (low resources) (Szathmary, 1993; de Visser and Elena, 2007). In his *mutational deterministic theory*, Kondrashov (1984, 1988) pointed out that strongly synergistic mutational effects are necessary to avoid excessive mutational load under deleterious mutations, and argued that a genomic mutation rate much greater than unity under this type of selection would allow sexual populations to overcome the two-fold cost of producing males.

It has also been pointed out that negative epistasis can be caused by genetic robustness (phenotypic stability in the face of repeated mutation or other perturbations), so that genetic robustness may favor recombination (Gardner and Kalinka, 2006; de Visser and Elena, 2007). In addition, since recombination increases the variability in genetic backgrounds experienced by a locus across generations, recombination may itself select for greater genetic robustness in sexually reproducing organisms, generating negative epistasis and under this type of selection would allow sexual populations to overcome the two-fold cost of producing males.

Finally, the shape of the fitness landscape experienced by individual alleles is important to how epistasis may shape the evolution of recombination. The presence of *sign epistasis* (where the positive or negative effects of an allele varies across genetic backgrounds) creates a 'rugged' fitness landscape, with local minima and maxima, constraining the possible pathways that evolution can take (Crona *et al.*, 2013). Studies of the effect of this type of fitness landscape on the evolution of recombination have led to contradictory results. In simulation studies, de Visser *et al.* (2009) showed that recombination may prevent populations from escaping local maxima by leading to the break up of 'escape' genotypes. In contrast, Watson *et al.* (2011) considered a model with explicitly modular genomes (viewing genes as necessarily modular units within the larger genome). Using individual-based simulations borrowing ideas from evolutionary computation theory and the genetic algorithm literature, they showed the same advantage of a faster evolutionary rate under recombination seen in the classic Fisher–Muller model, but also saw an additional benefit to recombination. Here, under intragenic epistasis that allowed for local fitness optima, sexual populations were able to access the globally optimal genotype and escape local optima that trapped asexual populations. They pointed out that, in the absence of recombination between loci, competition between

alleles at one locus is interfered with by competition between alleles at another locus, such that *clonal interference* (as defined by Gerrish and Lenski, 1998) acted not only to slow the rate of evolution, but also to limit the overall increase in fitness (Watson *et al.*, 2011). Nowak *et al.* (2014) found only a transitory advantage to recombination under a rugged fitness landscape when selection is strong relative to recombination (at the limit of strong linkage) – here, at longer timescales, it is the recombining populations that become trapped at local fitness peaks. The dependence on the strength of selection is due to the fact that, under relatively weak selection, recombination plays a greater role in breaking up linkage, and allows sexual populations to follow adaptive trajectories defined by 'allele frequency space' rather than 'genotype frequency space' (Watson *et al.*, 2011).

The timescale effect found by Nowak *et al.* (2014) makes clear that an important consideration is how the fitness landscape itself changes with time. At the limit of a constantly changing fitness landscape, the transitory advantage of recombination could continue indefinitely, as long as the timescale of fitness change is shorter than the timescale of advantage for asexual populations. In a model considering the evolutionary dynamics of recombination modifiers under environmental fluctuations, Carja *et al.* (2014) showed that the recombination rate evolved toward a nonzero value that decreased with increasing environmental period. These types of models have an obvious connection to the Red Queen family of models where selection varies across time and/or space.

As genomic data become more readily accessible for a wide range of organisms, further consideration of the form and extent of epistasis, as well as the global and local properties of fitness landscapes, may permit a clearer picture of the interplay between recombination and selection in real populations. However, it is also likely that a single, simple explanation will not suffice – the use of computer simulations and genetic algorithms by researchers seeking more realistic models of genomic evolution has shown the importance of considering both the details of genome structure and the timescale under which evolution is being considered.

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See also: Linkage Disequilibrium: Population Genetics of Multiple Loci. Recombination and Molecular Evolution. Selective Sweeps. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth

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Recombination in Bacterial Populations

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Glossary

Bacteriophage A virus that infects and replicates inside bacteria. Infection may be lytic, which results in the cell bursting and releasing progeny phage, or lysogenic whereby the phage genome integrates into the host DNA.

Exponential bacterial growth (log phase) The phase in the bacterial growth cycle where the number of cells doubles per unit time. The actual rate of growth depends on the conditions and the species in question.

Genome-wide association studies (GWAS) Attempts to identify individual genetic changes, typically single nucleotide polymorphism that is statistically associated with a given trait (e.g., the ability to cause disease).

Housekeeping genes Core genes encoding essential functions relating to central metabolism. These genes are typically assumed to be under strong stabilizing selection.

Lysis Refers to the bacterial cells bursting and releasing their contents (including DNA) into the extracellular milieu. May be brought about by osmotic pressure or the action of enzymes (e.g., lysozyme).

Microevolution Evolution occurring over short-time scales (months, years, and decades), typically within a single species. Contrasts with macroevolution which refers to the emergence of major taxonomic groups over thousands or millions of years.

mecA This gene confers resistance to beta-lactam antibiotics such as Penicillin and Methicillin. It is commonly carried by Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumonia*. It encodes a variant penicillin-binding protein (pbp2A) which enables normal cell wall synthesis even in the presence of these antibiotics.

Mobilome This refers to the total complement of mobile genetic elements, including phage, plasmids, and transposons, with the potential to incorporate into the genome of a given bacterial species.

Multilocus sequence typing (MLST) A molecular typing technique based on the nucleotide sequence of a set of seven core housekeeping loci. This approach was the first typing technique to successfully combine automated sequencing technology with the Internet.

Quorum sensing A regulatory system commonly used by bacteria to control gene expression according to population density.

Restriction-modification A system by which bacteria protect themselves against attack by phage. A restriction enzyme is produced which degrades DNA in a site-dependant manner, the modification enzyme protects the host chromosome.

Sporulation The process leading to a metabolically dormant cell (endospore) commonly as a result of stressful conditions such as low nutrient availability. Typically a property of Gram-positive Firmicutes.

Whole genome sequencing The generation of complete sequence data for a given genome. Recently developed next-generation sequencing platforms (such as Illumina MiSeq) provide the means for rapid resequencing of closely related genomes against a given reference.

Introduction

In 1881, Robert Koch first demonstrated the use of solid culture medium for the isolation and growth of pure bacterial cultures, and by the 1890s this new technology gave rise to the use of petri dishes and agar that has remained essentially unchanged ever since. Also unchanged, and understandably so, is a bias in research emphasis on pathogenic bacteria responsible for causing human diseases such as cholera or tuberculosis, over 'environmental' taxa that have less obvious relevance to man. This bias is also evident in this article. However, one fundamental shift has occurred since the days of Koch and Pasteur. The emergence of antibiotic resistance from the 1940s onwards provided an urgent incentive for thinking beyond the petri dish, and recalibrating our view of bacteria in nature as highly diverse, dynamic, and interacting populations. It was quickly appreciated that the rapid acquisition of antibiotic resistance reflects key aspects of the population biology of bacteria, most notably huge population sizes, short generation times, and the ability to share genes through recombination. The advent of large-scale molecular data in the 1980s led to the first serious attempts to incorporate bacteria into formal population biology and microevolutionary frameworks (Caugant *et al.*, 1981; Ochman and Selander, 1984; Guttman and Dykhuizen, 1994; Feil *et al.*, 1995). The rich and complex hues of bacterial molecular diversity uncovered by these initial works have, in recent years, exploded into a kaleidoscopic melange due to the deployment of whole genome sequencing (Whittam and Bumbaugh, 2002; Fraser-Liggett, 2005; Joyce *et al.*, 2002; Koonin and Wolf, 2012; Abby and Daubin, 2007).

Where does all this sequence diversity come from? How is it maintained? Can it be partitioned and cataloged along meaningful epidemiological, ecological, or phylogenetic grounds into 'strains,' 'clones,' 'populations,' or 'species'? Moreover, what are the practical implications of this diversity for combating pathogens, and for gauging the risks of emergence of new resistant or virulent variants? A deep appreciation of the mode and tempo of recombination – the process through which DNA can be re-assorted and redistributed between bacteria as a common resource – is central to addressing all these questions. Here the central characteristics and outstanding questions concerning bacterial recombination, with

an emphasis on comparisons with eukaryotic sex will be outlined.

What Is Bacterial Recombination?

Recombination is a broad term used, somewhat casually, in bacterial population genetics to refer to the transfer of DNA originating from one bacterial cell into another, thus resulting in a hybrid that has genetic contributions from both parental cells. Bacterial gene transfer through recombination is often prefixed as 'horizontal' or 'lateral,' to distinguish it from 'vertical' inheritance which occurs down through the generations from mother to daughter cell. Recombination can arise through multiple mechanisms, which are discussed in detail in a number of excellent reviews elsewhere (Bellanger *et al.*, 2014; Juhas *et al.*, 2009; Thomas and Nielsen, 2005; Narra and Ochman, 2006), and has a commensurate broad range of outcomes. A key distinction is to be made between those events resulting in the acquisition of new genes, typically *en masse*, and cases where existing genes are replaced by different allelic variants of the same genes (orthologs) (Vos, 2009). This latter process occurs much more readily between closely related cells belonging to the same named species, whereas novel genes can occasionally be transferred between unrelated species or genera. These processes occur at different rates within and between named species, and there is no clear evidence that the frequencies of these two types of recombination event are linked (Narra and Ochman, 2006).

There are three main mechanisms for the transfer of DNA between bacteria: transformation, transduction, and conjugation. Details of these can be found in any modern textbook of bacterial genetics. Briefly, transformation describes a process involving the direct uptake of DNA from the environment, followed by integration into the chromosome, a process that requires the cell to enter a state known as 'competence' (Chen and Dubnau, 2004). This is historically significant as the process that led Griffith in 1928 to demonstrate the presence of a mysterious 'transforming principle' (now recognized as DNA) which when released from dead, virulent *Streptococcus pneumonia* cells is able to imbue non-virulent cells with the ability to cause disease (Griffith, 1928). DNA can also be transferred from one cell to another via bacteriophage vectors, a process known as transduction. Given the high abundance of these viruses, particularly in the marine environment (Sime-Ngando, 2014; Weinbauer, 2004), this may well be the most common mode of horizontal gene transfer in nature. Plasmids and integrative conjugative elements transfer genes via conjugation, and transposons can 'jump' within and between chromosomes or plasmids resulting in deleterious consequences for the host through gene disruption, or alternatively conferring a selective benefit through the provision of new advantageous cargo genes (Siguier *et al.*, 2006; Touchon and Rocha, 2007). These and other semiautonomous genetic entities are collectively known as mobile genetic elements (MGEs).

Core and Noncore Genomes

The ability of bacteria to acquire novel genes through horizontal gene transfer is now recognized as playing a major role in rapid adaptation to environmental challenges (Feil, 2004; Young *et al.*, 2006). This is strikingly illustrated by the emergence of antibiotic resistance, which is typically observed a few years after a specific antibiotic is first administered (Laxminarayan *et al.*, 2013; Brandt *et al.*, 2014). Multiple resistances may be conferred through groups of resistance genes linked together on plasmids or transposons, which can be transferred within and between species by recombination. Specialized mobile cassettes can be implicated, such as the Staphylococcal cassette chromosome (*Scc*) in *Staphylococcus aureus* which carries a mecA gene conferring resistance to β-lactam antibiotics, and is the distinguishing genomic feature of methicillin resistant *S. aureus* (MRSA) (Shore and Coleman, 2013). These MGEs form part of the 'noncore' or 'accessory' genome, defined as those genes that are variably present or absent within different strains of the species, usually acquired through horizontal gene transfer and encode specialized, but nonessential, functions. Although resistance genes are typically associated with plasmids, resistance genes can also be carried by phage, along with genes encoding toxins and other virulence factors (Colomer-Lluch *et al.*, 2011). More generally, genes carried by MGEs tend to involve interactions with the environment, the host, or with other bacteria (Rankin *et al.*, 2011). MGEs, and their cargo genes, are themselves prone to high rates of genetic re-assortment, and are usually hybrid (or chimeric) in structure (Bellanger *et al.*, 2014). In contrast, 'core' genes are, by definition, common to all strains of a species, and include those encoding essential housekeeping functions (Feil, 2004). Although the precise repertoire of genes assigned as core and noncore depends on the number of genomes considered (Tettelin *et al.*, 2005), this broad dichotomy is a useful framework for exploring bacterial genomic diversity and adaptation; the core being analogous to the 'operating system' of the cell, whilst the noncore genes are more like plug-and-play software modules for specialized functions.

As discussed in more detail below, homologous recombination does not have equal impact throughout the core genome, and genes in certain functional categories appear to recombine at atypically high frequencies. For example, genes in pathogenic bacteria that encode proteins exposed to the host immune response are often under strong diversifying selection pressure (Evans *et al.*, 2010), and in these cases recombination can contribute significantly to the generation of selectively beneficial diversity. Specific motifs in the genome can also elevate local rates of recombination, leading to the identification of 'hotspots' (Yahara *et al.*, 2014). It has been argued that 'informational' core genes, which include those responsible for essential DNA processing, replication and repair, are likely to be completely recalcitrant to recombination due to multiple protein–protein interactions leading to strong selective constraint, the so-called 'complexity hypothesis' (Jain *et al.*, 1999). However, there are no clear signals in the genomic datasets that any genes, core or noncore, are completely immune to the effects of recombination, including 16s rRNA (Kitahara and Miyazaki, 2013).

Is Bacterial Recombination Akin to Eukaryotic Sex?

Bacterial recombination involves the fusion of DNA from multiple parents to result in a hybrid genome with a novel

genetic repertoire. As such, it is often considered as analogous to eukaryotic sex. Whilst this comparison can be informative, it is important to bear in mind how bacterial recombination fundamentally differs from, and in many ways is even more enigmatic than, genetic re-assortment during meiosis.

The Multiple Ways in Which Recombination Rates Vary

Most pertinently, bacterial recombination differs from eukaryotic sex as it is not tied to reproduction. Rather than being an obligate process that occurs at every generation, the rates and consequences of recombination are broad-ranging and dynamic (Didelot and Maiden, 2010; Vos and Didelot, 2009). The impact of recombination varies widely between different species and lineages. For example, transformable species such as *Neisseria meningitidis* and *S. pneumoniae* recombine at far higher frequencies than *S. aureus* (Feil *et al.*, 2001), though the genetic bases underlying these differences remain unclear. Despite the fact that many genes and pathways responsible for recombination have been identified, a comprehensive comparison of published recombination rates in different species failed to find clear correlates with differences in the repertoires of these genes (Rocha *et al.*, 2005).

In addition to genetic differences, it has also been suggested that ecology may also play a role in shaping the opportunity for gene transfer (Ragan and Beiko, 2009). For example, obligate endosymbionts such as *Buchnera aphidicola*, which can only survive within the body of an aphid and are transmitted between hosts vertically through the maternal line, are assumed to remain ecologically isolated, and therefore to abstain from recombination altogether (Bordenstein and Reznikoff, 2005; Moran and Baumann, 2000). However, other arthropod-associated bacteria such as *Wolbachia* (Baldo *et al.*, 2006) and *Orientia tsutsugamushi* (Sonthayanon *et al.*, 2010) recombine at very high frequencies. This presumably reflects occasional horizontal transmission between hosts resulting in mixed infection and providing the opportunity to acquire exotic DNA. These examples also illustrate how the line of inquiry can be reversed, and evidence concerning recombination rates can inform on the underlying ecology. Similarly, the intracellular, sexually transmitted human pathogen *Chlamydia trachomatis*, which was once thought to be asexual due to its highly specialized niche, is now known to recombine at high frequencies (Joseph *et al.*, 2011; Joseph and Read, 2012). This implies a high rate of mixed infection, thus simultaneously shedding light on the sex life of both the bacteria and of its host.

The apparent rate of recombination may also vary according the evolutionary timescale being considered. A recent study looking at rates of recombination within a clone of MRSA noted that short-term diversification is primarily driven by mutation, but recombination assumes relatively greater prevalence when considering more highly diverged genomes (Castillo-Ramirez *et al.*, 2012). This implies that in order to make meaningful comparisons in recombination rate between species, one should ideally attempt to control for divergence times. Rates of recombination can also vary within a species. Certain combinations of lineages are more biologically suited to DNA exchange than others, owing to the variable presence of capsule (Connor *et al.*, 2012), restriction-modification systems (Waldron and Lindsay, 2006) or other compatibility groups such as pherotypes in *Streptococcus* (Pozzi *et al.*, 1996; Zaccaria *et al.*, 2014). Finally, competence for transformation is rarely constitutive (turned 'on' all the time), but instead arises under certain conditions or phases in the growth cycle. For example, in *S. pneumonia* cells develop competence simultaneously during exponential growth via a quorum sensing mechanism (Lee and Morrison, 1999), and in *Bacillus subtilis* competence is linked to stress and sporulation (Schultz *et al.*, 2009).

Bacterial Sex Is Localized

Bacterial recombination is unidirectional, and asymmetrical in terms of the contribution from each parent. Rather than a new hybrid individual representing 50% from the mother and 50% from the father, bacterial recombination involves a 'recipient' (major parent) and a 'donor' (minor parent). The donor may contribute only very localized fragments of DNA, perhaps a few hundred base pairs, thus resulting in the addition or replacement of a single gene, or even a fragment of a gene (Smith *et al.*, 1991; Lin *et al.*, 2009). The size of imported DNA depends upon the underlying mechanism. Very broadly, transformation tends to result in highly localized replacements of a few hundred base pairs or a few kilobases of DNA. Transduction typically results in the transfer of 20–50 kb (the amount of DNA being restricted by the physical capacity of the phage head), whereas conjugation can result in the transfer of hundreds of kilobases, at least in the laboratory. The largest homologous recombination event so far described in natural bacterial populations was noted in a strain of MRSA, where the imported donor DNA replaced $>20\%$ of the genome (~ 625 Kb) in a single 'cut and paste' (Robinson and Enright, 2004; Holden *et al.*, 2010). Other large replacements have been noted in this species (Robinson and Enright, 2004), in other Gram-positive taxa (He *et al.*, 2010; Brochet *et al.*, 2008) and, most recently, in the Gram-negative species *Klebsiella pneumonia* (Chen *et al.*, 2014). The underlying mechanism for these large replacements remains obscure, as does their evolutionary significance regarding adaptation and virulence.

Recombination does not impact on the chromosome randomly with respect to genomic position. Studies in *Escherichia coli* and *S. aureus* have noted lower rates of recombination (and, curiously, lower GC content) around the terminus of replication (Touchon *et al.*, 2009; Everitt *et al.*, 2014), and elevated rates of core gene recombination around regions experiencing high rates of gene flux (gain and loss); in the case of *S. aureus*, these regions are associated with MGEs (Everitt *et al.*, 2014). The genomes of some species, most notably *Haemophilus influenza* (Mell *et al.*, 2012) and the pathogenic *Neisseria* (Frye *et al.*, 2013), contain short conserved uptake sequences that provide regions of localized homology and promote the integration of 'self' DNA at specific sites (Mell and Redfield, 2014). 'Hotspots' of recombination in the *S. pneumonia* genome are associated with selection for antibiotic resistance and serotype-switching (Chewapreecha *et al.*, 2014). Modeling and experimental evolution approaches has also been used to characterize the heterogeneity of recombination events in this species (Mostowy *et al.*, 2014; Croucher *et al.*, 2012).

Recombination and Evolutionary Analyses

Analytical approaches to reconstructing phylogeny or past demographic changes, calibrating rates of evolution, establishing genotype–phenotype associations (genome-wide association studies; GWAS) need to be carefully tailored to account for the possible confounding effects of too much, or too little, recombination. Phylogenetic analysis is a key case in point, as recombination acting on single gene loci will result in discordant trees when different genes are used (**Figure 1**). This formed part of the motivation for developing multilocus sequence typing (Maiden *et al.*, 1998), where multiple (typically seven) physically unlinked core loci are sequenced in order to define strains. The use of multiple loci provided additional informative sites, thus increased resolution, but also provided a buffer against the possible confounding effects of recombination acting on one of the seven genes. Whilst this reasoning acknowledged that recombination can result in conflicting and misleading trees, there is also an implicit assumption that the frequency of recombination has not been so great that all phylogenetic signal is eroded. In other words, by identifying and then excluding all regions of the genome that have been affected by recombination (typically all noncore regions and a certain fraction of the core), it should be possible to reconstruct a true evolutionary history of a set of strains based on the parts of the genome that remain, the so-called 'clonal frame' (Croucher *et al.*, 2013; Milkman and Bridges, 1990). This logic appears to be mostly sound, and forms the central tenet for a number of recent analytical developments aimed at detecting recombination from whole genome data (Croucher *et al.*, 2014; Falush *et al.*, 2003; Marttinen *et al.*, 2012).

However, as tends to be the way with bacteria, what is true for the majority of species is not necessarily true for all, and in some cases recombination may have been so rampant that there is no remnant of a clonal frame (Shapiro *et al.*, 2012). In such a scenario, any attempt to reconstruct the 'true' tree will be futile, and alternative approaches based on networks, such as splits decomposition (Huson, 1998), should be deployed (Kloepper and Huson, 2008). Conversely, it is possible that recombination detection procedures may be overly sensitive, thus raising the possibility of excluding potentially informative data. A recent short report (Hedge and Wilson, 2014) noted by simulation that tree topology based on whole genome sequences is in any case largely robust to the effects of recombination. However, it is clear that recombination can

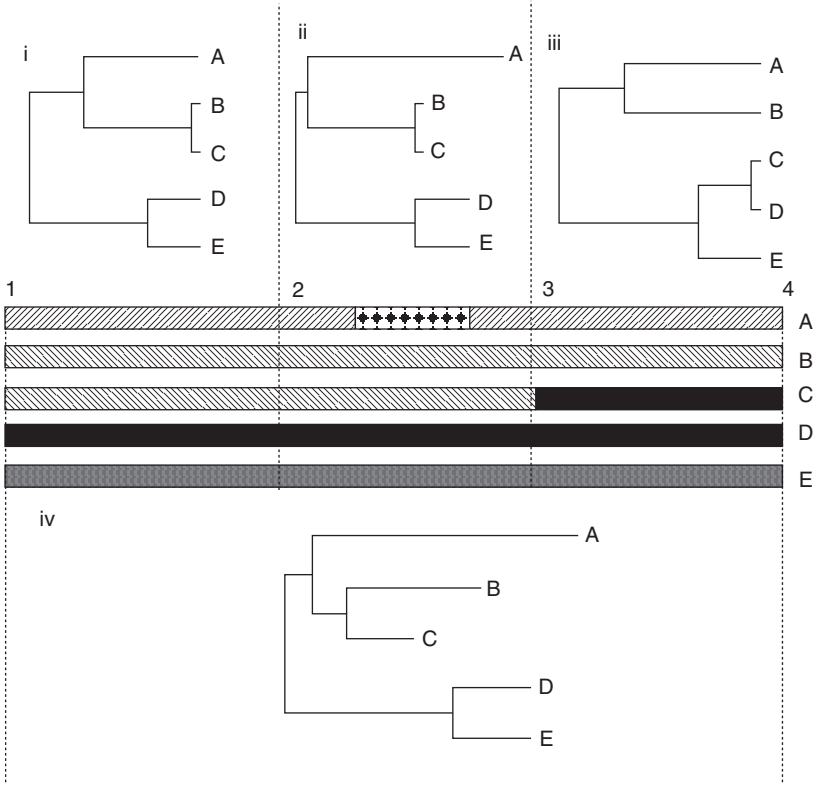

Figure 1 The effects of recombination on phylogenetic analysis. The patterned bars (A–E) represent aligned sequences which are divided into three regions (1–2, 2–3, and 3–4). Two recombination events are illustrated. Sequence 'A' has imported a diverged short block within the middle region (2–3). The donor of this sequence is not included in the sample. Sequence C is a hybrid of sequences B and D. Within regions 1–2 and 2–3, sequence C is identical to sequence B (sequence B is the 'major' parent). Within region 2–3, sequence C is identical to sequence D (sequence D is the 'minor' parent). Trees i–iii represent phylogenetic analyses based on regions 1–2, 2–3, and 3–4 respectively. Tree iv represents the tree obtained from the complete sequence (all regions). Note that tree i is based on sequences unaffected by recombination, and thus can be said to represent the 'clonal frame.' The topology of this tree is preserved in trees ii and iv, although the branch lengths are distorted. The topology of tree iii is discrepant from the clonal frame, as sequence C has imported DNA identical to sequence D in region 3–4.

seriously distort branch lengths, which is likely to compromise inferences concerning demographic changes and evolutionary rates. Intriguingly, rather than providing a solution to this problem, the authors of this study noted that the exclusion of recombined sites appears to make things worse.

GWAS refer to attempts to identify causal links between specific phenotypes with underlying genetic variation. These procedures were originally developed for use on data from sexual eukaryotes and in particular humans (Stranger *et al.*, 2011). Recently, GWAS has been successfully deployed on bacterial genome data, and helped to identify genetic changes associated with host adaptation (Sheppard *et al.*, 2013) and virulence (Laabei *et al.*, 2014). However, a key difficulty lies in the fact that the rate of recombination in bacterial genomes is typically far lower than for sexual species. This means that informative sites tend to be in a state of linkage disequilibrium, where the presence of one single nucleotide polymorphism (SNP), or allele, at locus X is strongly predictive of the presence of a second SNP or allele at locus Y. This makes it difficult to identify which site is primarily responsible for the observed phenotype, as any single phenotype might be associated with a large number of linked genetic changes. Recombination acts to break linkage, thus moderating this problem, and ongoing work is focussing on how best to tease apart individual genetic changes potentially responsible for a given phenotype in bacterial data where rates of recombination are relatively low (Yue and Schifferli, 2014).

Sex with the Dead

The contribution of the minor parent (donor) to bacterial recombination does not require this parent to be living or even extant, which is clearly a profound departure from eukaryotic sex. Transformation involves the uptake of DNA originating from lysed cells that can be preserved in the environment long after the bacterial cell itself has died (Nielsen *et al.*, 2007). Indeed, a recent report has even suggested that very short tracts of ancient DNA, bizarrely from extinct mammoths, could in principle be captured and utilized by bacteria in the wild, a process the authors called 'anachronistic evolution' (Overballe-Petersen *et al.*, 2013; Overballe-Petersen and Willerslev, 2014). Whilst the significance of this process remains unclear, it is surprisingly rare for the original donor of cargo genes carried by phage or plasmids to be unambiguously identified. Although this certainly in part reflects our patchy sampling of the bacterial biosphere, there is no reason to suppose that a single, original donor of these genes must actually exist. It is possible to imagine that genes and linked groups of genes could persist in perpetuity in a kind of nomadic limbo; circulating and recombining through the currents of the mobilome with occasional integration into amenable host chromosomes, but without a permanent residence. Such a view is awkward even for microbial taxonomists (who are easily disoriented). It is curiously both reductionist in the sense that complex genomes arise through combining simple genes, and at the same time holistic as it supposes that single genomes reflect a sample of a much larger gene pool (Frost *et al.*, 2005; Kunin *et al.*, 2005). This model also challenges the classical picture of the relationship between phage and their hosts; the long-term role of these MGEs as essential vehicles of gene transfer and adaptation being more akin to that of a benign pollinator than of a parasite.

Promiscuity and Specious Species

Eukaryotic sex has been proposed to represent both an underlying cause and a definition of species boundaries. The biological species concept (BSC) is a model whereby conspecifics are defined based on their ability to produce fertile offspring. Whilst the BSC is far from perfect even for eukaryotes (consider plant or bird hybrids, for example) it is more or less hopeless as a general rule for bacteria. Not only is there a broad range of recombination rates between different bacterial taxa, but DNA transfer may occasionally occur across phylogenetic distances so large that even the most ardent taxonomic 'lumper' could not accommodate donor and recipient into a single species. Similarly, taxonomic 'splitters' will take discomfort from observations that, even within freely recombining species defined though clinical, phenotypic, and genetic criteria (such as *S. pneumoniae* or *N. meningitidis*), gene flow does not always appear to be equally likely between all members of the population but, as discussed, can instead be structured according to subgroups. Again, this represents a significant departure from the assumptions of a truly sexual species where, by definition, there are no such biological restrictions to gene flow within species (**Figure 2**).

Despite these difficulties, the incorporation of relative rates of recombination remains a common feature of models attempting to explain the emergence and maintenance of ecologically relevant clusters (Nowell *et al.*, 2014; Cadillo-Quiroz *et al.*, 2012; Shapiro *et al.*, 2012; Cohan and Koeppel, 2008), although whether these clusters can be formally equated to 'species' remains an open question (Gevers *et al.*, 2005). Speciation models also draw on the role of the pan-genome, and the ecological opportunities afforded by the acquisition of new adaptive genes, as discussed earlier. The species *Bacillus anthracis* provides an illustrative example. In terms of genome-wide phylogeny, there is little reason to promote *B. anthracis* to species status, as it is highly related to the more benign soil dwelling bacteria *Bacillus thuringiensis* and *Bacillus cereus* (which are also not distinct from each other on the basis of phylogeny (Baillie and Read, 2001)). However, *B. anthracis* has a distinguishing ecological feature that is only too obvious, and one that can be clearly ascribed to recombination; that is, the ability to cause anthrax. *Bacillus anthracis* has acquired this ability courtesy of two plasmids (pXO1 and pXO2) that carry toxin and capsule genes respectively, and it is these plasmids that essentially define the species. Whilst in principle, the distribution of acquired genes (or MGEs), rather than phylogeny, could be used more broadly to define ecologically meaningful groups, it is typically very difficult to pin down the relevant ecological parameters, let alone identify the associated genetic changes. Perhaps it is a legacy of the petri dish that basic data pertaining to bacterial ecology, specifically the contribution to the selective landscape of abiotic factors (environmental conditions) and biotic factors (host adaptation, phage and competition, and cooperation with other bacteria), currently lags far behind the advances in genomics.

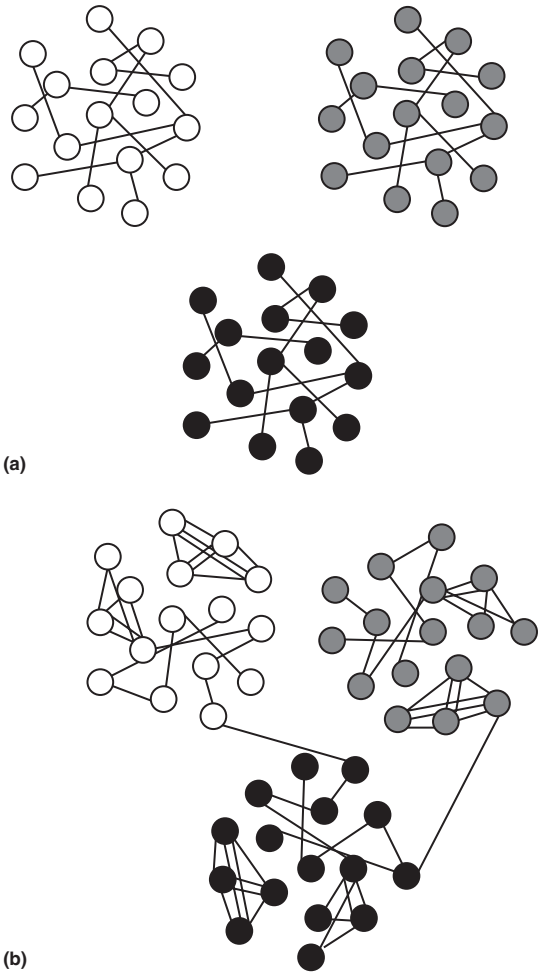

Figure 2 Gene flow and species borders. In sexual species (a), the biological species concept states that gene flow is equal and random with respect to members of the same species, and there is no gene flow between species. In asexual, but recombining, bacteria (b), gene flow may be nonrandom within named species, and can also occur between members of different species.

Why Do Bacteria Recombine?

In addition to mechanistic differences, comparisons between bacterial recombination and eukaryotic sex are also informative from an evolutionary perspective. The debate as to why bacteria recombine at all is often coined within the theoretical framework developed to explore the evolution of eukaryotic sex. The arguments have been outlined in a recent excellent review (Vos, 2009), and center on the fitness gains conferred through combining adaptive alleles from different lineages (clonal interference (Cooper, 2007)), the prevention of the inevitable loss of fitness in the absence of sex through mutation of the most adaptive class (Mueller's Ratchet (Andersson and Hughes, 1996)) and introducing adaptive mutations in optimal clonal backgrounds (sign epistasis (Weinreich *et al.*, 2005)). The ability to respond to rapidly changing biotic and abiotic pressures also forms a key component of models addressing the advantages of sex. The Red Queen hypothesis describes the requirement for the continual generation of diversity (diversifying selection) in order to maintain an adaptive genotype in response to dynamic selection pressures, such as from the host immune response or from coevolving phage. Thus, recombination represents an important mechanism to generate diversity in genes encoding surface exposed proteins that act as phage-binding sites (Betts *et al.*, 2014) or are immunologically relevant (Lipsitch and O'Hagan, 2007). Similarly, as discussed earlier, recombination provides an important response to strong directional selection pressure resulting from exposure to antibiotics.

Finally, the lottery model highlights the advantages of generating diversity through sex when selection pressures are unpredictable or conditions are particularly stressful. This is the most common method used to explain the broad observation in eukaryotes that sex is linked to dispersal; for example, winged aphids are sexual, wingless aphids are not. The notion that novel environmental challenges can at least in part be met through genetic variation driven by recombination also has some currency in bacterial world. Competence for transformation is often associated with stress and low nutrient availability (Antonova *et al.*, 2012), and the link between sporulation and competence for transformation in *B. subtilis* potentially provides a link between sex and dispersal.

On the flip side, bacterial recombination may also come at an evolutionary cost by disrupting adaptive genes and gene combinations. The dichotomy of bacterial genomes into a relatively stable core and a highly dynamic noncore may represent a strategy by which bacterial are able to rapidly diversify and adapt to new challenges through recombination, whilst simultaneously moderating this potential cost. This can help to explain why 'hotspots' of recombination and areas of high gene flux tend to be restricted to specific sites in the genome. The separation between the 'sexual' and 'nonsexual' components of the genome might be even more obvious in cases where the latter is represented by extrachromosomal elements. For example, the genome of *Borrelia* species is composed of a highly stable linear chromosome, and a multitude of small, highly dynamic, and largely uncharacterized plasmids (Qiu and Martin, 2014).

Other arguments have been put forward to explain why bacteria have evolved to take up and incorporate DNA that do not focus on the resultant generation of diversity (Michod *et al.*, 2008). The mechanism of homologous recombination is fundamentally linked to DNA repair, and the genes involved are thought to have evolved primarily for this function (Michod *et al.*, 1988). A further line of thought suggests that DNA uptake from the environment is a nutritional requirement, and DNA integration into the chromosome is simply an occasional by-product of eating DNA (Sinha *et al.*, 2013). Finally, the arguments above relating to the advantages of sex assume that selection is operating on the level of the host bacteria. It is, however, possible that gene transfer may reflect selection pressure operating on MGEs vectoring the genes, or even on individual ('selfish') genes themselves (Wagner, 2009).

Concluding Comments

In this article the author has attempted to sketch out the key properties of bacterial recombination, and compared the

process to eukaryotic sex both mechanistically and in terms of evolutionary relevance. Whilst the author has highlighted fundamental ways in which these processes differ, the comparison remains a useful one, and in the end one must simply draw on the analogy where appropriate, rather than seek an entrenched view in one camp or the other. One key point is that bacterial recombination encompasses a fantastically diverse range of mechanisms and consequences, and that generalizations, even with regard to the most fundamental questions, must only be put forward with extreme caution.

See also: Antagonistic Interspecific Coevolution. Bacterial Diversity, Introduction to. Bacterial Species Concepts. Genome Plasticity, Bacterial

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Regulatory and Coding Changes in Developmental Evolution, Roles of

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Glossary

***cis*-Regulatory elements or CREs** CREs are regions of non-coding DNA that regulate the transcription of their related genes. They comprise promoters directly upstream the start site of transcription, and enhancer that can reside within introns, 5′ and 3′ UTR of or up to tens of kilobases of the gene they regulate. They typically regulate gene transcription by functioning as binding sites for transcription factors.

Coding sequence A part of a gene that will be translated into a mature mRNA or a protein.

Duplicated genes A portion of DNA that comprise genes and/or its control region that are duplicated during evolution.

Epistasis It is a phenomenon that consists of the effect of one gene being dependent on the presence of one or more other genes.

Frameshifts The addition or deletion of a number of nucleotide(s) that causes a shift in the reading frame of the codons.

Immunofluorescence A technique that allow the *in situ* detection of protein by using an antibody against the protein of interest.

Indel The insertion or deletion of a DNA sequence.

***In situ* hybridization** A type of hybridization that use a probe (generally labeled RNA) to localize an express RNA of interest in a fixed tissue. In developmental biology, this technique is widely used to visualize expression patterns of genes in tissues

Non-synonymous substitution A point mutation that result in a change in protein sequence.

Pleiotropy The fact that a mutation or a gene is at the origin of multiple presumably not related phenotype. Consequently, a mutation in a pleiotropic gene may have an effect on some or all traits simultaneously.

Positive selection In population genetics positive selection is a mode of natural selection in which an extreme phenotype is favored over other phenotypes, shifting over the allele frequency of the genotype in the direction of that phenotype.

Synonymous substitution A point mutation that, thanks to the genetic code redundancy, do not affect the protein sequence.

Transcription factor It is a protein that blinds a specific CRE in the genome, modulating the expression of its related genes.

One central question in evolutionary biology is how species evolve, and more precisely, which mechanisms lead to such diverse phenotypes. Since the discovery of genes, biologists have long sought to understand the source of this variation, i.e., which genes and sequence changes are responsible for the evolution of morphological diversity (Carroll, 2008). Because organisms are mostly shaped during embryonic development, developmental genes became the most important candidates for variation. It has long been understood that changes in morphological evolution happened through the alteration of embryonic development (Gould, 1977; Raff and Kaufman, 1983). During most of the twentieth century, changes in protein coding genes (**Figure 1**) were thought to be the primary source of morphological evolution (Wittkopp and Kalay, 2012), essentially because they were easier to study. However, the discovery of *cis*-regulatory regions (CREs) that control the expression of genes through space and time revealed new sources of phenotypic variation (Jacob and Monod, 1961; Britten and Davidson, 1971; King and Wilson, 1975; Raff and Kaufman, 1983; Carroll, 1995; reviewed in Wray, 2007; or Stern and Orgogozo, 2008). At the beginning of the 1980s, the discovery of *Hox* genes and their regulation (McGinnis *et al.*, 1984; Scott and Weiner, 1984; Graham *et al.*, 1989; Duboule and Dollé, 1989 reviewed in Carroll, 2008) radically changed our vision of the evolution of genomes and fully introduced CREs as important players in morphological evolution as well as changes in protein sequences. The relative importance of coding versus CRE changes for the generation of novelties was a subject of some debate. In this article, author will review the main arguments on both sides using case studies will be reviewed.

Coding versus *cis*-Regulatory Sequences

Changes in Coding Sequences

Nature of changes in coding sequence

The coding region is the part of a gene that encodes the final gene product, which can be a protein or a mature RNA. Changes in the coding regions are all changes that alter the amino acid sequence or the mature RNA (**Figures 1(a)** and **1(b)**) but their importance depends on the mutation type. The impact of synonymous and nonsynonymous mutations is very different. In the first case, synonymous mutations do not give rise to a change in protein sequence but can affect transcription efficiency or mRNA stability (Stern and Orgogozo, 2008). The impact of these changes in morphological evolution is usually discreet and only a few cases have been reported (Stam and Laurie, 1996; Nackley *et al.*, 2006). In contrast, nonsynonymous mutations can give rise to drastic changes in the function of the protein (see Edar and Oca2 examples in the next section). A change in a functional domain could change protein structure, stability, activity, binding properties,

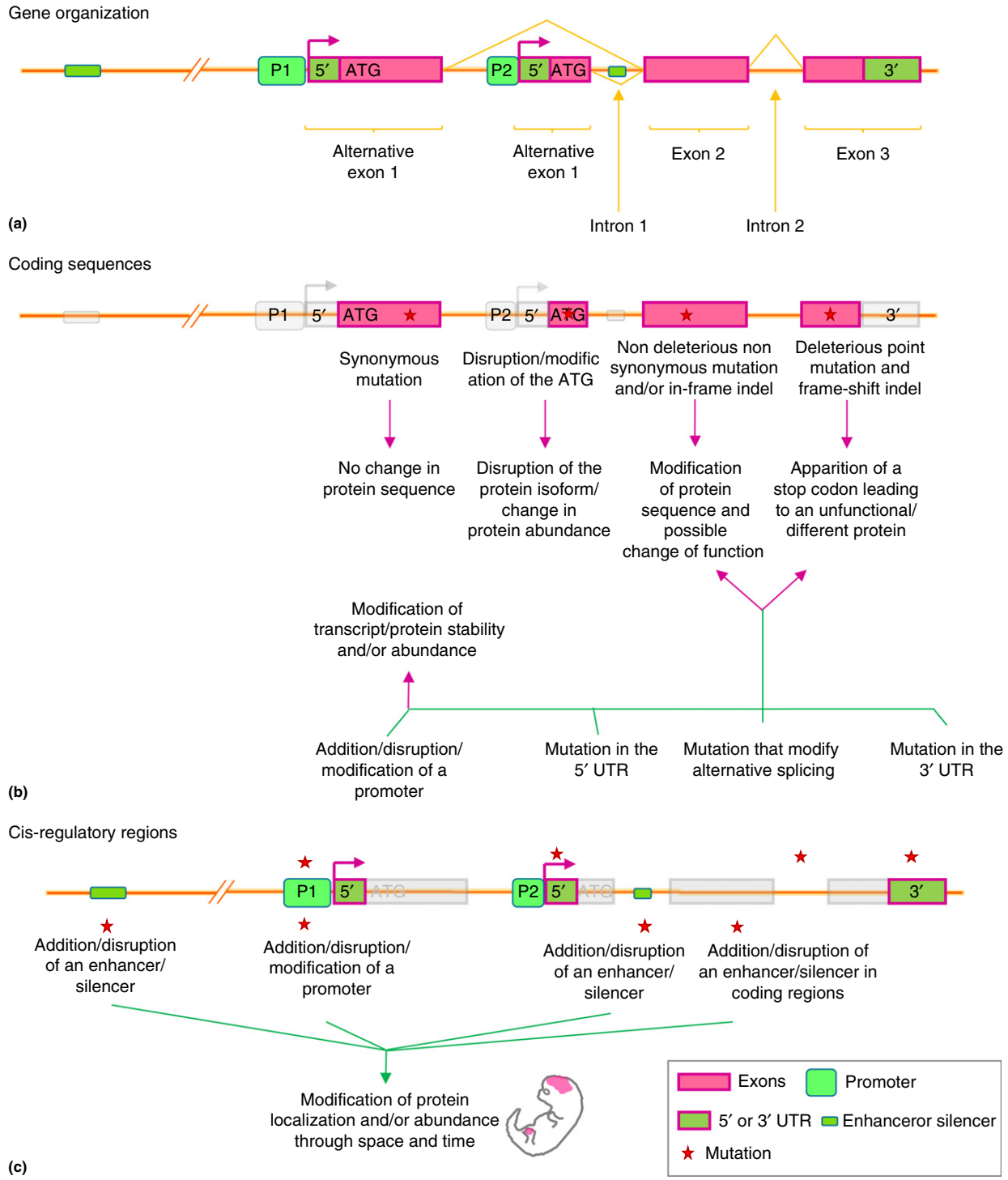

Figure 1 Organization of CRE and coding parts of a theoretical gene and the impact of different classes of mutations. (a) Organization of a theoretical gene with enhancers upstream and inside an intron, two alternative isoforms driven by two alternative promoters, and three exons. (b) Coding sequences and associated mutations. Synonymous mutations do not change protein product but nonsynonymous or in frame indel can change protein sequence as well as a stop codon or a frameshift. These changes can lead to a truncated or new or nonfunctional protein. Modification of the kozak sequence of an ATG can lead to changes in protein abundance or isoform disappearance. (c) *Cis*-regulatory sequences and associated mutations CRE mutation can be classified in two groups: mutations that change protein structure, stability, and abundance in a pleiotropic way and mutations that change protein expression and abundance through space and time. A mutation can add, remove, or modify a CRE leading to changes in protein localization and/or abundance. Mutations in promoters can modify protein localization and/or abundance as well as the apparition of a isoform. Modification in the 5′ and 3′ regions can modify the transcript stability and protein abundance. Mutations can also modify alternative splicing and lead to the generation of new isoform.

or whole function. Thus, nonsynonymous mutations are generally predicted to contribute more to phenotypic evolution than synonymous mutations (Stern and Orgogozo, 2008). A modification of coding sequence can also occur by frameshift mutations, which cause a shift in the reading frame of the codons and thus, a change in the whole sequence of the rest of the amino acids. Finally non-frameshift insertions or deletions could modify small domains or parts of the protein and can be at the origin of big changes in the protein function or properties as well as minor changes.

There are many examples of how changes in coding sequence lead to morphological evolution (Martin and Orgogozo, 2013). Many of them result in loss of function, modification of protein activity or abolition of the protein's function. In the next paragraph, the different impacts of coding changes in morphological evolution by reviewing two interesting cases studies will be highlighted.

The Edar mutation in Asian populations

First, as previously mentioned, some mutations can alter protein function. This case is highlighted by the receptor Edar from the EDA pathway (Figure 2(a)), a signaling pathway involved in the development of ectodermal appendages in vertebrates (including tooth, hair, nail, feather, and external glands such as mammary glands). This pathway has long been known to be involved in morphological evolution (Thesleff, 2002; Colosimo *et al.*, 2005; Sadier *et al.*, 2014), originally from studies that pinpointed its role in the establishment of tooth morphology. Interestingly, a mutation in Edar was identified by genome-wide scans as being under positive selection in humans (Sabeti *et al.*, 2007; Bryk *et al.*, 2008; Fujimoto *et al.*, 2008). The replacement of a conserved valine in the death domain of the protein, which is crucial for the establishment of the downstream signaling of the pathway (NF-κB, Figures 2(a) and 2(b)), by an alanine was selected for in Asian and Native American populations. A T to C substitution in the respective codon is at the origin of a gain of function that enhances NF-κB signaling and leads to thicker hair (Figure 2(c)). Other tests with this allele in mice showed that this mutation not only increases hair thickness but also alters the mammary gland branching pattern, and increases the number of sweat glands (Figure 2(d)), showing the impact of

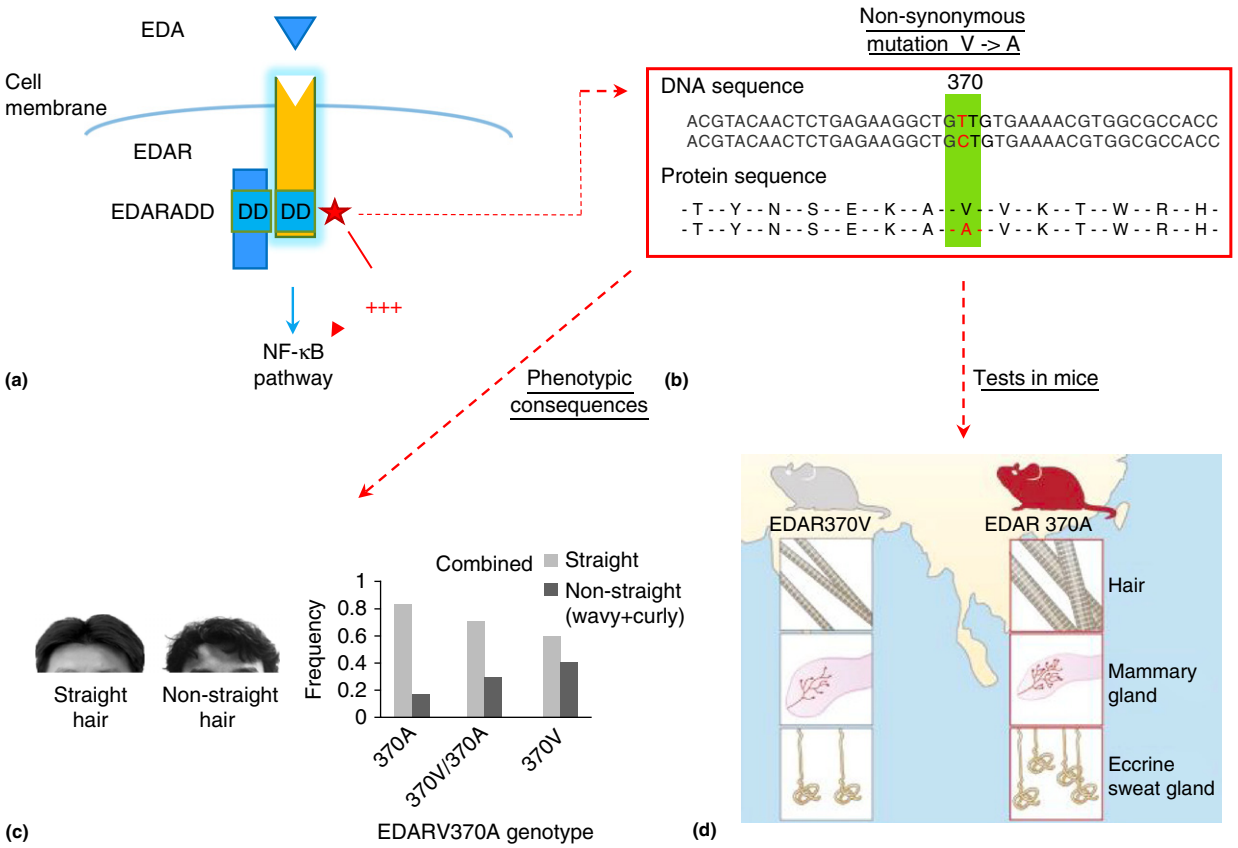

Figure 2 The Edar mutation in V370 is responsible for phenotypic changes. (a) The EDA pathway and the Edar mutation. The EDA pathway is composed by a ligand EDA, a receptor EDAR and an adaptor EDARADD. When EDA binds EDAR, the adaptor EDARADD is recruited and EDAR and EDARADD interacts by their death domains (DD) leading to the activation of the NF-κB pathway. The mutation V370 to A370 in human is located in the death domain and lead to an higher activation of the NF-κB pathway, knowing to be involved in phenotypic changes (Sadier *et al.*, 2014). (b) Genomic and protein sequence of the Edar mutation. (c) Phenotypic consequences of the 370V to 370A mutation, the A370 mutation is associated with thicker hair. The phenotype is shown on the left panel and the association between phenotypes and genotypes is indicated in the right panel. (d) Phenotypic consequences of the 370A mutation in mice, the human 70A mutation lead to thicker hair, highly branched mammary glands and more exocrine sweat glands showing the pleiotropy of changes in coding sequences.

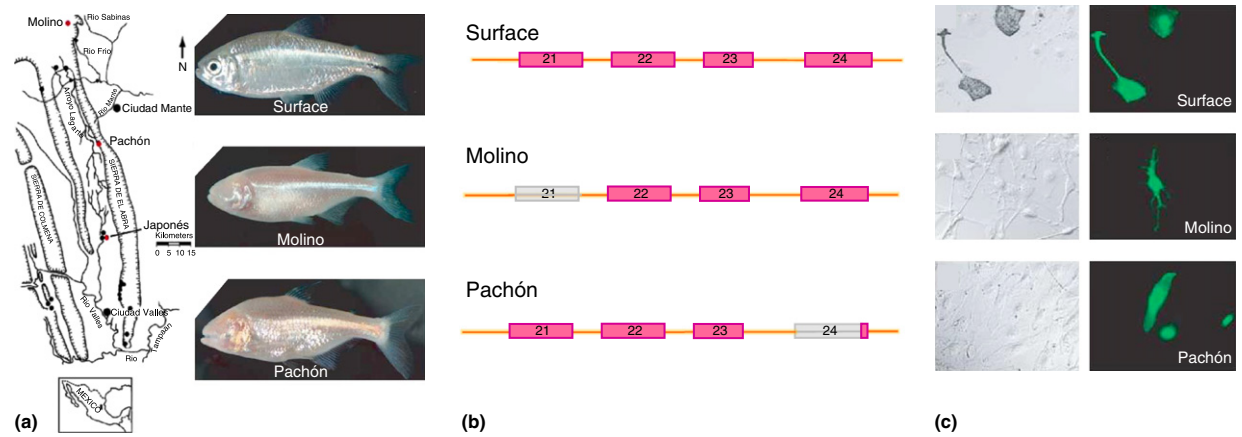

Figure 3 The *Oca2* locus is responsible for the cave fish albinism. (a): Map of the area in Mexico where the different cave populations are found. Caves with red dots are Molino and Pachón, all of which contain a majority of albino individuals. Inset map at bottom shows the location of the region within Mexico. Representative surface, Molino cavefish and Pachón cavefish are shown in right. (b) Coding changes at the origin of albinism. Organization of the last four exons of the *Oca2* gene in surface, Pachón and Molino fishes. Pachón nonsynonymous mutations are not shown as they are not implicated in albinism phenotype. (c) Complementation experiments in *Oca2*-/- melanocyte cells Surface, Molino and Pachón proteins were expressed into *Oca2*-/- melanocyte cells. Surface *Oca2* are able to rescue the pigmentation in melanocytes *Oca2*-/- cells, whereas Molino or Pachón *Oca2* protein failed. Transfection efficiency is vizualized by control with GFP in the right panel.

this mutation on various organs. This example perfectly illustrates the pleiotropic impact of mutations in developing genes as they are often involved in different processes.

The Oca2 gene in Astyanax populations and albinism

The *Oca2* gene in *Astyanax* (a freshwater characiform fish) populations provides a good example of modification of protein function by deletion of a part of a gene. *Astyanax* populations are a classic example of regression of certain traits in a particular environment. Compared to surface fishes, cave populations exhibit convergent losses of their eyes and pigmentation (**Figure 3(a)**). To identify possible loci at the origin of these losses, a genetic screen was performing by a genome-wide linkage map study between surface and cave populations (Protas *et al.*, 2006). In the two blind unpigmented cave fish populations that were studied in detail (Pachón and Molino), changes were identified in the coding sequence in *Oca2*, a gene implicated in pigmentation. In Pachón, two amino acid changes are observed (**Figure 3(b)**) as well as a deletion of a part of exon 23 and exon 24 that changes the protein sequence. In Molino, the entire exon 21 was missing. To test if these changes are at the origin of albinism, these mutations were functionally tested in a melanocyte cell line deficient in *Oca2* (**Figure 3(c)**). The results revealed that both deletions were unable to rescue the loss of pigmentation in cell lines whereas surface *Oca2* can, showing that both mutations are at the origin of albinism in *Astyanax*. This example thus shows how deletion of a coding sequence can be used repeatedly to produce a phenotype leading to parallel evolution and convergence.

Changes in *cis*-Regulatory Sequences

Regulatory sequences were discovered in 1961 by Jacob and Monod, who described the lac operon in bacteria. Soon, they pinpointed the importance of two distinct components for the proper function of all genes: the function of its product and the circumstances under which the product is produced (Wray, 2007). Thus, the discussion about potential evolutionary impact of mutations in what they called the 'operator' was introduced quickly after the discovery of CREs, even before the characterization of all types of CREs. The discovery of *Hox* genes in 1984 (reviewed in Carroll, 2008) highlighted the importance of regulatory changes in evolution as the remarkable conservation of the *Hox* coding sequence was not compatible with the diversity of body plan of vertebrate animals. Later, the spread of sequencing technology allowed a better understanding of genomes and the identification and characterization of many types of regulatory sequences.

Regulatory changes comprise all changes in regulatory regions of a gene (**Figures 1(a)** and **1(c)**), that controls where and when a gene is expressed. These changes are most often located in the promoter, enhancer, silencer, 5′UTR or 3′UTR regions of a gene, although in some cases, regions of coding sequence have been reported to act as transcription factor binding sites and thus act as CREs. Their organization around their related gene is generally modular, that is, each CRE can act more or less independently of the others. Indeed the modification of one given CRE will potentially affect gene expression only in a single developmental context through space and time. As for coding sequences, these changes could be due to a substitution of a nucleotide or an indel (i.e., insertion or deletion) of a DNA fragment. These changes could modify, add, or remove a CRE (see example below). Thus, unlike with coding sequence changes, the modification of CREs do not affect the function of the protein but its expression through space and time. In the next part, we will review some case studies of such variation to browse the role of such changes during evolution. Some other examples are given in Martin and Orgogozo (2013).

The yellow gene and the Drosophila pigmentation

This *yellow* gene is a historical example of CREs evolution using the fruit fly as a classical developmental model. Among the

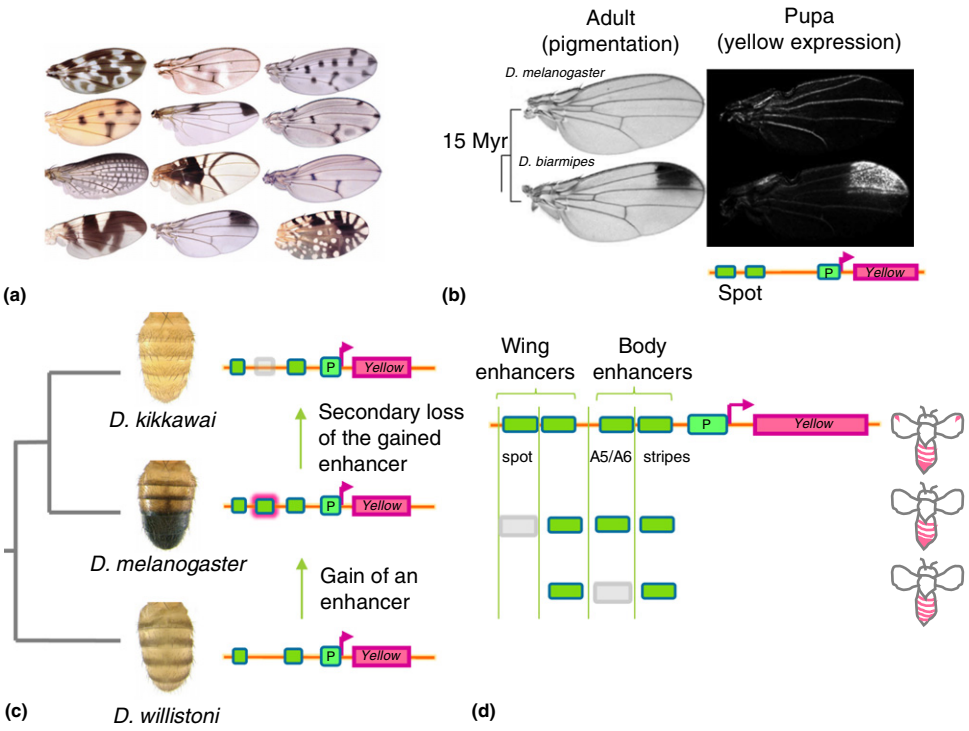

Figure 4 The evolution of the *Drosophila* pigmentation by *cis*-regulatory elements of yellow. (a) Wing pigmentation pattern diversity across higher Diptera. This plate illustrates the diversity of wing pigmentation patterns. (b) The expression of yellow prefigure wing pigmentation. The spot of dark pigmentation at the tip of the male wing of *D. biarmipes* is a new trait evolved among species of the *D. melanogaster* group superimposed on the ancestral pattern. Yellow is revealed by a specific antibody (right) in pupa. (c) Evolution of pigmentation pattern in A5/A6 segments in the *D. melanogaster* group in the *D. melanogaster* species group, the male abdominal pigmentation pattern is variable. In the ancestral situation, illustrated with *D. willistoni*, both sexes carry an identical segmental stripe pattern (green arrows). *D. melanogaster* males have evolved fully pigmented posterior segments (A5 and A6). This pattern has been secondarily lost in *D. kikkawai*. (d) The yellow locus and the resulting phenotypes. The two regions that control wing and body pigmentation are shown. In some species, the spot enhancer is not present or was secondary lost leading to the absence or loss of the wing spot. In other species, the spot enhancer is not present and the A5/A6 enhancer was secondary loss leading to unspotted and unpigmented species. The other wing enhancer as well as the one that control stripes in the body region are conserved. This example illustrates perfectly the modular organization of CREs of a gene.

well-known genes involved in pigmentation in *Drosophila melanogaster*, *yellow* is one of the most characterized. In the *D.* melanogaster species group, males of some species harbor dark spots at the anterior tip of their wings whereas those of other species have colorless wings (**Figures 4(a)** and **4(b)**; Prud'homme *et al.*, 2006). To decipher the genetic basis of these changes, immunofluorescence experiments in the spotted *D. biarmipes* and the unspotted *D. pseudoobscura* and *D. melanogaster* species were performed (Gompel *et al.*, 2005). These experiments showed that both spotted and unspotted species share a uniform low expression of *yellow* in the entire wing, except for a high expression zone in the spot of the spotted species. Because of these modifications of expression patterns, the authors concluded that a change in the sequences that control the expression of yellow occurred in spotted species, explaining this local high expression of *yellow*. The characterization of regulatory regions of *yellow* confirmed this idea: the expression of *yellow* is driven by a 800 bp long CRE in both species (**Figure 4(b)**). However, in spotted species, mutations gave rise to new binding sites for transcriptions factors that are controlling wing development, leading to the overexpression of *yellow* in spots. In conclusion, the gain of new CREs corresponding to transcription factors implicated in wing development lead to the generation of a new phenotypic pattern.

Interestingly, other studies at the *yellow* locus have revealed the importance of the modular organization of CREs for the evolution of phenotypes. Indeed, gain and loss of enhancers at this locus are also responsible for the differential pigmentation of the abdomen segment A5/A6 in different species (Jeong *et al.*, 2006). Immunofluorescence and transformation experiments demonstrate that the loss of *yellow* expression in the A5 and A6 segment of some Drosophila species is due to a disruption of another specific CRE that allows the expression of *yellow* in these particular regions. It is finally important to notice that both wing spots and abdomen pigmentation have been lost repeatedly in distinct lineages, both implicating the *yellow* locus. Thus, as for coding sequences, parallel evolution is likely to arise repeatedly for certain CREs.

In conclusion, the *yellow* locus illustrates perfectly the modularity of CRE for the expression of a gene at different places and times during development. Moreover, it shows that the modification of the expression of genes can be easily fine-tuned by the addition or removal of various enhancers or repressors elements around the gene (Prud'homme *et al.*, 2007).

Pitx1 enhancers in sticklebacks and the evolution of the pelvic region

The modification of the locus *Pitx1* in sticklebacks *Gasterosteus aculeatus* is also a case study of extreme morphological evolution. Three-spine sticklebacks have the ability to live both in freshwater and the ocean even if most of the population lives in the latter. Freshwater populations are supposed to have been isolated during the last glacial retreat in newly formed isolated lakes, thus becoming adapted to their novel environment (Bell and Foster, 1994). While ocean stickleback harbor full skeletal pelvic structures, some populations of freshwater stickleback exhibit a reduction or loss of skeletal armor (dorsal spine and pelvic girdle), a trait associated with reduced calcium and fewer large gap predators (Shapiro *et al.*, 2004; **Figure 5(a)**). To decipher the changes at the origin of these variations, the authors crossed marine and freshwater populations and mapped the responsible genetic locus. The *Pitx1* locus was shown to have the major effect of such variation. When looking at the sequence of *Pitx1* in each population, no protein coding changes were found in the freshwater one compared to the marine one showing that the casual mutation must be regulatory. Moreover, mRNA localization experiments by *in situ* hybridization (**Figure 5(b)**) showed that freshwater populations lack *Pitx1* expression in the pelvic region. Yet, the region was not fully characterized in this study, and the exact nature of the regulatory changes remained unclear. A few years later, another study carried on these experiments (Chan *et al.*, 2010). First, to avoid changes linking to a different transcription factor context, the authors crossed two freshwater populations that partially or totally lack the pelvic part of the sticklebacks and confirmed that the loss of expression is due to a modification of a CRE at the *Pitx1* region. Then, a high resolution mapping between marine and pelvic-reduced sticklebacks identified a 124 kb region of interest containing *Pitx1* and another gene. The study of the correlation of microsatellites with the absence or presence of pelvic phenotypes in natural population reduced this interval to 23 kb in the intergenic region of *Pitx1*, a region which is conserved in teleosts and may thus contain ancestral enhancers. Finally, a smaller putative 2.5 kb enhancer, *Pel* (**Figures 5(c)** and **5(d)**), cloned upstream *Pitx1* driven by a drhsp70 promoter was used in transgenic experiments to successfully restore pelvic expression in reduced pelvic fishes. The sequencing and analysis of this region in various fishes identified a 1868 bp deletion in this region, as well as other independent deletions of this locus in various independent freshwater populations of sticklebacks.

As a conclusion, this example clearly shows how a deletion of a CRE can lead to morphological changes and can also be selected repeatedly. It also perfectly shows the challenges involved in fully characterizing a CRE and the need to perform multiple experiments to decipher such changes in a natural population. Finally, this case highlights how the fine-tuning of CRE can produce different levels of expression leading to a

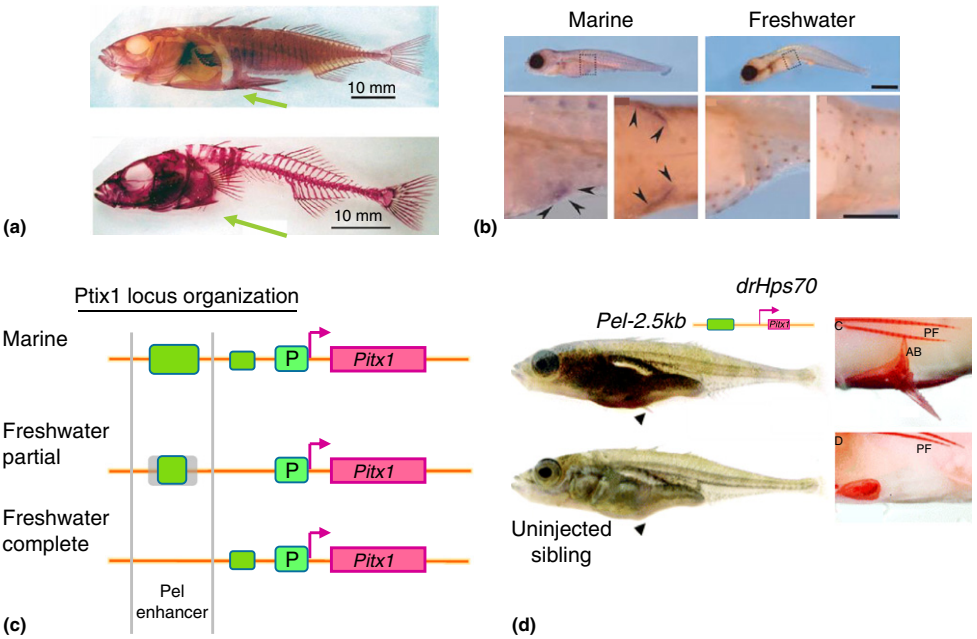

Figure 5 The loss of a Pitx1 enhancer in sticklebacks is responsible for loss of pelvic structures. (a) Three morph of three-spine sticklebacks. Marine population exhibit a complete morph whereas freshwater populations have a partial or complete reduction of this structure indicated with a green arrow. (b) *In situ* hybridization of the *Pitx1* gene in marine and Paxton freshwater sticklebacks, in marine sticklebacks, *Pitx1* gene is expressed in the pelvic region whereas no expression is detected for the Paxton freshwater stickleback. (c) Schematic region of the *Pitx1* locus Marine populations possess the enhancer that drive *pitx1* expression in the pelvic region whereas freshwater populations have partial or complete deletions of this enhancer leading to partial or complete reduction of the pelvic region. Others enhancers that drive *pitx1* expression in at different space and time during development are conserved in the two populations. (d) The Pel-2.5 kb that contains the enhancer is sufficient to rescue pelvic structure in freshwater population Juvenile pelvic-reduced stickleback expressing a *Pitx1* transgene driven by the Pel-2.5-kbSALR enhancer is compared with uninjected sibling. External spines form only in transgenic fish (arrowhead). Right panel: alizarin red-stained pelvic structures of adult transgenic fish compared with parental phenotype.

variation in phenotypes. Another interesting case of extreme morphological change was also observed in sticklebacks with the EDA pathway (see coding sequence, Colosimo *et al.*, 2005) that also exhibit a reduction of armor plate in a specific environment. The mutation is not yet identified but is thought to be linked to *cis*-regulatory mutation.

The Primacy of Coding versus CRE in the Generation of Novelties

The First Debate

Around 2006–07, as the evo-devo field started to be well established, many researchers tried to build a synthesis of what evo-devo was and what the important questions of the field were. Among them, the question about the nature of the genetic changes at the origin of evolutionary changes was central and debated (this subject was intensively reviewed in the following papers: Wray, 2007; Hoekstra and Coyne, 2007; Prud'homme *et al.*, 2007; Carroll, 2008; Stern and Orgogozo, 2008). Following the discovery of *cis*-regulatory sequences, many researchers argued that they represented most of the variation at the origin of evolutionary novelties. First, as transcription is viewed as a dynamic process that gives rise to different expression levels of a gene, it can be fine-tuned depending on the external context with less deleterious effects on the organism, whereas changes in coding sequences are often pleiotropic and could have dramatic effects. Second, the organization of CRE around a gene is modular and thus, the expression level of a gene can be modified at one point in space and time while the essential functions are maintained. Of course, it allows the evolution of a specific part without modifying the other parts. Third, the mutations in CRE are often codominant, as suggested by allele-specific studies of transcript abundancy. On the contrary, mutations in coding sequences that are often recessive and thus are supposed to play a lesser role as natural selection generally operate far more efficiently on codominant mutations. Four, developmental genes are often pleiotropic and share different roles at different spaces and times during development. Thus, a mutation that could give an advantage for one organ can be deleterious for another.

Some argued against this idea, stating that there is no valid reason to assume a preponderance of evolutionary relevant mutations in any particular part of the genome (Coyne and Hoekstra, 2007; Hoekstra and Coyne, 2007) and that some forms of protein evolution can avoid the negative consequences of pleiotropy, for example, by gene duplication. To reply to that comment, some authors have tried to estimate the impact of both types of changes on morphological evolution with their actual data and make predictions about their relative importance (Stern and Orgogozo, 2008), raising the idea of the importance of considering population and developmental studies simultaneously. They found that both types of mutations are important and are at the origin of morphological evolution but that the CREs and coding changes explain variations at different frequencies. More precisely, above the species level, the authors found that changes implicated in morphological variation are more common in CREs than in coding sequences. Thanks to the recent development of genomics, the debate has grown broader in scope.

The Coding and CRE Changes in the Actual View of Genotype/Phenotype Relationship

The question of the primacy of coding versus CRE changes in developmental evolution is part of the bigger question of the relationship between genotype and phenotype. In the last 10 years, the development of new generation sequencing, including transcriptomics, and modelization lead us to rethink our view about genotype/phenotype relationship toward an integrative view including all levels of variation such as the transcriptomic variation, the effect of population size or the influence of environment on the penetrance of a phenotype (Martin and Orgogozo, 2013; Orgogozo *et al.*, 2015). Moreover, some changes are at the frontier between coding and CRE changes and thus, are difficult to place in this debate. For example, during gene or genome duplication, both coding and regulatory region are duplicated and can then evolve differentially from the original copy and be sources of variation. The most famous case in evo-devo is of course the *Hox* genes that are present in four clusters in vertebrates, coming from a single locus before rounds of duplication. Another example is the mutations that arise in the coding sequence of transcription factors that are coding changes but that, due to the regulatory function for the expression of genes, lead to changes in the expression of genes such as CRE mutations. The impact of such changes is discussed in (Cheatle Jarvela *et al.*, 2014) who highlight that coding sequence changes in transcription factors can themselves be modular and affect only certain tissues. One reason for this is the generation of alternative isoforms that can lead to multiple versions of a gene depending on the cellular context. More broadly, many developmental genes present different isoforms (reviewed in Kalsotra and Cooper, 2011; Kelemen *et al.*, 2012) enhancing the evolutionary potential. These changes are both coding and CRE changes as they modify the protein while representing changes in gene expression. Some examples were already shown to have phenotypical implications, such as the *Edaradd* gene in mammals (Sadier *et al.*, 2015) or the BRANCHED1a gene in potatoes of which a new isoform controls the potato plant's architecture (Nicolas *et al.*, 2015).

Although the debate about the primacy of coding versus CREs change in morphological evolution remains, more and more studies are emerging view this question as a reductionist approach while recognizing its interest to identify such changes (Orgogozo *et al.*, 2015). As both coding and CREs are important players in morphological evolution, future syntheses would benefit from integrating all levels of interaction between developmental genes and the multiple environmental variables that interact to create phenotype.

See also: Developmental-Genetic Toolkit for Evolutionary Developmental Biology. Modularity and Integration in Evo-Devo

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Reinforcement

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Glossary

Allopatry Describes two organisms or species living or growing in two separate geographic areas.
Post-zygotic reproductive isolation A barrier to reproduction that occurs after the formation of a zygote; encompasses hybrid inviability and hybrid sterility.
Pre-zygotic reproductive isolation A barrier to reproduction that occurs prior to the formation of a zygote; encompasses habitat, behavioral, temporal, and mechanical isolation.
Reinforcement The process by which natural selection favors the increase in reproductive isolation (RI) in order to decrease costly hybridization and mating.
Reproductive isolating barrier A mechanism that impedes or prevents the interbreeding of two populations or species.
Sympatry Describes two organisms or species living or growing in the same geographic area.

Introduction

Reinforcement is the process by which natural selection favors the increase in reproductive isolation (RI) in order to decrease costly hybridization and mating. This is one of the important ways in which natural selection can contribute to the process of species formation. Reinforcement involves selection favoring a trait because it causes reproductive isolation. This process is in contrast with the more commonly invoked role of natural selection favoring locally adapted traits that, as a by-product of their primary selected function, happen to cause RI. Reinforcement is often thought of as a multi-step process, which starts with a period of allopatric divergence between taxa allowing for the accumulation of post-zygotic RI. When the two diverging taxa come into secondary contact in sympatry, hybrid mating is either costly, or it produces maladapted or sterile hybrids. This cost to hybridization creates selection for traits that decrease mating between the two taxa.

The History of Reinforcement

The conceptual roots of the theory of reinforcement can be traced back to Alfred R. Wallace and his efforts to explain the role of natural selection in the process of species formation (Wallace, 1889). In fact, historically the process was referred to as the 'Wallace Effect.' Wallace argued that selection should favor the increase of RI in its own right in order to prevent the swamping effects of intercrossing between species.

> It will occur to many persons that, as the infertility or sterility of incipient species would be useful to them when occupying the same or adjacent areas, by neutralizing the effects of intercrossing, this infertility might have been increased by the action of natural selection (Wallace, 1889, p. 173).

Although Wallace's general hypothesis that selection can favor reproductive isolation has been proven correct, the details in the argument have been improved and clarified in the ensuing decades. Specifically, it is now known that reinforcement favors RI mechanisms that act prior to the selective cost of hybridization, i.e., via pre-zygotic RI mechanisms.

During the Modern Synthesis, the notion that selection can favor an increase in RI simply to stop costly hybridization regained popularity. Theodosius Dobzhansky championed this hypothesis and argued for its importance during the process of speciation.

> Where hybridization jeopardizes the integrity of two or more adaptive complexes, genetic factors which would decrease the frequency or prevent the interbreeding would thereby acquire a positive selective value, even though these factors by themselves might be neutral (Dobzhansky, 1940).

In the 1950s, Frank Blair, who studied a hybrid zone of two narrow-mouthed toad species in central Texas, coined the term 'reinforcement.' He observed that these two species had more divergent mating songs in sympatric populations than in allopatric populations and hypothesized that this reinforced species boundaries (Blair, 1955). Blair suggested,

> The argument for reinforcement of isolation mechanisms through selection against hybridization is premised on the hybrids being at a disadvantage in competition with the parental types (Blair, 1955, p. 479).

Since the Modern Synthesis there have been countless studies, across the tree of life, identifying reproductive isolating barriers that likely evolved due to reinforcement. For example, reinforcement caused divergence of plumage color in sympatric populations of the European black and white *Ficedula* flycatchers (Saetre *et al.*, 1997). In allopatric populations, males of the two species have the same plumage color but in sympatric populations their plumage differs. Females in sympatry prefer their conspecific male plumage color and consequently there is less hybridization between species. A wealth of studies on insects has found evidence of reinforcement as well. In particular, *Drosophila* species from across the globe show evidence of greater RI in sympatry than in allopatry. In Australia, *Drosophila* species produce different

sexual pheromones in allopatric and sympatric populations (Higgie *et al.*, 2000). On the African island of São Tomé, sympatric *Drosophila* species have higher post-mating pre-zygotic reproductive isolation than allopatric individuals (Matute, 2010). And, in California, individuals from sympatric populations of *Drosophila* species show stronger conspecific mate preference than individuals from allopatric populations (Noor, 1999). Mammals have also diverged due to reinforcement. Two subspecies of the house mouse hybridize along a thin contact zone from Denmark through the Czech Republic. Mate-choice tests demonstrate that sympatric males prefer conspecifics and mate assortatively at higher rates than do allopatric males (Smadja and Ganem, 2005). This enhanced RI is likely caused by divergence of pheromone production in sympatry. Although less exhaustively studied than animals, plants also show evidence of reinforcement. For example, one of two co-occurring species of *Areneria* in the South Eastern United States has evolved high rates of autonomous self-fertilization, which has consequently decreased heterospecific pollen transfer (Fishman and Wyatt, 1999). The preponderance of studies suggests that reinforcement is widespread and that it is an important mechanism driving the evolution of RI during the process of speciation.

From Pattern to Process – Criteria for Reinforcement

The pattern of greater pre-zygotic RI in sympatry than in allopatry is often associated with reinforcement and therefore has been used to help identify possible examples of the process in nature. Of course, other evolutionary forces, such as local adaptation, can cause similar patterns of divergence so it is important to empirically demonstrate that the pattern is a result of the process. **Table 1** describes four criteria for showing that divergence is caused by reinforcement.

Each of these criteria addresses an important aspect of the reinforcement hypothesis, but rarely are all four investigated. Thoroughly investigating reinforcement is difficult in many systems because hybridization rates are hard to quantify, lifetime fitness is not always possible to assess, and isolating the effect of a single trait on reproductive isolation is challenging. Reinforcement in Texas *Phlox* represents one case study in which all criteria have been met (see **Figure 1**). *Phlox drummondii* has light-blue flower color throughout most of its range; but where it overlaps with the sister species *Phlox cuspidata*, which also has light-blue flower color, *P. drummondii* has dark-red flower color. The pattern of similar flower color in allopatry and different flower color in sympatry is consistent with reinforcement driving flower color divergence (Levin, 1985). A series of studies have investigated the criteria listed in **Table 1** and support the reinforcement hypothesis. Criterion 1 was investigated by performing crosses between the two *Phlox* species and determining that hybrid offspring are largely sterile (Ruane and Donohue, 2008). Multiple experiments have demonstrated that the evolution of *P. drummondii* flower color from ancestral light-blue to dark-red in sympatry decreases hybridization, thus satisfying criterion 2. Specifically, a common garden field experiment including both species of *Phlox* directly measured hybridization under natural conditions and found that dark-red flowered individuals hybridized 50% less than light-blue flowered individuals (Hopkins and Rausher, 2012). Furthermore, pollinator observation experiments showed that butterfly pollinators move pollen less frequently between the two species of *Phlox* when they have different colors than when they have the same color (Hopkins and Rausher, 2012). Results from common garden field experiments, pollinator observations, and population genetic modeling demonstrate that the benefit of reduced hybridization caused by having a dark-red flower color in sympatry outweighs the cost associated with this trait (Hopkins and Rausher, 2014; Hopkins *et al.*, 2014; Hopkins and Rausher, 2012). These studies address criterion 3 by showing that net selection favors the dark-red flower color in sympatry because of its role in decreasing hybridization. Criterion 4 was also tested using the common garden field experiment: other selective pressures that might effect fruit production or survival were quantified and shown to be insignificant (Hopkins and Rausher, 2012).

It remains difficult to prove that reinforcement is the cause of divergence in many species. Nonetheless, additional interesting questions involving the causes and consequences of reinforcement are beginning to be addressed. For example: Does divergence in sympatry cause reproductive isolation with conspecifics in allopatric populations? Is there a cost to reinforcement or does natural selection, or sexual selection, also favor traits under reinforcing selection? What is the genetic basis of reinforcement? Why is divergence due to reinforcement often asymmetric with only one sympatric species showing trait value changes?

Table 1 Criteria for demonstrating that reinforcement is responsible for trait divergence in sympatry

1. Hybridization has a selective cost
   When reinforcement drives the evolution of reproductive isolation, the selective mechanism is the cost of hybridization. Therefore it must be demonstrated that hybridization or hybrid mating is costly.
2. Trait divergence in sympatry decreases hybridization
   Not all divergence in sympatry causes an increase in reproductive isolation; It must be demonstrated that a novel phenotype actually decreases hybridization or mating between sympatric taxa.
3. The decrease in hybridization increases net fitness of individuals with the derived trait
   If reinforcement drives the evolution of trait divergence then individuals in sympatry with the derived trait should have a fitness advantage because of the reduction in hybridization. It should be shown that this reduction in hybridization outweighs any pleiotropic costs.
4. Other evolutionary processes do not explain divergence
   Evolutionary processes such as local adaptation to environmental variation and ecological character displacement can also cause patterns of divergence between allopatric and sympatric populations. It is therefore important to test alternative hypotheses about the mechanism of selection.

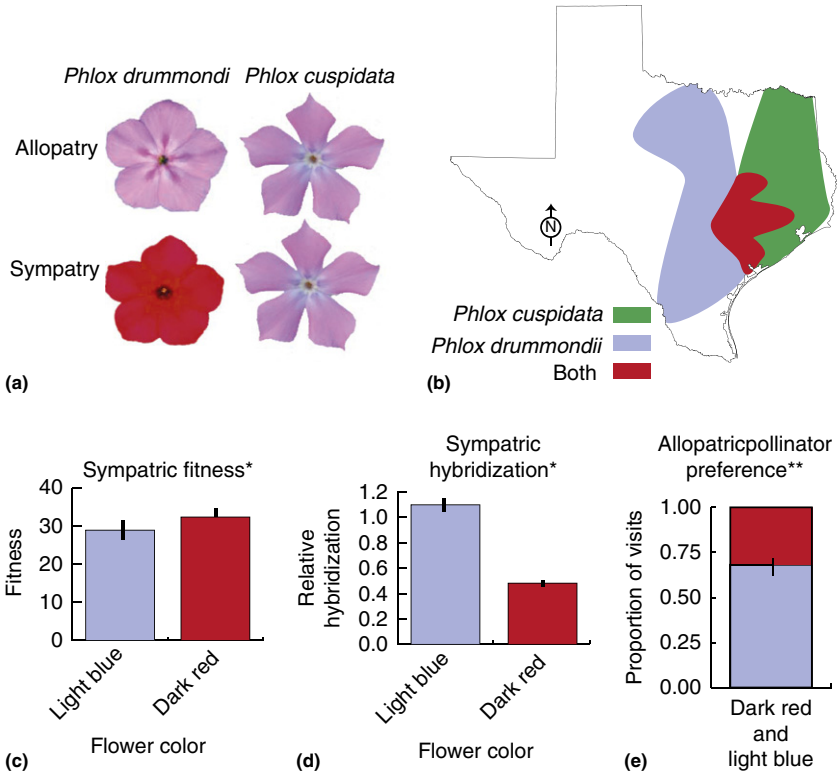

Figure 1 Case study of reinforcement in *Phlox drummondii*. (a) Pictures of *P. drummondii* and *P. cuspidata* flowers from allopatric and sympatric populations. Note the color divergence of sympatric *P. drummondii* flowers. (b) Schematic diagram of *Phlox* range in Texas showing sympatric area (in red) where *P. drummondii* has dark-red flower color. (c) *P. drummondii* fitness estimates from common garden field experiment in the sympatry showing no significant difference between dark-red and light-blue flowered individuals. (d) Relative hybridization rates of dark-red and light-blue flowered *P. drummondii* individuals with *P. cuspidata* showing the advantage of being dark-red in sympatric populations. (e) Pollinator preference for light-blue over dark-red *P. drummondii* flowers in allopatry showing a selective advantage for the ancestral light-blue flower color. * Indicates data derived from Hopkins and Rausher (2012). ** Indicates data derived from Hopkins and Rausher (2014).

Macro-Evolutionary Patterns

Scientists have been investigating reinforcement on a case-by-case basis in order to learn how, when, and why selection favors reproductive isolation. Despite the abundance of research, this strategy gives only a vague notion of the prevalence or importance of reinforcement during the process of speciation. Therefore, researchers have used comparative methods to look for macro-evolutionary patterns of divergence that might suggest the prevalence of reinforcement.

The rich history of empirical investigations of RI in *Drosophila* provided the first comprehensive dataset to evaluate the general importance of reinforcement in speciation. Coyne and Orr used a comparative approach based on the hypothesis that RI between taxa increases as genetic divergence between taxa increases (Coyne and Orr, 1989, 1997). Allopatric taxa are expected to evolve pre-zygotic and post-zygotic RI at equivalent rates with increasing genetic distance, but if reinforcement is acting, sympatric taxa are predicted to have a faster rate of pre-zygotic RI evolution than post-zygotic RI evolution with increasing genetic distance. These studies found strong evidence that pre-zygotic RI evolved faster than post-zygotic RI with increasing genetic distance in sympatric taxa but not in allopatric taxa, and therefore concluded that reinforcement contributed to speciation in *Drosophila*. Most subsequent comparative studies have also found evidence supporting the importance of reinforcement during speciation (Yukilevich, 2012; Grossenbacher and Whittall, 2011; Howard, 1993; Noor, 1997). In general, these studies find evidence of enhanced pre-zygotic RI in sympatric species that likely suffer from costly hybridization.

In the future, these large datasets used to understand the prevalence of reinforcement can also be used to investigate why and how reinforcement occurs. Are there particular groups of organisms more likely to diverge due to reinforcement than other organisms? Does reinforcement affect diversification or extinction rates? Are there particular types of traits that tend to respond to reinforcing selection? There is a wealth of knowledge to be gained from future macro-evolutionary studies of reinforcement.

Reproductive Isolation with Gene Flow

Despite the abundance of studies that suggest the prevalence of reinforcement, the feasibility of the process has been hotly debated. Reinforcement requires RI to evolve between two species that are still hybridizing. Hybridization between species can result in gene flow between the diverging species. Gene flow homogenizes differentiation and thus hybridization

can impede divergence and prevent reinforcement from evolving. Gene flow could cause new alleles conferring increased RI that arise in one species to recombine into the other species. Under this scenario, gene flow breaks down linkage disequilibrium between the alleles causing post-zygotic RI and the new alleles causing pre-zygotic RI. For example, in the *Phlox* system, gene flow could have recombined the derived alleles causing dark-red flower color in *P. drummondii* into *P. cuspidata*. If this had occurred, the two species would, once again, share the same flower color and hybridize.

The idea that gene flow would homogenize differentiation in sympatry led to the hypothesis that reinforcement could not evolve, was very rare, or only occurred if gene flow did not exist between the diverging species (Butlin, 1987). This belief sparked extensive theoretical work investigating the evolutionary conditions under which reinforcement can evolve (reviewed in Howard, 1993; Servedio and Noor, 2003; Kirkpatrick and Ravigne, 2002). The general finding from this body of work is that reproductive isolation can actually evolve in sympatry. Selection does have to be strong compared to gene flow and direct selection due to an ecological advantage can significantly aid the evolution of reinforcement. This theoretical work should stimulate empirical work that actually measures the strength of reinforcing selection and tests the model's predictions.

The Genetics of Reinforcement

The dilemma of reproductive isolation evolving in sympatry has also sparked theoretical work investigating the genetic basis of reinforcement. Specifically, it has been shown that the genetic architecture of the reproductive isolating mechanism can influence the success of reinforcing selection. If a mutant allele increases RI when it occurs in either or both of the two sympatric species, then reinforcement is more likely to be successful. This is referred to as a one-allele mechanism (Felsenstein, 1981). For example, an allele that increases 'choosiness' or reduces dispersal could cause both sympatric species to mate more frequently with conspecifics than heterospecifics. Despite much hypothesizing about the types of traits that could be one-allele mechanisms of reinforcement, it has only actually been identified once. *Drosophila pseudoobscura* individuals that co-occur with *Drosophila persimilis* show a greater reluctance to hybridize than individuals from allopatric populations. The sympatric allele at one locus, *Coy-2*, that increases assortative mating in *D. pseudoobscura*, has also been shown to increase assortative mating when it is occurring in *D. persimilis* as well (Ortiz-Barrientos and Noor, 2005). In other words, the same allele in either species will cause a decrease in hybridization. The one-allele mechanism overcomes the problems associated with gene flow because recombination of the derived assortative mating allele into the sympatric species will increase reproductive isolation instead of decrease it.

Besides some work on genetic architecture of trait variation caused by reinforcement, little is known about the genetics of reinforcement. In fact, *Phlox* is the only system for which the genes involved in reinforcement have been identified. Flower color variation in *P. drummondii* is caused by expression variation in two genes known to be involved in pigment production in flowering plants. Yet even in this case, we know little about the evolutionary history of these genes. Did a single mutation arise once and spread or are there multiple mutations causing the same changes in flower color? Did the alleles causing flower color variation arise under selection or were they segregating in the population prior to the two *Phlox* species hybridizing? Clearly, much more research is needed before we can determine if there are particular genetic or genomic patterns associated with reinforcement.

Future Directions

Unlike some other areas of speciation research, there is a rich history of both theoretical and empirical studies on reinforcement using many species as models. Many of these studies focus on determining whether reinforcement exists. Although it is still important to determine if divergence is driven by reinforcing selection, a growing amount of work is beginning to investigate more nuanced questions about how and why reinforcement occurs.

In order to understand how reinforcement occurs we must learn more about the genetic basis of trait variation caused by the process: What are the genes? What type of mutations? Are the mutations from standing genetic variation or novel mutations?

In order to understand why reinforcement occurs we must learn more about strength of the evolutionary forces acting during the process: How strong is reinforcing selection? How much gene flow is there between sympatric species? How is the cost of hybridization distributed across sympatric species?

Additionally, for many systems it is difficult to identify reinforcement, so determining if there is a genomic signature of reinforcement may aid in our understanding of the prevalence of this process. Answers to these questions will bring us much closer to understanding the role of natural selection in the origin of species.

See also: Reproductive Isolation, Postzygotic. Speciation Continuum. Species Concepts and Speciation

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Reproductive Isolation, Postzygotic

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Glossary

Allopatric speciation Form of speciation, wherein new biological species arise while inhabiting different geographical regions.
Epistasis An interaction between two or more genes.
Gene flow The free movement of genes (or alleles) between populations.
Gene transposition The movement of a gene from one position in the genome to another position in the genome, either on the same or different chromosome.
Polyploidization Whole genome duplication.
Reproductive isolation When individuals from two separate populations are unable to mate and/or produce fit offspring.
Speciation The evolutionary process wherein two separated populations are reproductively isolated from each other and are thus new biological species.
Sympatric speciation Form of speciation, wherein new biological species arise while inhabiting the same geographical region.
X-linked gene Genes located on the X chromosome.

The accumulation of genomic differences between diverging species can cause those genomes to become incompatible with one another. This can eventually lead to the termination of gene flow between those species when their hybridization yields only sterile or inviable offspring (Haldane, 1922; Coyne and Orr, 1989). This evolutionary phenomenon, referred to as postzygotic isolation, is a widespread and severe barrier to interbreeding, which, in the context of speciation, enables diverging species to form and subsequently become maintained as distinct reproductive communities. The prevalence of postzygotic isolation among sexual organisms is owed to the observation that any two species' genomes that evolve independently of one another will, given enough time, acquire genetic changes that interact unfavorably with each other within a hybrid background, causing the hybrids to be dysfunctional in some way (Coyne and Orr, 1997). If these dysfunctions are severe enough, the hybrids are among the first and last progeny for any prospective fusion of evolutionary lineages, allowing the persistence of each species as a unique and distinct type.

Hybrid dysfunction is mediated through either extrinsically and/or intrinsically manifested barriers. For extrinsic barriers, an incompatible genome × environment interaction occurs as a result of hybrids being phenotypically intermediate between divergent ecological optima (Grant and Grant, 1992; Hatfield and Schluter, 1999). Hybrids are competitively unsuited to either parental niche, and comparatively inferior to either parental species' pure offspring. These individuals are then selected against naturally and/or sexually and are eventually removed from the population. For example, among benthic and limnetic forms of stickleback fish, hybrids grow slower in a natural environment than in a lab environment, indicating that extrinsic factors (i.e., resource distribution), not intrinsic factors, are hindering their development in a way that does not seem to affect either parental species (Hatfield and Schluter, 1999, but see Rundle and Whitlock, 2001).

For intrinsic barriers, an incompatible genome × genome interaction results in a failure to form a fully viable and fertile individual (Forejt and Ivanyi, 1974). Male sterility has been observed to occur due to failures during spermatogenesis (e.g., no sperm, aberrant sperm head morphology, immotile sperm), abnormal testes development, failure to transfer sperm, or failure of sperm to survive within a female's reproductive tract (Koref-Santabañez, 1963; Forejt and Ivanyi, 1974; Kulathinal and Singh, 1998; Good *et al.*, 2008; Civetta and Gaudreau, 2015). Female sterility arises as failure of sperm to survive within the reproductive tract of hybrids, and failures in germ-line development (Engels and Preston, 1978; Santibañez *et al.*, 2009). Hybrid dysfunction resulting in inviability has been demonstrated to occur due to cellular errors in mitosis (Sawamura, 1997) and nuclear transport (Tang and Presgraves, 2009). There are certainly additional mechanisms of hybrid sterility and inviability beyond those listed above. The abundance of essential stepwise interactions during reproduction and development ensures that some form of hybrid dysfunction is bound to emerge over time as a consequence of structural (genomic) and/or functional (genic) evolutionary divergence (**Figure 1**).

Structural Genomic Variation and Postzygotic Isolation

From a structural (genomic) perspective, the rearrangement, transposition, and polyploidization of chromosomes are known to be integral players in postzygotic isolation. Indeed, genomic rearrangements are often predictive of reproductive isolation – the more heterokaryotic, the higher the degree of reproductive isolation (Basset *et al.*, 2008; Charron *et al.*, 2014). Chromosomal rearrangements, specifically inversions (inverted segments of the genome), may potentially play a pivotal role in speciation, especially when inversions segregate between a species pair (**Figure 2**). Inversions, such as those that differ between *Drosophila pseudoobscura pseudoobscura* and *Drosophila persimilis*, have been demonstrated to harbor loci for hybrid sterility (Noor *et al.*, 2001a,b). Inverted regions containing genes whose alleles give rise to hybrid sterility may persist due to linkage between the hybrid sterility allele(s) in

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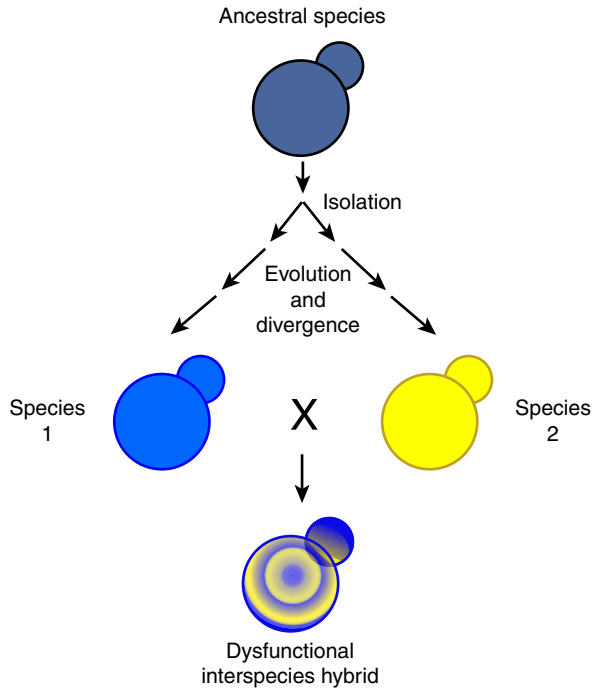

Figure 1 Isolated populations evolve and diverge from one another, eventually forming new species. When these species interbreed, the resulting hybrid offspring is dysfunctional due to divergent loci coming together within a single individual.

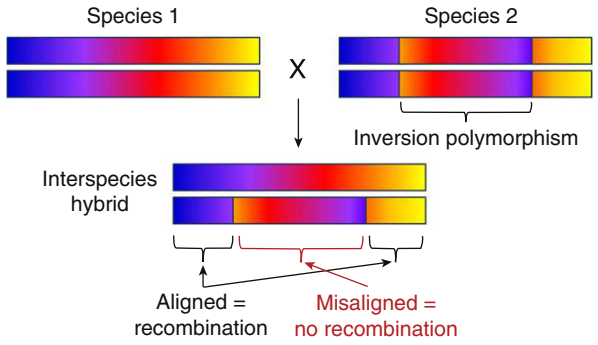

Figure 2 Inversion polymorphisms between species can prevent gene flow for genes that are within the inversion. Regions of the genome that are in the same order (e.g., blue and yellow on ends) can undergo recombination, shuffling the genomes of the two species in subsequent generations, allowing the alleles of one species to potentially be crossed back into the genome of the other species. The inverted region in the middle does not recombine, causing those alleles to be inherited only as a single unit.

either parental genetic background (Noor *et al.*, 2001b). As such, gene flow may occur between the species for those loci that fall within non-inverted regions, allowing selection to purge out any genes that confer hybrid sterility. Conversely, hybrid sterility genes within the inverted regions cannot be purged and persist. Inversions are also capable of triggering adaptive divergence and preventing lineage-defining polymorphisms from being separated by recombination – evolutionary processes that are both potentially capable of snowballing into eventual postzygotic isolation (Kirkpatrick and Barton, 2006; Noor *et al.*, 2001b). Lastly, the inversions themselves can induce meiotic errors, either disrupting or inducing inappropriate recombination leading to hybrid sterility and inviability (Noor *et al.*, 2001a; Zanders *et al.*, 2014). While these models have been shown to influence the speciation process in some species pairs, they do not apply to species that are homosequential and contain the same gene order.

In addition to within-chromosome rearrangements like inversions, other genome modifications have also been demonstrated to affect postzygotic isolation. One such mechanism is gene transposition. For example, the *JYalpha* gene is ancestrally located on the fourth chromosome in the *Drosophila melanogaster* subgroup, but has transposed (moved) to the third chromosome in *Drosophila simulans* (Masly *et al.*, 2006). Later-generation hybrids between *D. melanogaster* and *D. simulans* that possess a homozygous *D. melanogaster* third chromosome and a homozygous *D. simulans* fourth chromosome completely lack a copy of the *JYalpha* gene. These hybrid males produce mostly immotile sperm, suggesting that *JYalpha* influences a hybrid's fitness (Masly *et al.*, 2006).

Another path to postzygotic isolation is whole genome duplication (a.k.a. polyploidization), which is a frequently-occurring phenomenon in plants. These duplication events are capable of forming new species within a single generation (Johnston, 1980; Haig, 1991). This is due to the immediate presence of postzygotic isolation: diploid ($2n$) plants that undergo a genome duplication event (to tetraploid, $4n$) produce only inviable triploid seeds with their diploid ancestor, a phenomenon known as the 'triploid block' (Marks, 1966). The triploid block acts through abnormal development of the endosperm, a specialized ephemeral tissue that supports the developing embryo (Ramsey and Schemske, 1998; Li and Berger, 2012). The endosperm, a product of plant sperm cells (haploid) fusing with the central cell (diploid), ends up with a 2:1 maternal to paternal genome ratio, and any deviations from this ratio (e.g., through interploidy hybridization; Johnston *et al.*, 1980) affects endosperm development. A skew toward an increase in the maternal genome size inhibits the proliferation of the endosperm (Haig and Westoby, 1991; Scott *et al.*, 1998), whereas an increase in the paternal genome results in endosperm excess. This effect suggests that not only is the endosperm sensitive to ploidy imbalance, but the effect of the imbalance is dosage dependent and based on the inherited parental genome. Similarly, imprinted genes can play a role in the dosage-sensitivity of the endosperm, as seen with the paternally expressed *ADM* gene that causes seed abortion when over-expressed in triploid *Arabidopsis* seeds (Kradolfer *et al.*, 2013).

Genome duplication can also lead to postzygotic isolation through the presence of the duplicate copies (paralogs) of every gene in the genome. Either the original or duplicate copy of a gene often becomes nonfunctional, sub-functional, or acquires a new function. In the context of speciation, this can lead to postzygotic isolation if this process differs between two populations (reviewed in Sweigart and Willis, 2012). In the case of two strains of *Arabidopsis thaliana*, early embryo death is seen when there is a loss of function at duplicated copies of the histidinol-phosphate amino transferase genes, *HPA1* and *HPA2* (Bikard *et al.*, 2009). Similarly, within two subspecies of

rice, *Oryza sativa indica* and *Oryza sativa japonica*, pollen from their hybrids cannot germinate as a function of lost alleles for the duplicated genes, *DOPPELGANGER1* (*DPL1*) and *DOPPELGANGER2* (*DPL2*; Mizuta *et al.*, 2010).

Allelic Divergence, Genomic Conflict, and Postzygotic Isolation

From a genic perspective, hybrid dysfunction likely arises due to a genomic conflict that asymmetrically arose (and resolved) in one of the host species genomes but not the other (Hutter and Ashburner, 1987; Coyne and Orr, 2004). When these two genomes come into contact, the underlying conflict is uncovered, resulting in hybrid dysfunction. Thus, hybrid dysfunction arises due to negative interactions between two (or more) divergent genetic loci. If interspecies hybrids do survive and reproduce, it is often the case that hidden recessive incompatibilities will be increasingly unmasked, resulting in the eventual breakdown of the hybrid population. For example, the viability of hybridized cichlid fish degenerates after each generation with the fitness of first filial (F_1) hybrids, decreasing by 43%, and the fitness of F_2 hybrids decreasing by an additional 21% (Stelkens *et al.*, 2015).

Using traditional mapping techniques, several genes have been uncovered that contribute – to some degree – to hybrid dysfunction. Most of the sterility genes have been identified within *Drosophila* (reviewed in Presgraves, 2010). The first hybrid sterility gene to be identified was *Odysseus* (*Ods*), an X-chromosome gene that acts recessively to contribute to the sterility of later-generation male offspring between *D. simulans* and *Drosophila mauritiana* (Perez *et al.*, 1993; Sun *et al.*, 2004). The genetic interactors for *Ods* have yet to be identified, but it is known that its autosomal interactor(s) must also act recessively, and epistatic interactions with other nearby X-chromosome genes are required in order to fully induce hybrid sterility (Perez and Wu, 1995), indicating that the underlying mechanism by which this gene gives rise to sterility is complex.

The X-linked gene *Overdrive* (*Ovd*) was shown to cause both sterility and segregation distortion in F_1 hybrid males from *Drosophila pseudoobscura bogota* females mated to *D. p. pseudoobscura* males, suggesting a possible linked genetic basis between the two processes (Phadnis and Orr, 2009). Since this species pair is young in its divergence time, it can provide insight into the early stages of speciation since it is unlikely to have accumulated a high density of genetic incompatibilities – a dilemma that researchers face in many older species (Matute *et al.*, 2010). It is known that *Ovd* has undergone rapid evolution (Phadnis and Orr, 2009); however, the genes that interact with *Ovd* to give rise to both hybrid sterility, as well as segregation distortion, have yet to be determined. Additional mapping has been employed to identify regions along the X chromosome and autosomes that may interact with *Ovd* to induce hybrid sterility and segregation distortion in this species pair (Phadnis, 2011).

Sterile hybrid males produced from subspecies, *Mus musculus musculus* and *Mus musculus domesticus* have smaller testes and no sperm produced. *Hybrid sterility 1* (*Hst1*), located on chromosome 17, was identified as the region responsible for this spermatogenic failure (Forejt and Ivanyi, 1974; Good *et al.*, 2008). More than three decades later, the *Hst1* region was fine-mapped to the single locus *Prdm9*, which was found to be expressed in both the ovaries and testes of mice (Mihola *et al.*, 2009). *Prdm9* encodes a histone 3 lysine 4 (H3K4) trimethyltransferase, which activates genes that are necessary during meiosis. Null mutations of the *Prdm9* gene in nonhybrid male and female mice result in the arrest of spermatogenesis and oogenesis. Furthermore, sterility was rescued in hybrid males using an artificial chromosome carrying a functional *Prdm9* gene (Mihola *et al.*, 2009). To date, there have been no interacting genes identified.

Like hybrid sterility, a number of loci have also been identified that contribute to hybrid inviability. The first hybrid inviability loci to be identified are a pair of interacting genes that rescue male hybrid lethality between two species of *Drosophila*. Mutation of either the autosomal *Lethal hybrid rescue* (*Lhr*) gene in *D. simulans* (Watanabe, 1979) or the X-linked *Hybrid male rescue* in *D. melanogaster* (*Hmr*; Hutter and Ashburner, 1987; Hutter *et al.*, 1990) rescues inviable hybrid males. It was later discovered that lethal hybrid females of this same species pair can be rescued by either a mutation of the *D. simulans* autosomal *maternal hybrid rescue* (*mhr*) gene (Sawamura *et al.*, 1993), or by the *D. melanogaster Zygotic hybrid rescue* (*Zhr*) gene (Sawamura *et al.*, 1993; Sawamura and Yamamoto, 1997).

Using deficiency mapping, two nuclear pore proteins have also been identified as lethality factors in later-generation hybrid males between *D. melanogaster* and *D. simulans*. *Nucleoporin 160* (*Nup160*) and *Nup96* are both autosomal genes that are members of the nuclear pore complex, which serve to traffic molecules between the cytoplasm and the nucleus (Presgraves *et al.*, 2003; Tang and Presgraves, 2009). The *D. simulans Nup* gene, when homozygous, interacts with an as-yet unidentified *D. melanogaster* locus.

Recently, a new mechanism for the evolution of postzygotic isolation emerged in the study of hybrid yeast. Here, recessive genetic incompatibilities between the hybridized genomes of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, affect spore viability (Greig *et al.*, 2002). A recessively acting nuclear gene located on chromosome 13 of *S. bayanus*, AEP2, affects the translation of an *S. cerevisiae* ATP synthase subunit mitochondrial gene, OLI1 (Lee *et al.*, 2008). Specifically, AEP2 does not properly function alongside the mitochondria of *S. cerevisiae*, leading to defects during mitochondrial respiration and ultimately spore inviability. This provides evidence that, in addition to nuclear–nuclear interactions, hybrid dysfunction may also arise from nuclear–mitochondrial gene incompatibilities.

Meiotic Drive and Postzygotic Isolation

At its inception, meiotic drive was largely criticized but has regained some popularity due to compelling evidence put forward in the last few years (review in McDermott and Noor, 2010). Generally, meiotic drive is the phenomenon wherein unequal segregation of chromosomes or alleles during meiosis allow for the overrepresentation of that element within a population or species. Drivers (or selfish elements) arise as a

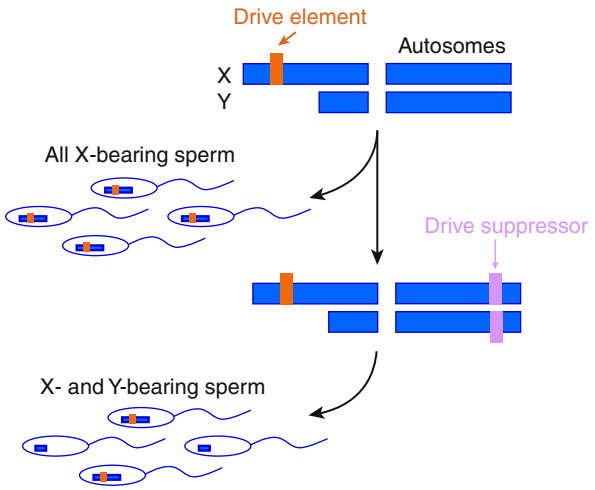

Figure 3 Meiotic drive leading to sex ratio skew. A meiotic drive element (orange) on the X chromosome causes all sperm to be X-bearing, and thus all resulting offspring to be female. A suppressor element that inhibits the drive element eventually arises on the autosomes, returning the offspring sex ratio back to normal.

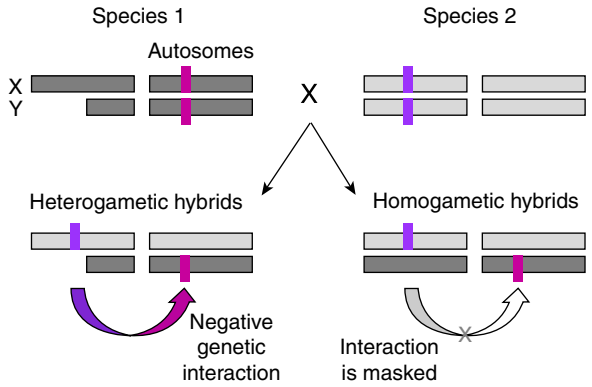

Figure 4 Dominant interactions and Haldane's Rule. A recessive allele from Species 2 on the X chromosome (purple) has a negative interaction with a dominant allele from Species 1 on the autosomes (pink) when placed together within a heterogametic (XY or ZW) individual, leading to hybrid dysfunction. The recessive allele on the X chromosome is masked by its corresponding homolog when it is instead within a homogametic individual, preventing hybrid dysfunction from forming.

means to increase the presence of that selfish element within the next generation. Drivers on the X chromosome may compete during the course of sperm development, leading to deviations from the normal representation of X- and Y-bearing sperm (Figure 3). A lack of Y-bearing sperm produced during spermatogenesis will affect the sex ratio within subsequent generations, which is detrimental for the population. As such, suppressor elements evolve (usually on autosomes; Hurst and Pomiankowski, 1991) to counteract the effects of these selfish elements. Separated populations will likely evolve unique sets of drivers and suppressors. If individuals from these two populations mate and produce offspring, a mismatch between drivers and suppressors will cause hybrid dysfunction to occur. To date, many lines of evidence have provided links between chromosomal drive and hybrid sterility, but there has yet to be any link for hybrid lethality (reviewed in McDermott and Noor, 2010).

Haldane's Rule

Despite the myriad means postzygotic isolation may come about, there is nonetheless a widespread consistency as to which sex these incompatibilities arise in first. This principle, known as Haldane's rule, states: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterogametic sex" (Haldane, 1922). Thus hybrid dysfunction is expected to occur first among the XY or ZW bearing sex of the species before their homogametic counterpart (Coyne, 1985). Additionally, the 'large X effect,' another sex chromosome centric pattern, notes that the contributions of the X chromosome to postzygotic isolation between species are disproportionately larger than their autosomal partners (reviewed in Presgraves, 2008). Studying the genetic basis of speciation via sex chromosomes has been governed by both the interaction between the X and Y chromosomes (Haldane's rule) and the clearly 'large X effect' exhibited by the X chromosome (Coyne, 1985, 1992; Coyne and Orr, 1989; Charlesworth *et al.*, 1987; Masly and Presgraves, 2007). It is obvious that the sex chromosome plays an integral role during speciation (Masly and Presgraves, 2007; Presgraves, 2008), and explanations of how this effect entangles with Haldane's rule is currently addressed by two primary theories.

Genetic Incompatibilities and the Dominance Theory

The issue of how species can accumulate differences that do not negatively interact with their own genetic background, but are nonetheless deleterious in a hybrid background, has been addressed by the Bateson–Dobzhansky–Muller incompatibility (BDMI) model (Bateson, 1909; Dobzhansky, 1934; Muller, 1940). In this model, each species acquires a new non-deleterious allele at a different genetic locus, and thus these alleles are able to sweep to fixation within their respective local populations. However, while each of these new alleles on its own is not deleterious, they do not interact well with one another, giving rise to dysfunction when in contact within an interspecies hybrid. Over time, additional loci that would have a negative epistatic interaction within a hybrid are likely to arise.

The Dominance theory has been proposed to explain how these incompatible genetic factors within hybrids could explain the trend noted by Haldane's rule. In the simplest form of the Dominance theory, hybrid sterility and inviability are the result of an incompatible interaction between one or more recessive X-linked factor(s) and one or more autosomal-linked factors (Figure 4; Orr and Turelli, 1996). For two species able to produce a hybrid male (XY), the X-chromosome is inherited from one species (e.g., Species A), whereas one set of autosomes is inherited from both species (Species A and B). An improper interaction (BDMI) between the recessive X-linked gene(s) of Species A and the autosomal gene(s) of Species B affects the male's development, rendering it sterile or inviable. Alternatively, hybrid females (XX) do not suffer from the same fate, as they inherit an X-chromosome from both parental

species, thus allowing the recessive X-linked gene(s) of Species A to be masked by the X-linked gene(s) of Species B. This prevents (or at least reduces) the interaction between foreign genomes of Species A and Species B, enabling hybrid female development to occur without the emergent compromises to fertility or viability present within hybrid males.

While the Dominance theory is conceptually robust, over the past few decades, geneticists working toward the identification of speciation genes have encountered controversial and sometimes conflicting data that have muddied the predictive and explanatory powers of the Dominance theory as originally presented, particularly as an explanation for hybrid sterility (Perez *et al.*, 1993, 1995; Presgraves *et al.*, 2003; Masly *et al.*, 2006; Tang and Presgraves, 2009; Presgraves, 2010). Part of the difficulty in resolving the theory is the paucity of loci that have been identified as contributing to hybrid dysfunction in the first generation, as most of the loci that have been identified can only act in later-generation hybrids. Additionally, given that genetic incompatibilities between species continuously accumulate over time, there currently exists no obvious and easy means of determining (1) the sequence and types of barriers as they arose and (2) their relative contributions to isolation (if at all). The second question is particularly important as barriers that arise after total speciation has been achieved are redundant and bear no impact on species formation or maintenance (Orr, 1995; Matute *et al.*, 2010).

Postzygotic Isolation Contributing to Increased Behavioral Isolation

Geographically, postzygotic isolation is expected to occur in any allopatric scenario if given enough time for divergence (Coyne and Orr, 1997). However, if contact is resumed between incipient species, partial postzygotic isolation may accelerate speciation in an evolutionary process referred to as reinforcement (Dobzhansky, 1975). In this scenario, selection against matings that would produce hybrids occurs as a means of preventing the production of these unfit offspring. For example, hybrid males produced between two species of chorus frogs have reduced fertility, and these males also experience reduced acceptance by courted females, resulting in a fourfold greater reduction to gene flow than would result from reduced fertility alone, reinforcing the isolation between the two species (Lemmon and Lemmon, 2010). Reinforcement appears to be prevalent among sympatrically diverging species, often leading to earlier-occurring and stronger reproductive isolation (Coyne and Orr, 1997).

See also: Speciation Continuum. Speciation Genes. Species Concepts and Speciation

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Reproductive Isolation, Prezygotic

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Glossary

Allochronic isolation Reproductive isolation arising due to differences in the timing of reproduction between two nascent species.

Assortative mating The tendency of individuals to mate with similar individuals, particularly of the same species.

Crossbreeding Matings between individuals of two nascent species; synonymous with interbreeding or intercrossing.

Gametic isolation Reproductive isolation arising due to interactions between the gametes of nascent species (for example, less efficient fertilization of ova by sperm of the other type).

Mechanical isolation Reproductive isolation arising due to differences in the shape of male and female genitals between two nascent species.

Pollinator isolation Reproductive isolation arising due to differences in the pollinators used by nascent species.

Prezygotic isolation Reproductive isolation arising due to any factor which reduced the opportunity for sperm and eggs of nascent species to meet and fuse.

Reinforcement The exaggeration of sexual isolation between nascent species in order to prevent the production of deleterious hybrids.

Reproductive character displacement The exaggeration of sexual isolation between good species in order to prevent the production of deleterious hybrids, usually due to increased divergence in mating traits and preferences in sympatry.

Sexual isolation Decreased sexual attractiveness between potential mating partners of nascent species.

Sexual selection Selection arising due to differences in mating success.

Prezygotic Isolation

Reproductive isolation represents a breakdown in the ability to reproduce successfully with sexual partners of another type of organism, and speciation requires a build up of reproductive isolation between diverging types of organism until gene flow is sufficiently rare or ineffective that the entities are considered 'good species.' Traditionally this was thought to require complete or near complete cessation of gene flow, though increasingly absolute reproductive isolation is thought to be too stringent a criterion (Mallet, 1995; Wu, 2001). Factors which influence prezygotic isolation are those that come into play before gametes of the different types meet and form zygotes. After this point postzygotic isolation occurs, and this simple classification of categories of reproductive isolation based on pre- and post-gametic fusion has been widely adopted since Dobzhansky originally categorized major factors influencing the origin of species into various 'reproductive isolating mechanisms' (Dobzhansky, 1937). However, it is important to appreciate that all factors influencing reproductive isolation act in combination. If cross-matings between males and females of different types are half as likely as within types we say their isolation index (I) is 0.5. If the viability of their offspring is also around 50% these are equally effective barriers to gene flow, and acting together will produce a combined I of 0.75, though prezygotic isolation will have made a greater contribution to the overall isolation only because it occurs first (Coyne and Orr, 2004).

Causes of Prezygotic Isolation

Many aspects of an organism's ecology, breeding system, and behavior will influence the potential for crossbreeding, and these will diverge under the influence of a wide range of sources of natural and sexual selection, as well as historical contingencies. Courtship behaviors of animals and pollination strategies of plants are probably the most obvious and widely researched factors influencing prezygotic isolation, but it is important to appreciate that these occur within a much broader context of divergent ecological adaptations.

Allopatry, the occupation of geographically disjunct locations, will inevitably stop the opportunity for crossbreeding. While it is trivial to say that few African and Asian elephants have the opportunity to crossbreed due to their historical biogeography, smaller-scale changes in spatial or temporal distribution provide an important setting for evolutionary divergence due to reduced potential for crossbreeding. Plants will adapt to different habitats, for example, occupying wetter versus drier regions of a landscape, or specialize to different regions of altitudinal gradients. If pollen dispersal is restricted between these habitats, some prezygotic isolation will inevitably follow (see later description of *Anthoxanthum*). Phytophagous insects which feed upon such plants, or lay eggs in their fruit within which larvae develop, may also start to show assortative mating. This is the statistical tendency for like-with-like matings to be more common, and need not be due to mate choice, so if mating takes place on or around the preferred plant, assortative mating can arise without any divergence in courtship behaviors. A famous example of this (because it played an important role during debates about the probability of sympatric speciation) is the fruit fly *Rhagoletis* (Dambroski *et al.*, 2005). Even if there is free migration between habitats but migrants perform less well in one habitat, statistically this will lead to a form of assortative mating due to 'immigrant inviability,' and a particularly well-worked recent example of this is provided by the walking stick *Timema*

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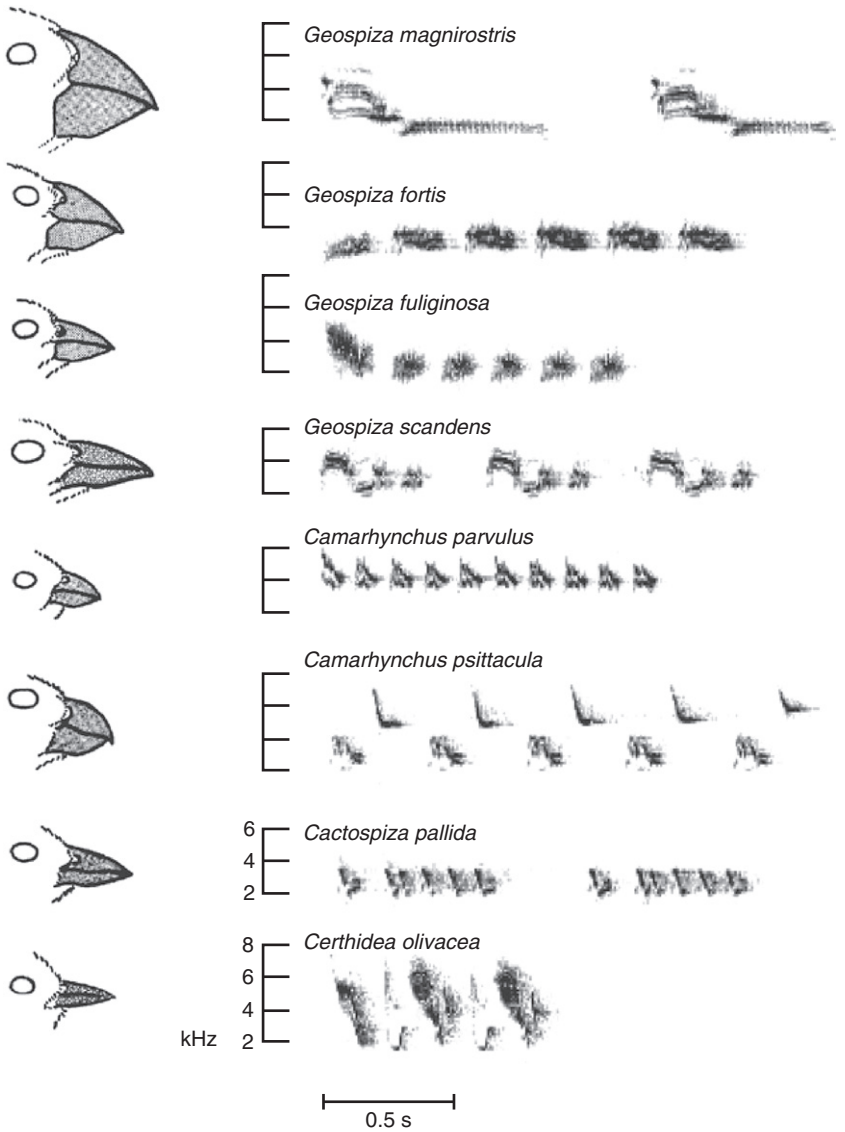

Figure 1 Beak morphology and song spectrograms from eight species of Darwin's finches. Interspecific variation is evident in both morphology and song structure. Birds with larger, more cumbersome beaks tend to produce songs with lower rates of syllable repetition and with more narrow frequency ranges. Reproduced from Podos, J., Nowicki, S., 2004. Beaks, adaptation, and vocal evolution in darwin's finches. Bioscience 54, 501–510, with permission from Oxford University Press.

(Nosil, 2004). Any system of ecological specialism which leads to a reduction in mating opportunities between individuals with different specializations may lead to pre-mating isolation as an indirect consequence of ecological or habitat divergence. Indeed, the traditional division of prezygotic isolating factors into different subcategories (e.g., temporal, ecological, sexual, pollinator) could all be seen as simply different forms of ecological specialization (Sobel *et al.*, 2010; Nosil, 2012).

There are now several examples where the potential relationship between ecological selection, assortative mating, and prezygotic isolation has been investigated. Different ambient light environments in water can select for different colors and color preferences in stickleback, and lead to assortative mating (Boughman, 2001). The extent to which ecological specialization will directly affect prezygotic isolation depends on how strong a functional link there is between biological features selected upon by the ecology and those which influence assortative mating. A classic example of ecological selection, specialization, and speciation is that of Galapagos finches adapting to specialize on different forms of seed, which alters beak morphology. Each species of Galapagos finch has a different song (**Figure 1**) and there is a relationship between beak morphology and song structure even within bimodal populations (Huber and Podos, 2006). Because song in turn influences female mate choice, there is a route where ecological specialization could link to assortative mating (Podos and Nowicki, 2004). A more direct link is provided by the mimetic wing patterns of *Heliconius* butterflies. Mimicry patterns diverge under ecological selection – in this case selection due to Mullerian mimicry – but as these wing patterns also influence

mate choice, assortative mating would directly follow wing pattern divergence (**Figure 2**; Merrill *et al.*, 2012, 2014). Theoretical studies have demonstrated that such exceptional functional linkage can more rapidly produce speciation by prezygotic isolation (Smadja and Butlin, 2011), and such traits have sometimes been called 'single gene' traits because of probable pleiotropy underlying their genetic control, or even 'magic traits' (Servedio *et al.*, 2011). If different traits involved in adaptation and assortative mating are caused by independent genes, the evolution of prezygotic isolation may be more difficult because selection would have to create strong associations between these genes, for example, by genetic linkage (Felsenstein, 1981; Gavrilets, 2003; Smadja and Butlin, 2011).

Although there are a multitude of ways in which ecological adaptation could influence assortative mating, two aspects of ecological specialization deserve special mention, both because of their likely importance, but also because they provide fantastic examples of adaptation and speciation.

Allochronic Isolation

When an organism chooses to breed can be as important to prezygotic isolation as where or how an organism reproduces. Divergence of flowering time in plants is a widespread cause of reduced gene flow between diverging species. The grass *Anthoxanthum* has adapted to heavy metal polluted soil around mines in wales, and differences in flowering time have evolved between adjacent populations. Reduced potential for cross-pollination due to this restricts gene flow by almost 50% (I = 0.46) between populations adapted to polluted soil from unadapted meadow populations. Remarkably, a series of studies show that these patterns have remained stable for around 40 years (**Figure 3**), strongly suggesting that these are maintained by a balance between selection against hybrids and gene flow (Antonovics, 2006). In animals, breeding period can also greatly reduce gene flow. One of the most spectacular examples of temporal isolation in insects is the periodical *Magicicada* cicadas of North America (**Figure 4**). Different broods of these cicadas emerge synchronously from their underground larval stage as adults in dramatic numbers, and it is thought that predator satiation is one of the most important selective factors favoring co-emergence. In different geographic regions broods appear in different years. Periodical cicadas have the longest life cycles of insects and different broods have life cycles based on prime numbers of years, and two well-studied species groups emerge either every 13 or 17 years. Sympatric broods of these types would therefore only have the opportunity to hybridize once every 221 years. Remarkably, following some detailed detective work, it has been demonstrated that a relatively newly described 13 year species *Magicicada neotredecim* seems to be derived from a brood of the 17 year species *Magicicada septendecim*, which 'accelerated' its life cycle to emerge synchronously with a local 13 year brood of *Magicicada tredecim*, so changes in the periodicity of the life cycle can occur, and will allochronic isolation between *M. neotredecim* and its ancestor (Simon *et al.*, 2000; Cooley *et al.*, 2001). Could these changes occur due to developmental plasticity, or could they provide an example of a major change in prezygotic isolation due to only a key change in a single gene or a gene regulatory network? While the mechanisms of life history divergence in cicadas will be extremely challenging to work out, phylogeographic studies suggest that changes in

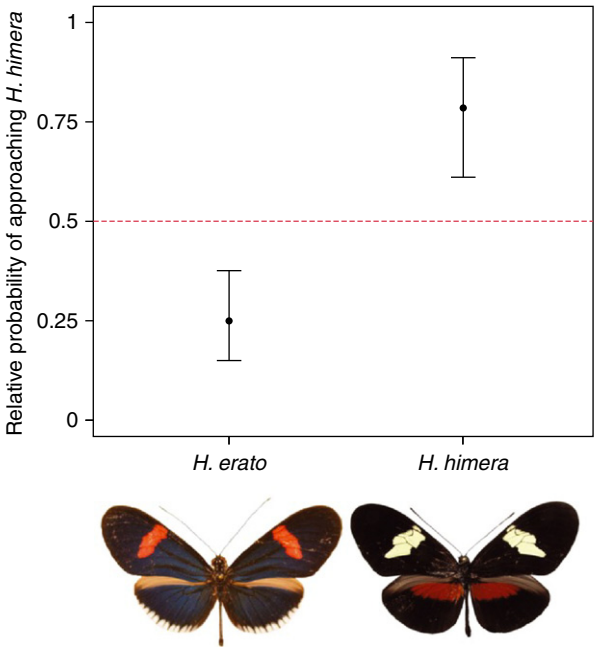

Figure 2 The relative probability of approaching *Heliconius himera* mounted females by *Heliconius erato cyrbia* and *H. himera* males (below left and right, respectively), where one would indicate a complete preference for *H. himera* and zero a preference for *H. erato cyrbia*. Dashed red line represents a relative probability of 0.5 (i.e., no preference). Reproduced from Merrill, R.M., Chia, A., Nadeau, N.J., 2014. Divergent warning patterns contribute to assortative mating between incipient Heliconius species. Ecology and Evolution 4, 911–917, with permission from John Wiley and Sons.

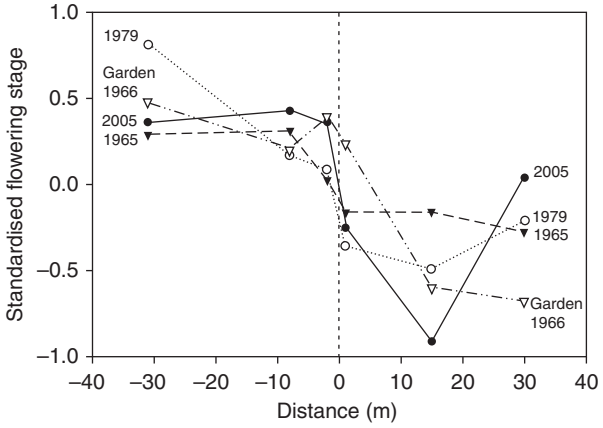

Figure 3 Mean flowering stage at specific transect positions across the mine boundary (at '0') of *Anthoxanthum odoratum* in 1965, in common garden conditions in 1966 and in the field in 1979 and 2005. Results are remarkably consistent across this time period. Reproduced from Antonovics, J., 2006. Evolution in closely adjacent plant populations x: Long-term persistence of prereproductive isolation at a mine boundary. Heredity 97, 33–37, with permission from Nature Publishing Group.

Figure 4 The periodical cicada *Magicicada septendecim*. Photo courtesy of Chris Simon.

life cycle have occurred several times, favored by selection for co-emergence (Sota *et al.*, 2013). Less spectacular examples of allochronic isolation are more amenable to genetic investigation, and do suggest that allochronic isolation has the potential to be influenced by a changes in a few key genes. Diurnal mating periods differ between closely related species of *Drosophila* and therefore potentially cause assortative mating (Sakai and Ishida, 2001). Diurnal cycles and other aspects of behavioral and physiological rhythmicity of flies are regulated by only a few 'clock' genes, and moving *period*, one such clock gene, between different species of flies can change their diurnal pattern of mating activity. *Drosophila melanogaster* flies carrying the *period* gene of *Drosophila pseudoobscura* show a *D. pseudoobscura*-like activity cycle, being more active at dusk rather than dawn. Interestingly, such *D. melanogaster* flies show assortative mating in the laboratory with respect to the origin of the introduced gene (*melanogaster* or *pseudoobscura*), probably due to associated changes in sensitivity to different behavioral rhythms (Tauber *et al.*, 2003), providing an experimental example of how a change in a key clock gene could influence prezygotic isolation.

Pollinator Isolation

If plants evolve to utilize different pollinators, prezygotic isolation will build up due to reduced cross-pollination (Grant, 1981). A spectacular example of such divergence is provided by the monkeyflowers *Mimulus cardinalis* and *Mimulus lewissii*, which are pollinated by hummingbirds and honeybees, respectively. Changes in floral morphology associated with attraction of and pollination by these distinct pollinators seems to show relatively simple segregation and early studies mapped some large effect quantitative trait loci for these floral differences (Bradshaw *et al.*, 1995). Introgression of a single one, effecting floral color, between species changed patterns of pollinator visitation (Bradshaw and Schemske, 2003). Ramsey *et al.* (2003) examined components of reproductive isolation between these species in the field and found that cross-pollination hardly ever occurred. Both ecological differences and pollinator isolation were the most important forms of isolation, though pollinator effects were almost sufficient to provide complete isolation where plants coexisted. Orchids are a famous example of insect pollination (Darwin, 1877) and many species are sexually deceptive with morphology and chemical cues 'fooling' insects into attempted copulations with the flowers. Whitehead and Peakall (2014) neatly demonstrated that high (>90%) levels of prezygotic isolation between sympatric *Chiloglottis* orchids was due to variation in attractive volatile compounds present on the flowers. Genetic analysis failed to find any introgression between the species in the field, yet forced crosses between them showed hybrid vigor. Hence, once more, the prezygotic isolation due to pollinator specialization seems to be the primary cause of speciation in the field. However, the extent to which prezygotic isolation is a cause or effect of such specialization is an important question, but hard to disentangle from such studies.

Sexual Isolation in Animals

Of all the features of organisms that can influence prezygotic isolation, the most studied and debated is sexual isolation in animals. Put simply, the most obvious reason why animals fail to crossbreed is because males are less likely to court females of another species, and females should be even less likely to accept the courtship of heterospecific males. It is striking that elaborate male courtship displays and morphologies can show dramatic divergence between closely related species (Darwin, 1871). Controlled quantitative comparative studies suggest that speciation rate is higher in lineages in which sexual selection might be heightened, such as those with more polygamous mating systems or showing higher sexual dimorphism. However, such evidence is not especially strong (Kraaijeveld *et al.*, 2011). There is more convincing evidence that sexual selection may act alongside ecological adaptation, as discussed earlier for some mating traits (Ritchie, 2007; Maan and Seehausen, 2011).

There are broadly two ways in which mating behaviors may diverge and cause prezygotic isolation, due to sexual selection operating within a species, or due to direct selection for prezygotic isolation. Sexual selection is a very prevalent force in animal evolution. In a typical mating system, male–male competition and female mating preference can act strongly on male morphology and behavior to produce elaborate courtship traits which help to outcompete other males or to induce females to mate (Andersson, 1994). Genomic comparisons show that sex-biased genes evolve more quickly between species, probably due to sex-specific selection including sexual selection (Ellegren and Parsch, 2007), and considerable progress is being made in identifying the genetic changes

responsible for sexual isolation in model organisms such as *Drosophila* (Chertemps *et al.*, 2007; Fan *et al.*, 2013). One of the superficially simplest ways in which sexual selection might lead to rapid differences of traits and preferences between populations is the dynamic coevolution of female preferences and male traits that is thought to occur due to potential 'Runaway Selection' (Fisher, 1958; Lande, 1981; Andersson, 1994). If traits evolve together in different directions in different populations, it is widely expected that assortative mating will arise as an indirect consequence of this form of sexual selection (Lande, 1982; Uyeda *et al.*, 2009). At one level, this seems obvious if traits and preferences change in a coordinated manner due to coevolution, then mismatches between males and females from different areas of the (phenotypic or geographic) distribution should lead to assortative mating (floral morphology reflecting differences in pollinator shape, such as moth probosces and floral tube length may be a loosely analogous pre-fertilization barrier in plants). However, enhanced sexually selection may not always increase speciation rates. Increased male–male competition may lead to a diminution of the importance of female preferences, increasing gene flow (Parker and Partridge, 1998). In a particularly interesting model, Servedio and Burger (2014) found that when species make secondary contact following allopatric divergence of traits and preferences, strong female preferences can actually inhibit divergence even when male traits are under some ecological selection. This happens because introgression of strong preference genes across hybrid zones can lead to greater effective gene flow because they enhance the mating success of males with the locally maladaptive trait. These males have a relatively high reproductive success simply because of the presence of these females, due to immigration. The authors argue that the role of the runaway or coevolutionary models of sexual selection in speciation is overestimated and that other forms of selection on male traits and female preferences must be more important. Females are under selection to choose with the best males available, which may be those with the most elaborate traits if they are condition dependent, therefore providing reliable indicators of male genetic quality, or more simply to gain proximate rewards or reduce costs of mating. Potentially more complex processes incorporating condition-dependent signaling of local adaptation, again linking sexual isolation with ecological or habitat selection, may be more effective (van Doorn *et al.*, 2009).

Another way in which sexual isolation could evolve quickly in animals (and preygotic isolation in plants) is under direct natural selection to avoid the production of interspecific hybrids. If postzygotic isolation were complete, there would be strong natural selection to favor increased assortative mating to prevent gamete wastage and other potential costs of cross-mating. We have seen earlier that the periodical cicada *M. septendecim* accelerated its life cycle to produce the 13 year *M. neotredecim*, which gained protection by co-emergence with *M. tredecim*. However, this also created the opportunity for hybridization between these two now co-emerging species. The mating system of cicadas is dominated by the characteristic loud calls of males and *M. neotredecim* has evolved a change in fundamental song frequency which distinguishes its song from that of *M. tredecim* in sympatry (Marshall and Cooley, 2000). This is a nice example of reproductive character displacement, an increase of isolation mechanisms where species overlap geographically (though in the case of *Magicicada* the source of selection against crossbreeding is not entirely clear (Cooley *et al.*, 2006)).

More contentious than reproductive character displacement is whether prezygotic isolation can increase if postzygotic isolation is not complete, in the face of ongoing gene flow between forms with partially unfit hybrids. In other words, could prezygotic isolation evolve in order to prevent the production of partially fit hybrids? This idea, usually termed 'Reinforcement' and credited to Alfred Russell Wallace, has proven to be remarkably controversial (Howard, 1993; Butlin, 1995; Noor, 1999; Servedio and Noor, 2003) (unfortunately, the terms reinforcement and reproductive character displacement are not always used consistently, but the key issue is distinguishing the evolution of prezygotic isolation in the face of gene flow versus increasing assortative mating between 'good species' (how to phrase this relies on cross-referencing?)). The controversy arises because (like sympatric speciation) it requires reproductive isolation to build up and become complete against the countervailing effects of gene flow and recombination breaking down associations between genes that underlie assortative mating and postmating isolation. Current consensus seem to be that such reinforcing selection can be successful in both animals and plants, but that this is relatively less important than intraspecific selection due to sexual selection or pollinator competition.

Mechanical Isolation

If males and females of different animal species do meet and attempt to copulate, there can be an additional barrier to insemination. Often the most dramatic sexually dimorphic differences between animal species are male genitalia, and correctly identifying many closely related insect species may require an intimate knowledge of male genital shape. Almost 200 years ago the 'Lock and Key' hypothesis proposed that male and female genitalia correspond in shape in order to prevent hybridization (Dufour, 1844; Shapiro and Porter, 1989; Masly, 2012). Detailed studies show complex morphological interactions occur between male and female genitalia during copulation (Jagadeeshan and Singh, 2006). However, as with sexual isolation, there are several reasons why dramatic changes in genital shape are thought not have evolved primarily in order to cause prezygotic isolation, but rather under intraspecific sources of selection such as sperm competition or antagonistic sexual interactions. Male–male competition may commonly drive genital shape, with males competing for strategic placement of sperm in the female reproductive tract or overcoming female reluctance to mate (Arnqvist, 1998; Eberhard, 2004). The remarkable corkscrew morphology of the male duck penis and female reproductive tract may arise as a counter-strategy to coercive mating (Brennan *et al.*, 2010), and detailed analyses of coevolution of genital morphology of waterstriders have suggested that sexually antagonistic selection may predominate in their evolution (Arnqvist and Rowe, 2002). A bizarre recent discovery of a group of Brazilian cave insects where females have intromittent organs, and these differ between species, seems to have arisen due to genitalic sex-role reversal following

behavioral sex-role reversal (Yoshizawa *et al.*, 2014). However, there are one or two potential examples of reproductive character displacement for genital shape (e.g., Hollander *et al.*, 2013), so interspecies interactions is one potential source of selection on genital morphology. Also, it has been suggested that the importance of genital differences of animals to prezygotic isolation may be underappreciated if the differences are sensory, such as tactile, rather than simply differences in physical structures (Coyne and Orr, 2004). Finally, it could be extremely difficult identifying the precise role of genital divergence in the evolution of prezygotic isolation, because strong mechanical incompatibilities would presumably exert strong selection for rapid pre-mating isolation on both sexes (heterospecific mating can have strong direct fitness costs, including lethality, for females (Sota and Kubota, 1998; Ting *et al.*, 2014)).

Gametic Isolation

Events occurring after insemination or pollination can still cause prezygotic isolation (sometimes termed PMPZ, for postmating prezygotic isolation). Pollen can germinate less effectively on the styles of other plant species, and pollen tube growth can be slower or blocked by the host flower tissue (**Figure 5**). Such barriers can be remarkably effective, in cross-pollination experiments between the sunflowers *Helianthus petiolaris* and *Helianthus anuus*. Rieseberg *et al.* (1995) showed that there was almost complete isolation even if 1:9 mixtures of homo- to heterospecific pollen was introduced to the styles. The mechanism was unknown, but acted post-germination and pollen tube growth. The evolution of mechanisms of self-incompatibility in plants may predispose some plant species to PMPZ isolation and genes involved in, for example, pollen tube interactions with the female genotypes are being identified (Shimizu and Okada, 2000). In animals there is a very dynamic source of selection post-insemination, with sperm competition, potential cryptic female choice of sperm and antagonistic interactions between male semen components and female reproductive physiology all occurring within this arena. The latter is particularly likely to drive complex coevolution between both intersexual and intrasexual gene interactions within the female reproductive tract. Because cross-mating between diverging populations will be rare, such interactions may not have been subject to consistent selection. The outcomes of rare interactions are hard if not impossible to predict, but almost certainly include less efficient insemination of sperm encountering reproductive tracts they have not previously coevolved with. There is now an abundance of evidence that PMPZ barriers to cross mating are common in animals. Interestingly, there are very few if any examples of alien sperm being more successful, which might be predicted under coevolutionary models of sperm-reproductive tract interactions.

The final step before zygote formation is fertilization. Once more coevolution is a predominant feature of sperm–egg interactions (Frank, 2000) as egg membranes are usually under strong selection to resist deleterious polyspermy, whereas sperm are obviously strongly selected to permeate egg surfaces, and additional features can contribute to rapid evolution of molecules involved in gamete recognition (Palumbi, 2009). A failure to pierce the membrane of eggs from another population may be an important barrier to cross mating between divergent races of *D. melanogaster* (Alipaz *et al.*, 2001), and genes involved in gametic isolation show particularly rapid divergent evolution in marine spawning organisms (Moy *et al.*, 2008). Some form of direct sperm–egg barrier seems to exist in a wide range of organisms (Howard *et al.*, 2009). Their importance to overall prezygotic isolation will depend on the relative strength of other barriers which act earlier in reproduction, so, for example, gametic effects are likely to be most important in wind pollinated plants or those with generalist pollinators, or broadcast spawners.

Relative Rates of Evolution

The relative importance of the various forms of pre- and postzygotic isolation to speciation differs between organisms and ecologies. While there is a general belief that pre-mating isolation may evolve more quickly than postmating incompatibilities in many groups, especially under strong sexual selection in animals, controlled quantitative comparisons of the rate of appearance of pre- and postmating isolation are rare. In sexually dimorphic darter fish, sexual isolation does evolve more quickly than postzygotic isolation (Mendelson, 2003). A quantitative analysis of 19 plant species pairs found several

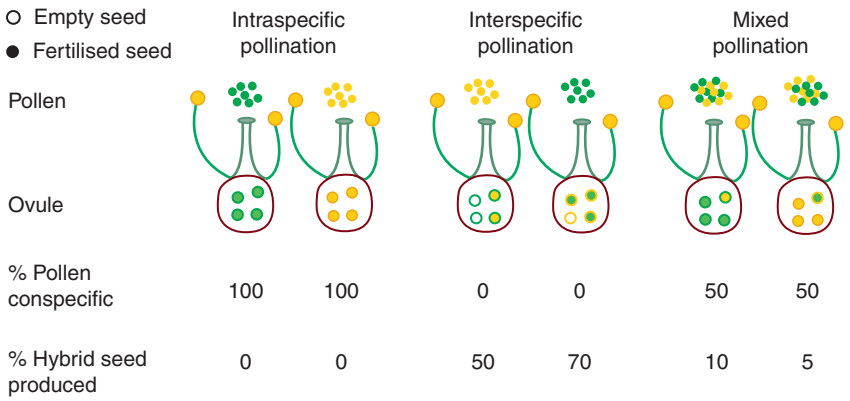

Figure 5 Illustration of PMPZ (postmating but prezygotic isolation) in plants. Reproduced from Baack, E., Melo, M.C., Rieseberg, L.H., Ortiz-Barrientos, D., 2015. The origins of reproductive isolation in plants. New Phytologist 207, 968–984, with permission from John Wiley and Sons.

components of prezygotic isolation were stronger than any hybrid incompatibilities (though the authors only consider homoploid comparisons) (Lowry *et al.*, 2008). The most influential comparative studies were initiated by Coyne and Orr (1989, 1997) who compiled comparative data on pre- and postzygotic isolation scaled by estimates of divergence times from studies of *Drosophila*. These showed two striking patterns. Firstly, there was a broad correspondence between the evolution of pre- and postzygotic isolation, especially in allopatry. However, the second pattern was an accelerated rate of evolution of prezygotic isolation between sympatric species, especially at low levels of genetic divergence. This has been interpreted as evidence in favor of reinforcement of prezygotic isolation (the absence of strong prezygotic isolation between allopatric species of similar divergence implies that sympatry accelerates the evolution of prezygotic isolation). Perhaps most convincingly, asymmetries in pre- and postzygotic isolation follow the pattern predicted by reinforcement (Yukilevich, 2012). For example, if crosses between males of species A and females of species B produce more poor hybrids than the alternate cross, prezygotic isolation is likely to be stronger in this direction. These data provide the most compelling case for the importance of prezygotic isolation in early speciation in animals (Turelli *et al.*, 2014) and the possibility of speciation in sympatry or parapatry (Yukilevich, 2014). Postzygotic isolation will be low in the early stages of divergence, so perhaps additional factors about sympatry such as ecological character displacement also select for assortative mating (note also that all of these estimates of isolation in *Drosophila* are lab-based, so assortative mating could be much higher within natural ecologies). In a related study, Funk *et al.* (2006) showed that ecological divergence is associated with reproductive isolation in a wide range of organisms.

Conclusions

Multiple factors have the potential to influence prezygotic isolation during evolutionary divergence. Most commonly, prezygotic isolation will arise as an indirect response to ecological divergence acting on where, when and how organisms mate, but direct selection on male–female interactions due to sexual selection or antagonistic selection, and pollinator–plant coevolution will also cause prezygotic isolation to evolve. Direct selection for sexual isolation can also occur. We need more comparative studies making controlled comparisons of types of reproductive isolation between species showing a wide range of levels of divergence to assess the relative importance of these different forms of reproductive isolation.

See also: Reinforcement. Speciation Continuum. Species Concepts and Speciation

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Responses to Climate Change, Evolution and

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Glossary

Common-garden experiment An experiment in which a number of genotypes are raised in a common environment in order to quantify the genetic component of phenotypic variation.

Phenology Timing of seasonal life-history events, such as reproduction, migration, or emergence from winter dormancy.

Phenotypic plasticity The capacity of one genotype to produce multiple phenotypes when exposed to different environments.

Quantitative genetics The study of continuous traits and the statistical analysis of the relative contributions of genetic and environmental effects on phenotypic variation.

Introduction

Climate change may put as much as 35% of the earth's biota at risk of extinction (Thomas *et al.*, 2004; Maclean and Wilson, 2011). Species in a changing environment can escape extinction through shifts in geographic range, phenotypic plasticity, or evolutionary adaptation (Williams *et al.*, 2008). All three of these processes will probably occur simultaneously in most populations, but initial projections of climate-driven extinction risk focused mainly on changes in the availability of habitat (Thomas *et al.*, 2004), and tended not to incorporate evolutionary thinking. However, recent work has shown that evolution can happen on rapid (ecological) timescales, and could play an important role in rescuing some species and populations from extinction (Bell and Gonzalez, 2009; Hoffmann and Sgrò, 2011; Hendry, 2013; Munday *et al.*, 2013). Studies of evolutionary responses to climate change seek to understand both past responses to climate and the role that evolutionary adaptation might play in increasing species' resilience to the effects of future change.

Evolution by natural selection depends on the presence of heritable variation and selection (variation in fitness). If heritable variation is present, changing climate is expected to drive evolution by altering selection regimes in a variety of ways. Increases in maximum temperatures will favor increased heat tolerance (Somero, 2012), while changes in mean temperatures will favor new temperatures of optimal performance (Higgins *et al.*, 2014). In many terrestrial habitats, climate change will be associated with changes in rainfall patterns (Dai, 2012) favoring increased desiccation tolerance in some taxa (Eads *et al.*, 2012). In many terrestrial species, phenology (the timing of seasonal events) is driven by day length as well as temperature, and so climate change will impose selection on response to day length (e.g., earlier spring emergence) to better match the timing of seasonal events to temperature (Bradshaw and Holzapfel, 2008). Indeed, many studies have documented earlier flowering times (Anderson *et al.*, 2012) and earlier migration and breeding in birds (Charmantier and Gienapp, 2014), but it is also difficult to document whether these represent evolutionary or purely plastic responses. Increased atmospheric CO_2 may also select for increased efficiency of photosynthesis, to take advantage of available CO_2, although there is little evidence that wild populations have actually been able to do this (Collins and Bell, 2006; Leakey and Lau, 2012; but see Grossman and Rice, 2014). Climate change may also select for changes in dispersal patterns, and the ability to take advantage of new patterns of resource availability (Pulido and Berthold, 2010; Buckley *et al.*, 2012; Bridle *et al.*, 2014). Finally, and maybe most importantly, climate change will impose new patterns of selection by altering species interactions (Karell *et al.*, 2011; Gilman *et al.*, 2012; Urban *et al.*, 2012; Herstoff and Urban, 2014). In this article, we will discuss the evidence that species have already evolved in response to climate change and methods for assessing potential for future adaptation.

Retrospective Studies: Responses to Recent Climate Change

Across the globe, species have undergone shifts in phenology and shifts in geographic range. These have now been documented in thousands of species in diverse habitats, and represent a clear global footprint of ongoing climate change (Parmesan, 2006; Chen *et al.*, 2011). But to what extent do observed responses involve evolutionary change? For most traits, an organism's phenotype is the product of both its genotype and its environment, and it can be difficult to disentangle environmental and genetic effects. If a population shows phenotypic change over time in response to a changing environment, that change may be due to adaptation (a change in the genetic makeup of the population in response to selection), plasticity (a change in the phenotypes expressed by the same genotypes in response to different environments) (West-Eberhard, 2003), or both. In order to document that a population has evolved in response to climate change, researchers must be able to show not just that phenotypic change has occurred, but that the change in phenotype was driven, at least in part by a change in the genetic makeup of the population. Relatively few studies have been able to demonstrate conclusively that a change in phenotype observed in response to climate change has a genetic basis (Merilä and Hendry, 2014).

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Table 1 Methods for assessing past evolutionary change

Approach	*How it works*	*Examples*
Resurrection ecology	For species with seeds or with dormant eggs, researchers rear offspring from past and present populations in a common environment to test for evolutionary change	Rearing seeds from different years in a common environment revealed that an annual plant (*Brassica rapa*) had evolved earlier flowering time in response to a drought (Franks *et al.*, 2007)
Multiyear common garden experiments	Researchers rear organisms under common conditions across multiple years	Between 1972 and 1996, the pitcher plant mosquito (*Wyeomyia smithii*) evolved earlier emergence times (Bradshaw and Holzapfel, 2001)
Animal model analyses	If researchers have pedigree data for the population they are studying, quantitative genetic statistical techniques can be used to estimate the relative contributions of genetic and environmental effects on the trait of interest. If applied through time, animal model analyzes can be used to understand whether the genetic contributions to a trait have changed over successive generations	In a long-term study of laying dates and fitness in great tits (*Parus major*), females varied in the strength of their response to warmer temperatures, and this variation in plasticity was heritable. Furthermore, the degree of plasticity was correlated with lifetime reproductive success, especially in later decades (Nussey *et al.*, 2005)
Molecular genetics	Researchers genotype organisms from the same population over successive generations at molecular markers thought to be tied to climate adaptation	Over 20 years, a cline in the alcohol dehydrogenase polymorphism in Australian *Drosophila* shifted, so that southern populations came to resemble the allele frequencies that had been found in more northern populations 20 years earlier (Umina *et al.*, 2005)

There are several methods for disentangling environmental and genetic influences on traits through time (Table 1). These include resurrection ecology, common-garden experiments replicated through time, quantitative genetic animal model analyses, and the use of molecular genetics to measure changes in allele frequencies through time and space. Below we will discuss three case studies attempting to document evolutionary responses to climate change, each using a different technique: a study of spring emergence times in pitcher plant mosquitoes, a study of breeding date in songbirds, and a study of changing allele frequencies in Australian *Drosophila*.

Emergence Date in Pitcher Plant Mosquitoes

For many organisms in temperate environments, the timing of emergence from winter dormancy is a key component of survival and reproductive success: those that emerge too early may be killed by late spring cold-snaps and those that emerge too late may lose out in the race to acquire resources and mates. The mosquito *Wyeomyia smithii* occurs throughout much of eastern North America, and breeds exclusively in pitcher plant *Sarracenia purpurea* (Figure 1). *Wyeomyia* undergo winter dormancy, and emerge in the spring in response to lengthening days. The day length that triggers emergence varies among northern and southern populations, with longer days required to trigger emergence in northern populations. In the last 40 years, *Wyeomyia* have begun to emerge earlier in the spring, especially in more northern populations. Researchers Christina Holzapfel and William Bradshaw were able to show that this shift was due to a genetic change by rearing mosquitoes under common conditions in the laboratory (Bradshaw and Holzapfel, 2001). Mosquitoes collected and reared in the laboratory in 1996 emerged at a shorter day length than those that had been collected from the same populations and reared under the same conditions in 1972. Common-garden experiments replicated through time in this way can be a powerful method for demonstrating evolutionary change. However, they depend on being able to accurately replicate past experimental conditions in current experiments.

Figure 1 Between 1972 and 1996, the pitcher plant mosquito (*Wyeomyia smithii*) evolved earlier emergence times in response to warming temperatures (Bradshaw and Holzapfel, 2001).

Breeding Date in Birds

A wide variety of bird species are migrating and nesting earlier in the spring in response to warming temperatures, but no

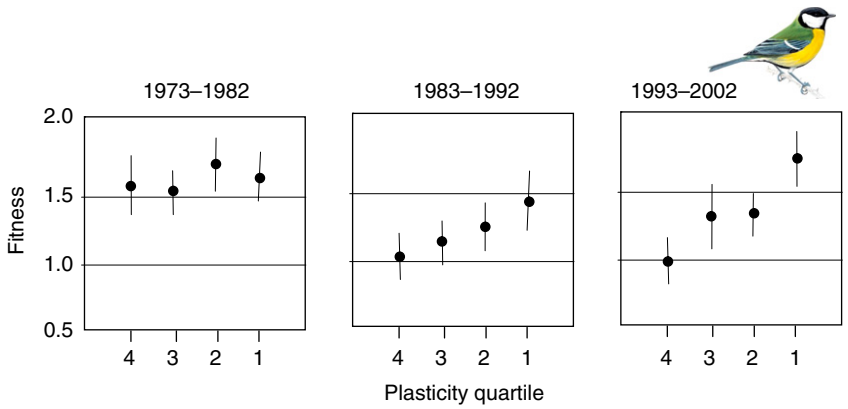

Figure 2 In the great tit (*Parus major*) plasticity of laying date in response to temperature is positively related to fitness, and the strength of this relationship has been increasing through time. Modified from Nussey, D.H., Postma, E., Gienapp, P., Visser, M.E., 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310, 304–306.

study has conclusively demonstrated that these shifts represent an evolutionary, rather than purely plastic change in phenology (Charmantier and Gienapp, 2014). One of the most extensive datasets on breeding dates and fitness in birds is a long-term study of great tits (*Parus major*) in the Hogue Veluwe, a national park in the Netherlands. In the last four decades, the emergence of this population's caterpillar prey has shifted earlier in the spring while laying dates of this population have not, resulting in a mismatch between peak food abundance and the timing of reproduction (Visser *et al.*, 1998). Nussey *et al.* examined data collected on laying dates for hundreds of female birds between 1973 and 2004. They found that females laid earlier in warmer springs, but also that individual females varied in the strength of their response to warmer temperatures, and that this variation in plasticity was heritable. Furthermore, the degree of plasticity was correlated with lifetime reproductive success, especially in the second two decades of their study (**Figure 2**). The presence of both selection and heritable variation in plasticity predict an adaptive response, however, an evolutionary change in plasticity has not yet been detected in this population. The researchers in this study used quantitative genetic animal model to estimate genetic variation. This approach is powerful, because it specifically quantifies genetic shifts while controlling for plasticity (Kruuk, 2004). However this approach also requires pedigree data, which is usually available only when animals can be individually marked and followed through multiple generations.

Shifting Allele Frequencies in Australian *Drosophila*

Many organisms exhibit geographic clines in allele frequencies at particular loci, presumably as a result of local adaptation. If clines in allele frequencies are driven by latitudinal variation in temperature, we can expect a shift in those clines through time with climate warming, so that pole-ward populations shift toward the allele frequencies previously found in equator-ward populations. Umina *et al.* used molecular markers to characterize allele frequencies at the alcohol dehydrogenase locus along the coast of Australia in 2002 and 2004, and compared their results to previous studies documenting allele frequencies in the same populations 20 years earlier. They found that the cline had shifted by about 4° latitude, so that southern populations had come to resemble the allele frequencies that had been found about 4° further north 20 years earlier (Umina *et al.*, 2005). A benefit to this approach, in terms of documenting evolutionary change, is that the use of sequence data eliminates the need to confirm that the observed change had a genetic basis. A drawback to this approach is that the connection between DNA sequence data and the actual phenotype under selection is typically unclear.

Prospective Studies: What Is the Potential for Future Adaptation?

Given evidence that evolution has already occurred in response to climate change, researchers would also like to understand whether adaptation will play a role in increasing some species' resilience to future change. Evolution can occur on relatively short timescales and may decrease extinction risk during periods of rapid environmental change (Bell and Gonzalez, 2009; **Box 1**). However, evolution does not always occur rapidly enough to outpace population decline, and current rates of environmental change may dramatically exceed rates of niche evolution that have occurred in the past (Quintero and Wiens, 2013). The key question then is how rapidly contemporary evolution will occur. Evolution over short timescales can occur from preexisting variation or from new mutations. Predicting the likelihood of new beneficial mutations is difficult, as it involves estimating rates of events that are extremely rare. Although quite labor intensive, the process of quantifying existing genetic variation for a trait is relatively straightforward. Three methods for assessing the capacity for future adaptation include quantitative genetic breeding experiments, artificial selection, and experimental evolution (**Table 2**). Importantly, these three methods also allow researchers to estimate correlated responses to selection and trade-offs, which may alter the rate of adaptation to a changing environment (Chirgwin *et al.*, 2015; Duputié *et al.*, 2012; Etterson and Shaw, 2001).

Box 1 Evolutionary Rescue

Evolutionary rescue can be defined as a species' ability to evolve quickly enough to adapt to a rapid change in the environment, thereby preventing its extinction (Gonzalez *et al.*, 2013). Understanding evolutionary rescue depends not just on understanding adaptation, but also its demographic effects (Tallmon *et al.*, 2004; **Figure B1**).

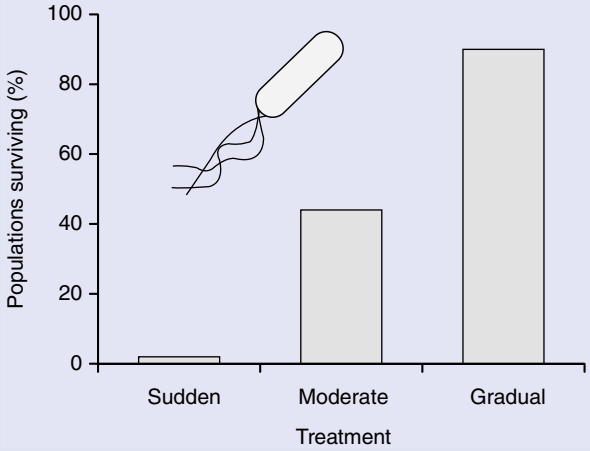

Figure B1 Percent of *E.coli* populations surviving, when given sudden, moderate, and gradual increases in an antibiotic treatment. Redrawn from Lindsey, H.A., Gallie, J., Taylor, S., Kerr, B., 2013. Evolutionary rescue from extinction is contingent on a lower rate of environmental change. Nature 494, 463–467.

Evolutionary rescue depends on the presence of some genotypes able to grow sustainably under the strong selective stress provided, and therefore on absolute, rather than relative fitness. Rescue is complicated by the fact that a response to selection comes with demographic costs. For example, Haldane showed that the number of deaths required for a single allele to be fixed in the population could be 10–30 times the size of the population. Thus, adaptation may not occur if a population cannot replenish its lost individuals fast enough, or if juveniles with the favorable allele do not survive to reproduce (Haldane, 1957). As a result, for both genetic and demographic reasons, evolutionary rescue is more likely in large populations (which will contain more variation) and when populations are exposed to gradually increasing stress (Bell, 2013; Bell and Gonzalez, 2009).

Lindsey *et al.* (2013) studied the precise effect of the rate of environmental change on populations of *Escherichia coli*. An original population was subcultured into three groups, which were treated with the antibiotic rifampicin. The 'sudden' stress group was immediately given the lowest lethal concentration of rifampicin, the gradual group was given doses in increments up to the maximum, and the medium group was given the maximum in half the time of the gradual group. The overall survival of the sudden group was much lower than that of the medium and gradual group, and genetic analysis showed that the mutations shown by the small surviving population were both radically different and smaller in number than the gradual and medium groups. A slow rate of change of environment allowed the accumulation of adaptive mutations, whereas a sudden one did not (Lindsey *et al.*, 2013).

Quantitative Genetics

The degree to which a population can respond to selection depends on the amount of additive genetic variation for a particular trait. Therefore, to predict evolutionary responses to climate change, researchers must estimate the additive variance present for the trait(s) under selection. One way to do this is through quantitative genetic breeding experiments (Shaw and Etterson, 2012). Quantitative genetics is the study of continuous traits and the statistical analysis of the relative contributions of genetic and environmental effects to phenotypic variation. For example, Kelly *et al.* measured the capacity to adapt to ocean acidification in the purple sea urchin (*Strongylocentrotus purpuratus*) by rearing 64 families (individual male–female crosses) under both ambient and elevated CO_2 conditions (Kelly *et al.*, 2013; **Figure 3**). Families varied in their sensitivity to high CO_2, and this allowed the researchers to estimate the amount of genetic variation present for the high CO_2 response. When the researchers incorporated this estimate into models of future population growth under high CO_2, they found reductions in population growth rates that were less severe than those predicted under the 'no evolution' scenario, suggesting that adaptation might ameliorate (but not erase) future effects of high CO_2 on this species.

Experimental Evolution

Experimental evolution probably provides the most realistic glimpse into potential evolutionary responses to high CO_2. In these studies, experimental populations are exposed to future conditions, such as warming temperatures, or increasing CO_2. After multiple (sometimes thousands) of generations of evolution under these conditions, populations are compared to controls under common conditions to quantify any genetically based phenotypic change. Interestingly, after 1000 generations of exposure to elevated CO_2, the green alga *Chlamydomonas* failed to evolve any specific adaptations to these conditions, suggesting a constraint on the response of photosynthesis to increased CO_2 (Collins and Bell, 2004). By contrast, 500 generations under high CO_2 conditions led to substantial adaptive evolution in the calcification response of the coccolithophore alga *Emiliania huxleyi* (Lohbeck *et al.*, 2012). Although calcification rates were not completely restored under high CO_2 conditions, they were still up to 50% higher in adapted as compared to nonadapted strains. A major strength of experimental evolution is that it can demonstrate capacity for evolution in response to a specific environmental factor; a limitation is that its application is typically limited to small organisms with short generation times.

Artificial Selection

Because the response to selection is proportional to the amount of genetic variation, another way to estimate capacity for adaptation is to expose a laboratory population to a predefined amount of selection and measure the response in the next generation. To measure the capacity to adapt to increasing temperatures, Kelly *et al.* exposed populations of a tide pool

Table 2 Methods for measuring potential for future change

Approach	*How it works*	*Examples*
Quantitative genetic breeding experiments	Researchers rear offspring from many families (single male–female crosses) under experimental conditions. By measuring variation among and within families, researchers can use statistical techniques to estimate additive genetic variance in traits important for responses to future environmental change	Purple sea urchins (*Strongylocentrotus purpuratus*) were shown to have substantial capacity for adaptation to ocean acidification (Kelly *et al.*, 2013) Sea urchins (*Centrostephanus rodgersii*) are not constrained in their ability to adapt to ocean warming and acidification simultaneously (Foo *et al.*, 2012)
Experimental evolution	Experimental populations exposed to future conditions, such as warming temperatures, or increasing CO_2. After multiple (sometimes 1000s) generations of evolution under these conditions, populations are compared to controls under common conditions to quantify any genetically based phenotypic change	500 Generations under elevated CO_2 led to partial recovery of calcification rates in the coccolithophore alga *Emiliania huxleyi* (Lohbeck *et al.*, 2012) Green algae failed to evolve specific adaptations to take advantage of high CO_2, after 1000 generations of evolution under high CO_2 conditions in the lab (Collins and Bell, 2004)
Selection experiments	Researchers expose a population of organisms to artificial selection in the laboratory. The response to selection in the next generation is proportional to the amount of genetic variation present for that trait	A marine crustacean showed limited ability to evolve increased heat tolerance, after 10 generations of selection in the laboratory (Kelly *et al.*, 2012) The rainforest endemic *Drosophila birchii* showed variation in desiccation resistance among populations, but the most tolerant population lacked the ability to evolve any further resistance after 30 generations of laboratory selection (Hoffmann *et al.*, 2003)

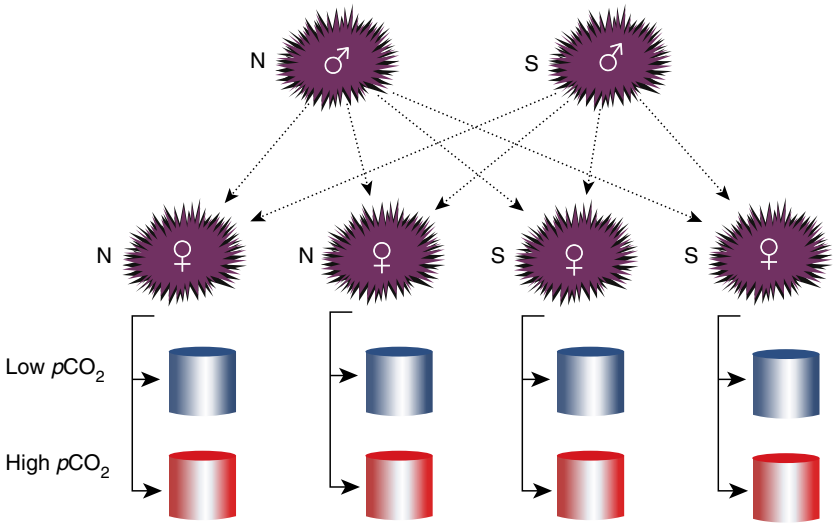

Figure 3 Controlled crosses raised under experimental conditions can be used to estimate genetic variation for traits involved in the response to environmental change. Modified from Kelly, M.W., Hofmann, G.E., 2012. Adaptation and the physiology of ocean acidification. Functional Ecology 27, 980–990.

copepod to 10 generations of selection for increased heat tolerance (Kelly *et al.*, 2012). After five generations of selection, populations evolved up to half a degree of additional heat tolerance. However, the response to selection plateaued rapidly, with no additional increase in heat tolerance between generations five and ten of selection in most populations. Although populations varied in their heat tolerance across the species, individual populations effectively 'ran out' of genetic variation after five generations of selection, suggesting limited capacity to adapt to increasing temperatures without gene flow from more tolerant populations.

Will Evolution Matter?

Theoretical models suggest that evolution might buy time for carbon emissions standards to take effect. For example, theoretical models that do not incorporate evolution predict the

collapse of coral reefs by the middle of this century, while models that incorporate adaptation predict the persistence of reefs into the next century under moderate greenhouse emissions scenarios (Baskett *et al.*, 2009). Incorporating evolutionary change into theoretical models also changes predictions about the probability of negative events. In Australia, climate change is expected to increase habitat suitability for *Aedes aegyptii,* the mosquito that carries dengue fever. Accounting for the potential evolution of higher desiccation resistance in expanding populations increases the projected risk that dengue fever will become established in a major urban area (Kearney *et al.*, 2009).

Incorporating Evolutionary Thinking into Management Decisions

There are a number of ways that evolutionary thinking can be incorporated into management efforts aimed at minimizing biodiversity losses from climate change. If populations of a species are locally adapted to a current climatic gradient, management efforts might focus on maintaining corridors of gene flow among populations, with the understanding that populations currently adapted to the warmest/driest conditions within a species range are an important source of variation for adaptation to future conditions in other populations. By contrast, in highly panmictic species, management efforts might focus on maintaining large local population sizes, to maximize potential for evolutionary adaptation *in situ.* Evolutionary thinking may also lead to changes in assessments of species' relative vulnerabilities to climate change. For example, evolutionary change in some populations may render them less vulnerable than would have been predicted from that populations' current range of environmental tolerances (Lohbeck *et al.*, 2012; Kelly *et al.*, 2013). On the other hand, in species with high levels of natural fragmentation, each population may contain a small subset of the environmental tolerances present in the species as a whole, so that individual populations will be more vulnerable to environmental change than would be predicted from the species-level range of tolerances (Kelly *et al.*, 2012; Schiffers *et al.*, 2013).

At this point, relatively few studies have measured evolutionary responses to climate change or the potential for future change in natural populations. Even fewer empirical studies have addressed the relative magnitudes of plastic versus evolutionary changes or the potential rate of evolution relative to the expected rate of environmental change. Evolutionary thinking can make important contributions to management decisions aimed at minimizing biodiversity losses, but more data are needed to better understand the role that evolution will play in species' responses to climate change.

See also: Climate Change, Quantitative Genetics and. Life-History Evolution, Human Impacts on. Quaternary Biogeography and Climate Change

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Ring Species

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The perfect demonstration of speciation is presented by the situation in which a chain of intergrading subspecies forms a loop or an overlapping circle, of which the terminal forms no longer interbreed, even though they coexist in the same localities. (Mayr, 1942, p. 180)

With the above quote, the great evolutionary biologist Ernst Mayr concisely described the phenomenon now termed 'ring species' (Cain, 1954) and pointed out their importance to the study of speciation, the process by which one species evolves into two or more. Ring species are fascinating to biologists because they illustrate in geographic space a central principle of evolutionary biology that we usually conceive of occurring in time: that species can evolve via small, stepwise changes into new species (**Figure 1**). In a ring species, two distinct forms can be found in the same geographic region, such that most biologists would consider them different species; yet these forms are connected by a chain of populations through which traits change gradually from one species to the other (Mayr, 1942; Cain, 1954; Moritz *et al.*, 1992; Wake, 2001; Irwin *et al.*, 2001b). The two forms can be considered simultaneously as members of the same species (if one looks at the chain of intergrading populations) and as two distinct species (if one looks at the reproductive isolation in the overlapping region). As Mayr (1942, p. 180) stated, ring species are "disturbing to the orderly mind of the cataloguing systematist, but they are welcome to the student of speciation." The ring species concept has inspired much thought about speciation, both with regard to specific case studies (see below) and in development of theory (Gavrilets *et al.*, 1998; Ashlock *et al.*, 2010; Martins *et al.*, 2013).

A wide variety of ring species have been proposed (reviewed by Mayr, 1942, 1963, 1970; Irwin *et al.*, 2001b; Irwin and Irwin, 2002; see also Bowen *et al.*, 2001; Bensch *et al.*, 2009; Mulcahy and Macey, 2009; Patten and Pruett, 2009; Cacho and Baum, 2012; Eastwood *et al.*, 2014), but there is controversy over whether specific cases should be described as ring species. This is a result of wide variation among biologists in concepts both of what a ring species is and of how they form, and ultimately in the ways ring species are used as evidence for various evolutionary processes. Here we review these distinct uses of the ring species concept, discuss the various types of ring species and the ways they could form, examine two ring species in depth, and then look toward future investigations of ring species.

Uses of the Ring Species Concept

The literature reveals a striking characteristic of the history of thought on ring species: the basic concept has been used as illustrations of and support for evolutionary processes in at least four distinct ways:

Illustrating the Fact that Species Evolve

Ring species are often used as a simple yet powerful illustration that distinct species can be connected by a series of small stepwise changes. While ring species provide a taxonomic conundrum (should we call a ring species a single species or two (or more) species?), they in fact illustrate in space the fundamental way that a single species can evolve into two over time (**Figure 1**). It is primarily for this reason that ring species are used so often in textbooks on evolution: they illustrate the continuity between evolution within species and between species so clearly.

Showing the Importance of Geography in Speciation

In the earliest apparent clear articulation of the ring species concept, in a quote attributed to Leonhard Stejneger (as reported by Jordan, 1905), the concept is used to explain how two closely related forms might come to live in the same area,

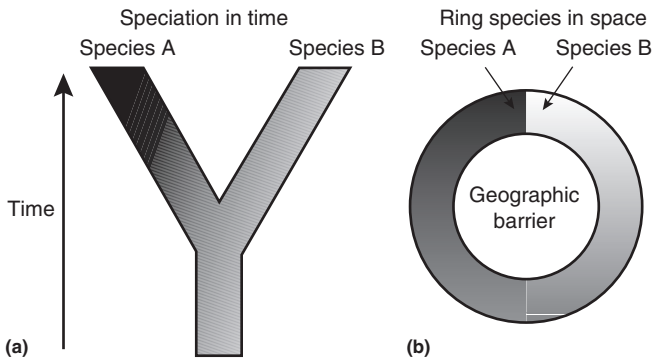

Figure 1 Speciation (a) is the process by which a single species diverges into two (or more) over time. Ring species (b) show a similar pattern over space, allowing geographic variation to serve as a possible proxy for divergence from a common ancestor over time.

by expansion from an ancestral area in two directions around a geographical barrier, the end points coming into contact and behaving as distinct species. In this context, the concept was first developed as support for the idea that geographic differentiation plays a key role in speciation: closely related species may be found living in the same area, but they diverged in different geographical areas and then expanded into the same region. Mayr (1942, 1963, 1970), who did much to popularize the concept, also emphasized this aspect of ring species, writing that "a more dramatic demonstration of geographic speciation than cases of circular overlap cannot be imagined" (Mayr, 1970, pp. 292–293; see also Cain, 1954). More recently, Newton (2003), Price (2008), and Coyne and Orr (2004) also emphasized ring species as illustrating various forms of geographic speciation.

Reconstructing the History of Changes During Speciation

Geographic variation in a ring species can sometimes be used to reconstruct how speciation occurred in time (Wake, 2001; Irwin *et al.* 2001a,b). This requires several assumptions: (1) the ancestral population is well represented by one of the intermediate forms in the ring, (2) we have a good idea which population is that source ancestral population, and (3) the current pattern of variation well represents the changes that occurred in time. A variety of genetic and biogeographic analyses can in some cases provide support for these assumptions, increasing confidence that such reconstruction of the history of speciation is valid.

Illustrating that Speciation Can Occur Despite Gene Flow

Finally, and more controversially, the ring species concept has sometimes been considered to demonstrate that speciation can occur without complete geographic and genetic isolation. The standard model of 'allopatric speciation' holds that speciation occurs following division of a single population into two geographically separated populations that do not exchange genes. Another theoretical possibility is 'speciation by distance,' in which divergence to the point of species occurs between the end points of a long chain of populations – gene flow can occur between neighboring populations, but the distance between the end points is so much greater than the movement of individuals that the two end populations are effectively isolated. Mayr (1942) initially emphasized both the geographic-speciation and speciation-by-distance aspects of the ring species concept, but later abandoned the speciation-by-distance idea as an important part of his concept of circular overlaps, given the "major gaps in nearly all of these chains of populations or at least evidence for the former existence of such gaps" (Mayr, 1970, p. 320). Nonetheless, he continued to hold up circular overlaps as prime examples in which "the process of geographic speciation can be followed step by step" (Mayr, 1970, pp. 292–293).

Dobzhansky went further than Mayr in emphasizing gene flow as an important feature of ring species, describing the chain of populations as a 'genetic bridge' between forms that are otherwise reproductively isolated (Dobzhansky, 1958; Dobzhansky *et al.*, 1964). His prime example, however, was based on various forms within the *Drosophila paulistorum* complex, in which gene flow was inferred using laboratory breeding experiments, and he acknowledged that "it is questionable whether [gene flow] is actually taking place" (Dobzhansky *et al.*, 1964).

Given these varying ways that the ring species concept has been used, it is unsurprising that there is debate over proposed cases in terms of whether they can be described as ring species. The debate can be simplified by clearly separating questions about pattern from questions about process. We can organize our thinking by considering two basic questions: First, what patterns have been described as ring species? Second, what processes lead to their formation?

Types of Ring Species

Scenarios described as circular overlaps or ring species come in a wide variety of flavors. The ideal case is illustrated in Figure 2(a), in which there is a complete ring of gradually varying populations, the end points of which (in blue and red) act as reproductively isolated species where they meet. There have never been actual proposed examples that fit such an ideal scenario; rather all cases that have been proposed (reviewed by

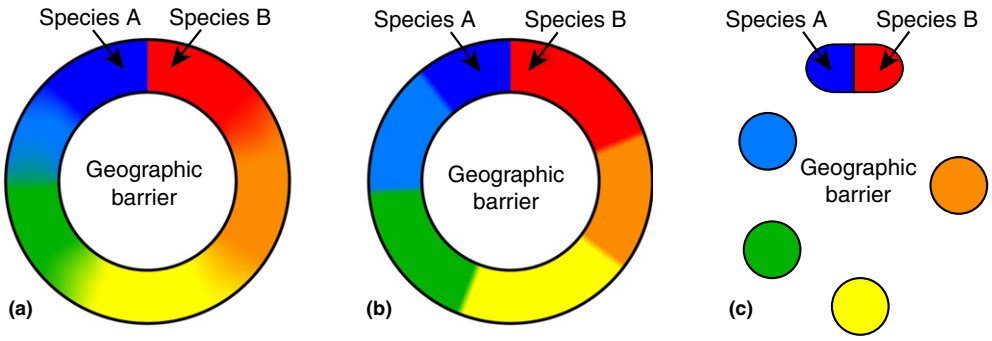

Figure 2 The types of situations that have been described as ring species. The ideal situation (a) is one in which a continuous ring of populations, through which traits and genes change gradually, except at a single location where distinct forms live side-by-side. All known cases of ring species fit less ideal situations in which there are stepwise, rather than completely gradual changes around the ring (b) and/or large gaps in distribution around the ring (c). Nonetheless, those situations illustrate the central concept of a ring species: a single species boundary through a ring of populations.

Mayr, 1942, 1963, 1970; Stebbins, 1949; Irwin *et al.*, 2001b; see also Bowen *et al.*, 2001; Bensch *et al.*, 2009; Mulcahy and Macey, 2009; Patten and Pruett, 2009; Cacho and Baum, 2012; Eastwood *et al.*, 2014) have imperfections of one sort or another. These real-world cases usually have a mixture of two other patterns, illustrated in **Figures 2(b)** and **2(c)**. In these cases, variation around the ring is not completely gradual (2(b)) and/or continuous (2(c)). Nonetheless, the overall pattern is one in which a series of progressively intermediate forms is arranged in a rough geographic order between the co-occurring and most divergent terminal forms (shown in blue and red). Another common complication is that reproductive isolation between the terminal forms can be incomplete, with some direct hybridization and introgression occurring between them (Brown, 1974; Wake *et al.*, 1989; Devitt *et al.*, 2011; Alcaide *et al.*, 2014). However, it is now generally accepted that reproductive isolation between distinct species is often incomplete (Coyne and Orr, 2004), such that a small amount of direct introgression is not inconsistent with the ring species description. The unifying characteristic common to all of these types of ring species is that there is a single species boundary around the ring: species A and B are considered two species where they meet, but there is no species boundary through the chain of populations connecting them around the ring (Cain, 1954).

How Do Ring Species Form?

Because ring species have generally been used as evidence for evolutionary processes, we have a fairly good understanding of various authors' ideas regarding how ring species form. In the ideal scenario (**Figure 3(a)**), which could be used for all of the purposes referred to above, an ancestral form expands from one side of the ring along two pathways around a geographic barrier, with progressive differentiation occurring before the terminal forms meet each other on the other side of the ring, where they are reproductively isolated. During this differentiation process, individuals can move a short distance during their lives in any direction, such that gene flow is continuous, although low in magnitude, throughout the ring. As mentioned above, we have no good examples of fully gap-free and completely gradual rings, so empirical evidence for such an ideal scenario is lacking. However, there is much evidence for cases in which populations expanded from a common ancestor along two pathways, colonizing a series of habitat patches in each pathway, with the final colonization event in each chain bringing the end points together again (**Figure 3(b)**). Such a scenario was outlined for many of the examples provided by Mayr (1942, 1963, 1970), as well as the recently proposed example in plants (Cacho and Baum, 2012). Finally, such a colonization process can be followed by expansion of each population into the gaps in the ring (**Figure 3(c)**), resulting in contact between previously separated populations and resulting in broad blending (**Figure 2(a)**) or the formation of narrow hybrid zones (**Figure 2(b)**).

In reality, most species complexes have had very complex histories, involving periods of geographic isolation and re-expansion as well as divergence-with-gene-flow (Mayr, 1942, 1963, 1970; Wake, 1997; Alcaide *et al.*, 2014), such that particular ring species may have formed via a complex mix of the processes illustrated in **Figure 3**. Regardless, the resulting pattern of two mostly reproductively and co-occurring forms being connected by a chain of progressively intermediate forms illustrates the evolutionary continuity between species and in some cases allows the inference of processes important in the generation of reproductive isolation.

Example Ring Species

There have been a large number of proposed ring species, but evaluating many of them is difficult due to lack of detailed research on specific cases as well as to the variety of ring species concepts. We propose that the emphasis should be not on declaring whether particular cases are or are not ring species, but rather on understanding the particulars of each proposed case and what we can learn from it. Specific cases should be viewed as lying somewhere on a spectrum between the ideal ring species (**Figures 2(a)** and **3(a)**) and clear examples of non-ring species.

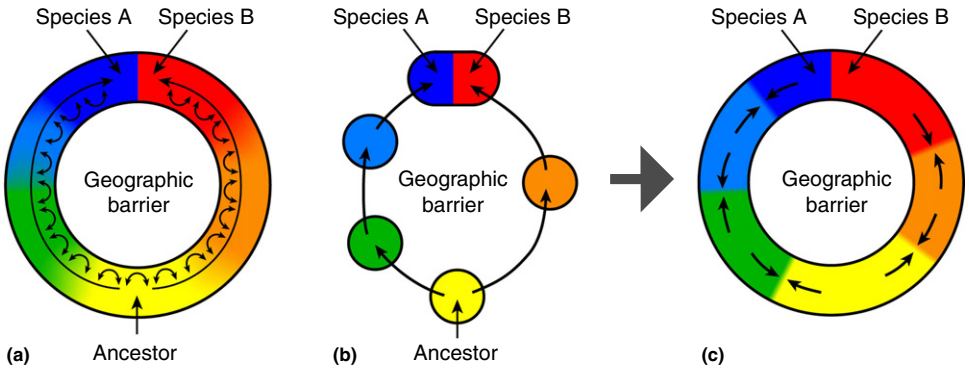

Figure 3 Scenarios by which ring species might form. In (a), an ancestral form expands around a geographic barrier, diverging gradually despite continuous gene flow at all stages of the process. In (b) populations colonize new areas and differentiate in a stepwise manner but there are major gaps in distribution, and divergence eventually proceeds through the chain of populations to the level of distinct species. In (c), the populations expand to meet each other again in narrow zones of intergradation. In each case, divergence to the level of distinct species has occurred only between the terminal forms.

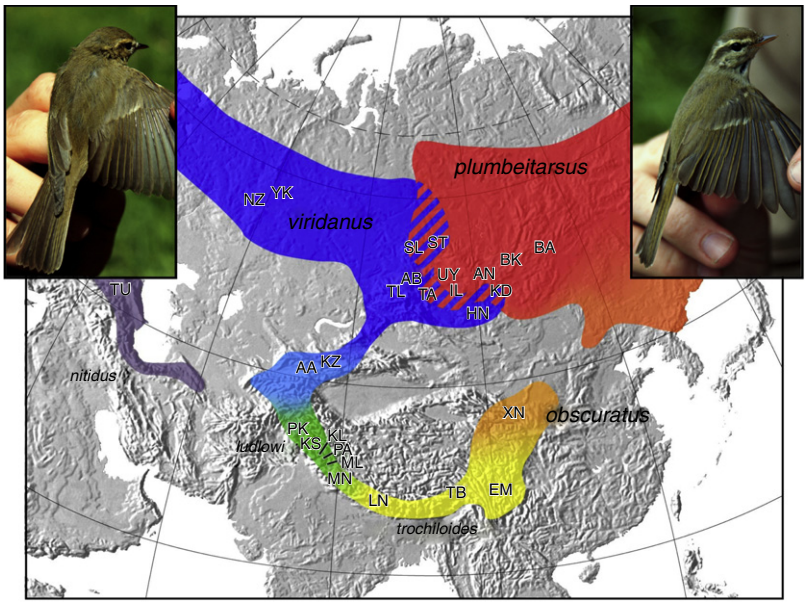

Figure 4 The breeding range of greenish warblers (*Phylloscopus trochiloides*) in Asia. Subspecies designations according to Ticehurst (1938) are shown with different colors: *viridanus* in blue, *ludlowi* in green, *trochiloides* in yellow, *obscuratus* in orange, *plumbeitarsus* in red, and *nitidus* (outside of the main ring) in purple. Photos show the difference in wing bars between *viridanus* (upper left, with a single wing bar) and *plumbeitarsus* (upper right, with two wing bars).

Of all of the cases that have been proposed, two in particular, the greenish warblers in Asia and the *Ensatina* salamanders in California, have stood out as meeting the ring species definition fairly well in terms of being a chain of intergrading forms with distinct coexisting terminal forms. We discuss each in some detail, before mentioning other proposed cases.

Greenish Warblers

In 1938, Claude Ticehurst conducted a detailed taxonomic reassessment of the *Phylloscopus* genus of Old World warblers (Ticehurst, 1938), based on examination of morphological variation in museum skins (e.g., body shape and size, as well as plumage color variation). He provided an intriguing description of six subspecies within the greenish warbler (*Phylloscopus trochiloides*) species complex: two subspecies, *viridanus* in west Siberia and *plumbeitarsus* in east Siberia, differed in plumage but co-occurred in central Siberia without intermediates there, suggesting reproductive isolation. But these forms were apparently connected by a ring of progressively changing forms: *viridanus* extended south into central Asia, where it met *ludlowi*, which Ticehurst described as a transitional form leading to *trochiloides*, the southern form spanning across the Indian and Nepali Himalayas and into southern China. Next came *obscuratus* in central China, which appeared intermediate between Himalayan *trochiloides* and east Siberian *plumbeitarsus*. Based on this description, Mayr (1942) featured the greenish warblers as an illustrative example of circular overlap. Ticehurst hypothesized that the situation arose when an ancestral species in the Himalayas (i.e., in the south) expanded along two pathways northward, separated by the high-altitude Tibetan Plateau.

Both the ring-species description and the proposed parallel northward expansion have now been tested by examining a variety of genetic, morphological, and behavioral traits, and the patterns are broadly consistent with Ticehurst's hypothesis (**Figure 5**; Irwin, 2000; Irwin *et al.*, 2001a, 2005, 2008; Wake, 2001; Alcaide *et al.*, 2014). Vocalizations (both songs and calls), plumage characteristics (mainly wing bar size), and genetic markers (including mitochondrial DNA, microsatellites, AFLPs, and genomic SNPs) differ strongly between *viridanus* and *plumbeitarsus*, supportive of there being strong reproductive isolation between them in central Siberia. Genetic data are supportive of two northward expansions into Siberia, as west Siberian *viridanus* is most related genetically and phenotypically to central Asian *viridanus* and *ludlowi*, and east Siberian *plumbeitarsus* is most related to *obscuratus* in China and *trochiloides* in the eastern Himalayas. Phenotypic traits such as songs, plumage, and migratory behaviors show gradual change around the ring (**Figure 5**). The pattern of song variation is particularly interesting: songs are simple in the south but become increasingly long and complex toward the north, but the form of complexity that has evolved during the two northward expansions differs dramatically between them, resulting in highly divergent songs in west and east Siberia (Irwin, 2000; Irwin *et al.*, 2008), where songs appear to play a role in reproductive isolation (Irwin *et al.*, 2001a).

Complicating the ring species scenario is evidence for a zone of steep genetic transition in the western Himalayas near the boundary between Kashmir and Himachal Pradesh, in a region where there is little change in song or plumage. This genetic transition was first observed as a narrow overlap area between the two major mitochondrial clades (Irwin *et al.*, 2001a). Later, patterns in AFLP markers were interpreted as supportive of gradual genetic variation through this region as

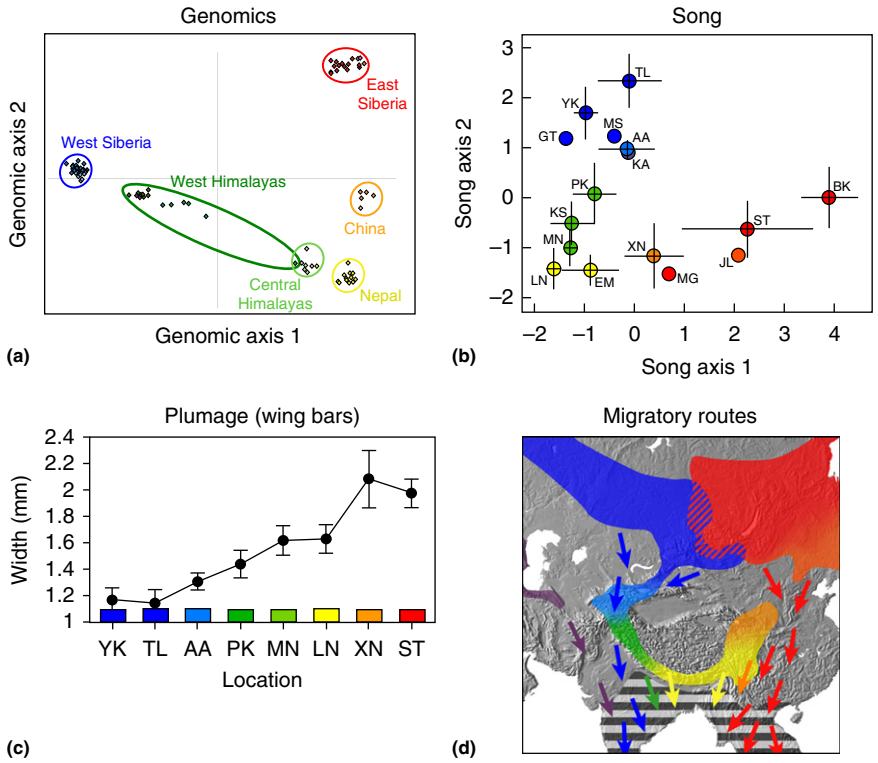

Figure 5 Patterns of genomic and phenotypic variation in greenish warblers. Colors correspond to the subspecies groups in **Figure 4**. Genomic variation (based on 2334 single nucleotide polymorphisms; Alcaide *et al.*, 2014), songs (Irwin, 2000; Irwin *et al.*, 2008), wing bars (Irwin *et al.*, 2001a,b), and migratory routes (Irwin and Irwin, 2005) all show strong differences between the two Siberian forms (*viridanus* and *plumbeitarsus*), but gradual or stepwise variation through the chain of populations to the south. These patterns are supportive of Ticehurst's (1938) and Mayr's (1942) description of the greenish warbler as a ring species, with two separate expansions northward into Siberia.

well as the entire ring, except between the highly divergent *viridanus* and *plumbeitarsus* (Irwin *et al.*, 2005), and this interpretation of isolation-by-distance was confirmed in subsequent analyses of the same data by Novembre and Stephens (2008), Zhang *et al.* (2009), and Martins *et al.* (2013). Finally, analysis of thousands of markers spread throughout the genome has revealed strong evidence for secondary contact in the western Himalayas of previously separated populations (**Figure 5(a)**; Alcaide *et al.*, 2014), within the range that Ticehurst described as the subspecies *ludlowi*. A further complication is that the genomic data shows strong evidence for a small amount of hybridization and introgression between *viridanus* and *plumbeitarsus*, such that reproductive isolation between those forms is not absolute (Alcaide *et al.*, 2014). There is also evidence for some individuals in central Siberia singing songs that mix elements of the songs of *viridanus* and *plumbeitarsus* (Irwin *et al.*, 2012b; Kovylov *et al.*, 2012). Nonetheless, those forms display highly divergent genetic clusters, indicating that the limited hybridization has not blended the two forms and suggesting that there is selection against introgressed genes.

The greenish warblers display the essential characteristics of a ring species: two highly differentiated forms that co-occur in one region (central Siberia) while being connected by a long chain of forms through which traits change gradually (e.g., song) or in a more stepwise fashion (e.g., genetics). However, the evidence for periods of geographic separation and secondary contact implies that greenish warblers do not provide a clear example of speciation by distance (Alcaide *et al.*, 2014), counter to a previous interpretation based on more limited genetic data (Irwin *et al.*, 2005).

Ensatina Salamanders

In 1949 R.C. Stebbins at the University of California, Berkeley, published a monograph on the *Ensatina* genus of salamanders that had a surprising conclusion: what had been treated as a genus with four distinctively colored species distributed along the west coast of North America was reduced by Stebbins to a single species comprised of 7 subspecies distributed in the form of a geographic ring (**Figure 6**; Stebbins, 1949). The subspecies all occur in California, where they are wrapped around the inhospitable Great Central Valley and extend southward both along the coast and inland into northern Baja California, Mexico. A form with the most generalized color pattern occurring in the historically most stable part of the overall range, in northwestern California, was hypothesized to have spread inland and southward through the Sierra Nevada, in one leg of its distribution, and separately along the coast southward through diverse coastal uplands. The forms were thought to have adapted to local and regional habitats and climates as they migrated southward, with coastal forms becoming more or less uniformly, and brightly, colored, and in the central coastal area they became aposomatically colored as part of a mimicry complex involving the dangerously poisonous news, *Taricha* (Kuchta, 2005; Kuchta *et al.*, 2008). In contrast, in the inner mountains

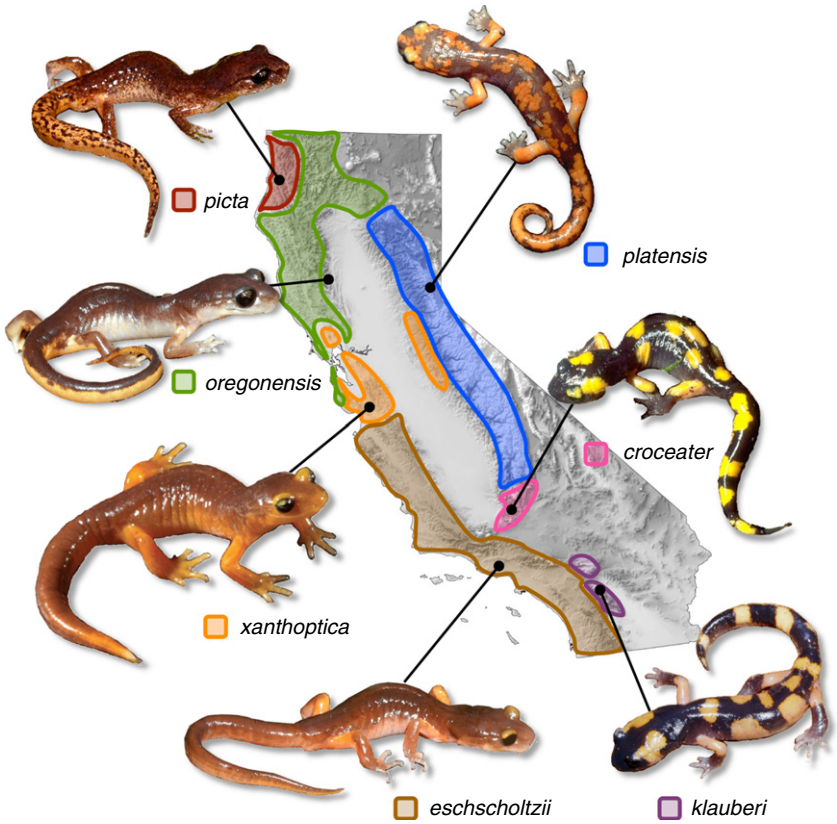

Figure 6 Geographic variation among races in the *Ensatina eschscholtzii* ring species in California. Reproductive isolation is stronger between the southern forms *klauberi* and *eschscholtzii* than elsewhere around the ring. Reproduced from Pereira, R.J., Monahan, W.B., Wake, D.B., 2011. Predictors for reproductive isolation in a ring species complex following genetic and ecological divergence. BMC Evolutionary Biology 11, 194, with permission from BMC Evolutionary Biology.

the salamanders evolved cryptic coloration and behavior, becoming spotted and blotched (such that they match the leaf litter well). The end points of these adaptively divergent pathways appeared to be different species.

Stebbins, who was renowned for his artistic abilities as a painter as well as being a serious scientist, argued that the forms intergraded with each other at various points in the ring. His critical insight was that the two major types came into near sympatry in Southern California, and he recognized signs of hybridization and intergradation between forms elsewhere around the ring. Nowhere was the ring hypothesis seen in more vivid detail that in the central Sierra Nevada, where two remarkably distinct forms, the aposematically colored *Ensatina eschscholtzii xanthoptica* and the cryptically colored, blotched, *Ensatina eschscholtzii platensis*, met and hybridized extensively, the result of a 'mid-valley leak' that had brought coastal forms into contact with Sierran forms (essentially a 'test' of the ring species concept mid-way through the ring); this contrasted with the much stronger reproductive isolation between distinct forms *Ensatina eschscholtzii klauberi* and *Ensatina eschscholtzii eschscholtzii* at the southern end of the ring. Stebbins did not think that the ring resulted from smooth expansion without any geographic breaks in continuity around the ring, but rather as something that had developed in fits and starts, with many interruptions and long periods during which regional adaptation occurred.

Subsequent research has added much detail and texture to the *Ensatina* example, and in recent years a heavy emphasis on molecular traits has shown that the ring of forms is old (millions of years) and deeply differentiated (Wake and Yanev, 1986). Both intergradation (based originally on color analysis, now backed up by allozyme data) and hybridization are well documented at various places around the ring (Wake and Schneider, 1998; Kuchta *et al.*, 2009). It is now thought (Jackman and Wake, 1994; Pereira and Wake, 2009; Pereira *et al.*, 2011) that the blotched forms were separated from other members of the complex for a long time before moving back northward and meeting the unblotched forms in secondary intergradations north and west of Lassen Peak, in northeastern California. Some hybridization has been observed between the terminal forms in southern California (Brown, 1974; Wake *et al.*, 1989; Devitt *et al.*, 2011), but it is more restricted than in the central Sierra Nevada (Alexandrino *et al.*, 2005), and at the southern-most possible point of meeting of the two main lineages, in the Cuyamaca Mountain of inner San Diego County, California, there is sympatry with no evidence of local hybridization (Wake *et al.*, 1986). While some have questioned the ring species description of the *Ensatina* complex (e.g., Highton, 1998) no primary researchers focused on the group has felt their results constituted a refutation of Stebbins' original hypotheses concerning intergradation, hybridization, and sympatry at different places within the ring,

nor, especially, of his broad biogeographical hypothesis. Despite the complexities, there is a single place in the ring where genetic and phenotypic differentiation are much stronger than elsewhere around the ring.

Other Proposed Cases

At least two dozen species complexes have been described as 'circular overlaps' or 'ring species' at one time or another (Mayr, 1942, 1963, 1970; Bowen *et al.*, 2001; Irwin *et al.*, 2001a,b; Patten and Pruett, 2009; Cacho and Baum, 2012; Eastwood *et al.*, 2014), but many of these are far from the ideal. Most of them are cases cited by Mayr (1942, 1963), containing large geographic gaps in distribution (e.g., similar to **Figure 2(c)**). Several cases that have been commonly used as example ring species in the popular literature have recently been called into question; these include the herring gull (*Larus argentatus*) complex surrounding the Arctic Ocean (Crochet *et al.*, 2002; Irwin and Irwin, 2002; Liebers *et al.*, 2004), the great tit (*Parus major*) complex (Kvist *et al.*, 2003; Päckert *et al.*, 2005), and the crimson rosella (*Platycercus elegans*) complex (Joseph *et al.*, 2008; Ribot *et al.*, 2009). In each of these cases, molecular analysis has revealed sizeable discontinuities and a lack of support for previously articulated biogeographic scenarios, leading some authors to declare that they are not ring species. During the same period of time that these classic cases were being called into question, a few other potential cases have been proposed. These include the first convincing example of a possible ring species in plants, in the Caribbean slipper spurge (*Euphorbia tithymaloides*; Cacho and Baum, 2012), the ring-like evolutionary pattern described for Night Snakes (*Hypsiglena*) around the Gulf of California (Mulcahy and Macey, 2009), an apparent 'incipient ring species' in willow warblers (*Phylloscopus trochilus*) surrounding the Baltic Sea (Bensch *et al.*, 2009; Irwin, 2009), a possible although complex example in song sparrows (Patten and Pruett, 2009), and a case in trumpetfish (*Aulostomus* sp.), although in the latter case the terminal forms of the ring appear to be blending together (Bowen *et al.*, 2001). Particularly intriguing is the first proposed case of a ring species in a pathogen (Eastwood *et al.*, 2014), in beak and feather disease virus infecting the crimson rosellas, which have themselves been proposed as ring species (see above). Most of these cases have strong discontinuities around the ring, such that the cases recently proposed as ring species ironically have many of the characteristics of those recently declared not to be ring species. This is a reflection of the fact that biologists use a variety of meanings of the word 'ring species,' and why we advocate that the term be used for any current situation in which a loop of populations has a single species boundary, avoiding definitions that include a statement about the process of formation of such a situation.

Conclusion

Both the greenish warblers and *Ensatina* salamanders are ring species in the sense that two forms that have diverged to the species level are connected by a chain of populations in which there is gradual or stepwise change, such that there is no clear species-level boundary through that chain. They both beautifully illustrate the first two purposes for which ring species have been used: as demonstrations that species differences are a result of evolution and that geography often plays an important role in the evolution of separate species. Species are not fixed entities, and the barriers between them are fluid and context-dependent. These cases also have been used to infer the history of changes during speciation. This is perhaps most clear in song variation of greenish warblers (**Figure 5(b)**), which provoked the working hypothesis that short, simple ancestral songs gradually evolved into the long, complex, and divergent songs of the two Siberian species. Both cases are however not good examples of speciation by distance, and the quest will have to continue for any examples that fit the ring species concept in that most ideal form (**Figures 2(a)** and **3(a)**). We note however that there is strong evidence from a variety of other sources for divergence-with-gene-flow (Pinho and Hey, 2010), and increasing evidence for speciation-with-gene-flow (Martin *et al.*, 2014), such that evidence for these phenomena from ring species is not essential to the validity of those ideas. In most of the literature on speciation-with-gene-flow, however, reproductive isolation is envisioned as evolving directly within the chain of populations connecting the diverging forms. The ring species concept differs in that reproductive isolation evolves not within the chain but rather between the overlapping end points.

One reason that the ideal ring species scenario (**Figures 2(a)** and **3(a)**) has proven so elusive is that it requires an unusual geographic arrangement of ecologically suitable and unsuitable areas of just the right geographic sizes, such that the central barrier is large enough to prevent movement of individuals across it while the ring is small enough for gene flow to keep neighboring populations similar. Furthermore, a period of ecological stasis long enough for the ring to form is necessary. It is likely this last requirement that is not often satisfied on this complex Earth with its frequent climatic shifts. Divergence to the level of species tends to occur on a scale of hundreds of thousands to millions of years, whereas climate shifts occur on a scale of hundreds to thousands of years, such that species distributions are undergoing frequent shifts. Hence it may be unlikely that we will find any cases that fit the most ideal scenario, but the search should still go on, as the Earth is filled with many possible barriers around which ring-like distributions may form (Irwin, 2012a; Monahan *et al.*, 2012).

See also: Speciation Continuum. Speciation-with-Gene-Flow

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RNA Viruses, Evolution of

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Glossary

Capsid Protective protein coat that surrounds the viral genomic nucleic acid and possessed by all true viruses.
Clonal interference Competition (interference) between advantageous mutations as they go to fixation in asexual populations.
Complementation Where a defective virus particle is rescued by utilizing the functional genes/proteins of a virus that co-infects the same cell.
Defective interfering (DI) particle Defective virus genome, usually because of deletion mutations, that can compete (interfere) with co-infecting functional viruses.
Endogenous virus element (EVE) Partial (usually) or complete (occasionally) viral genome that is integrated into the host genome and transmitted through the germ line. RNA, DNA, and retroviruses all give rise to EVEs.
Epistasis Mutational effects that are different in combination than individually. Antagonistic epistasis reduces the fitness effect of combined mutations, whereas synergistic epistasis increases this effect.
Incomplete lineage sorting It occurs when there has been insufficient time for allele fixation (in the viral genome) between host speciation events and can lead to a mismatch between virus and host phylogenies.
Molecular clock A model in which molecular evolution proceeds at an approximately constant rate.
Muller's ratchet Progressive decrease in fitness due to the accumulation of deleterious mutations in asexual populations of finite size.
Multiplicity of infection (MOI) Technically defined as the ratio of viruses to the number of infected cells, but can more simply be thought of as the number of viruses infecting an individual cell.
Overlapping reading frame (overprinting) Translation starts on different positions within a codon leading to more than one protein product from a single codon region.
Persistent infection A relative term, that usually reflects an RNA virus infection that lasts for many months or years, or for most of the life span of the host. In contrast, acute infections may only last for a few days.
Phylodynamics Subject describing how the combination of evolutionary, epidemiological, and immunological processes determines the structure of phylogenetic trees.
Polymerase Essential, but error-prone, replication protein common to all RNA viruses. RNA viruses employ an RNA-dependent RNA polymerase, while retroviruses employ reverse transcriptase in which DNA is made from an RNA template.
Reassortment Form of recombination in segmented viruses in which viruses with different ancestries are packaged into the same virion leading to hybrid progeny.
Robustness The ability to maintain phenotype in the face of deleterious mutation.
Segment Independently replicating genomic molecule. Some RNA viruses carry a single segment (such that they are unsegmented), while others possess multiple segments. Viruses with multiple segments are able to reassort.
Sequence space All possible mutational combinations in a nucleotide or amino acid sequence.
Virion The mature virus particle containing the RNA genome and the full set of proteins.
Viroid Small circular RNA molecules that cause a variety of plant diseases and which do not encode proteins. Because they do not possess a coat protein they are not considered true viruses.

The Origins and Evolutionary History of RNA Viruses

The Origins of RNA Viruses

Determining when and how viruses originated is one of the most difficult topics in the study of viral evolution. The central challenge is that viruses likely originated so long ago that the signal of evolutionary history has been largely eroded and there is no fossil record. Despite this intrinsic difficulty, two theories dominate discussions of viral origins: that viruses have a pre-cellular origin, such that they arose before the last universal cellular ancestor, or that they evolved later as 'escaped' host genes that acquired protective capsid (coat) proteins and the ability to replicate autonomously.

According to the pre-cellular theory, RNA viruses are likely to be descendants of replicating elements that existed in a hypothetical and primordial 'RNA world.' Modern day viroids from plants, which share many similarities with RNA viruses, have been proposed to be remnants of this ancient time in earth's evolution (Flores *et al.*, 2014). Similarly, DNA viruses would be remnants of the first DNA replicators, with retroviruses' descendants of the first molecules that made the major evolutionary transition from RNA to DNA. Although difficult to test, there is growing support for the pre-cellular theory, particularly in the guise of protein structures that are conserved among highly divergent viruses (Koonin and Dolja, 2014). One of the most compelling examples is the jelly-roll capsid, a protein structure that forms the major capsid subunit of viruses with icosahedral shaped virions. Remarkably, the jelly-roll capsid is found in diverse RNA and DNA viruses that exhibit no clear sequence homology (Bamford *et al.*, 2005), which is strongly suggestive of ancient common ancestry.

In contrast, the escaped gene theory proposes that viruses originated from host cells on multiple occasions, and perhaps

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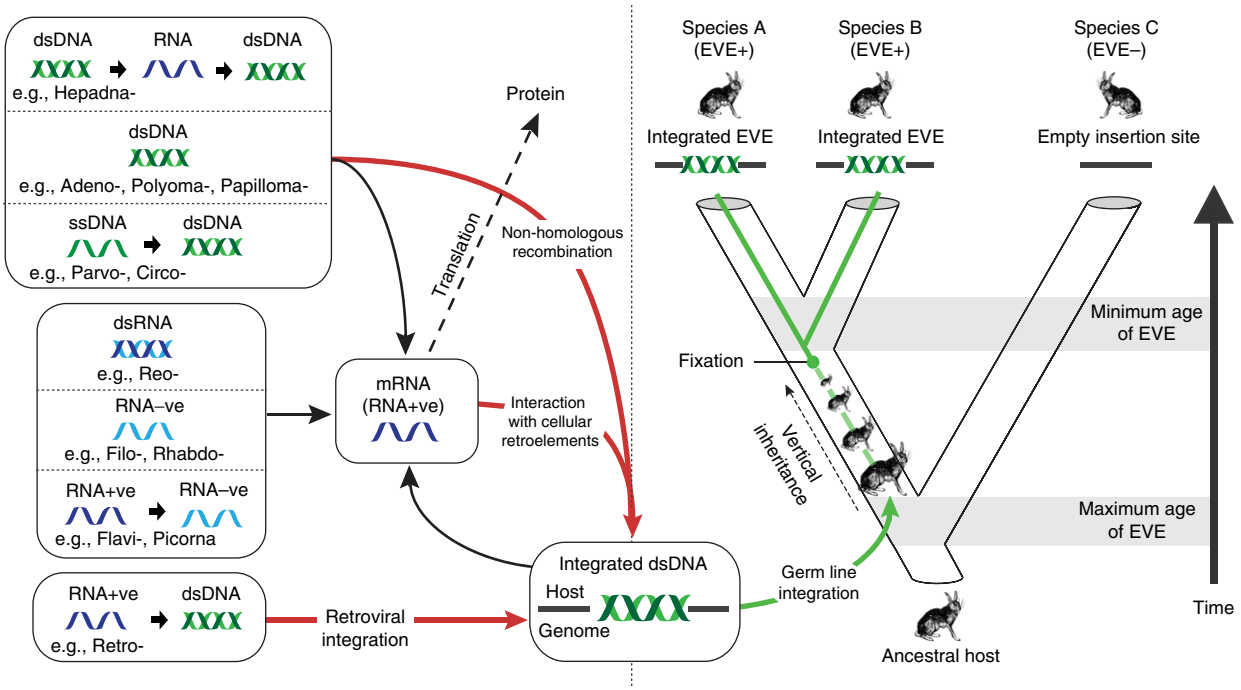

Figure 1 The evolution of endogenous virus elements (EVEs), which can occur in both RNA and DNA viruses, of which different types are shown. Importantly, EVEs allow estimates of the timescale of viral evolution. The presence of orthologous EVEs in host species A and B means that this EVE integration event occurred prior to the divergence of these two species, but subsequent to species C, such that its age can be estimated if the divergence time of the host species is known. Reproduced from Katzourakis, A., Gifford, R.J., 2010. Endogenous viral elements in animal genomes. PLoS Genetics 6, e1001191. Freely available through Creative Commons.

from cellular lineages that are now extinct. Hence, it is possible that RNA, DNA, and retroviruses represent independent episodes of host gene escape, which may also be the case for single-stranded (ss) and double-stranded (ds) RNA and DNA viruses. However, many escapes are proposed, the underlying theory is that a host gene that possessed or acquired the ability to self-replicate escaped from the cell, acquiring a protein coat on the way, eventually evolving into an independently replicating entity. A simple prediction of this theory is that key virus proteins (capsid, polymerase) have a host origin, although high levels of sequence divergence make this theory difficult to test. Although the escaped gene theory has fallen out of favor, it may have happened on occasion, with human hepatitis delta agent a possible example (Salehi-Ashtiani *et al.*, 2006).

The Timescale of Virus Evolution

Although RNA viruses are likely to be ancient replicators, it has proven difficult to provide a resolved timescale of their evolution because high divergence and frequent host jumping, and there are ongoing debates over the time of origin of major virus groups. Some information is provided by the match between the phylogenies of viruses and those of their hosts, which suggests that they have experienced long-term co-divergence (Sharp and Simmonds, 2011). A well studied example is that of the retrovirus simian foamy virus which has co-diverged with primates for at least 30 million years (Switzer *et al.*, 2005). More evidence for the deep timescale of virus evolution comes from the presence of endogenous viral elements (EVEs) that are a common component of eukaryotic genomes, particularly in the form of endogenous retroviruses (Katzourakis and Gifford, 2010). Once integrated into the host germ line they assume the low evolutionary rates of their hosts and can be used to estimate divergence times, particularly if orthologous ERVs are shared between species (**Figure 1**). Molecular clock dating using EVEs suggests that some human retroviruses diversified relatively early on in mammalian evolution. For example, there is evidence of endogenous foamy viruses in mammals for more than 100 million years (Katzourakis *et al.*, 2009).

Evolutionary Relationships among RNA Viruses

The evolutionary relationships of RNA viruses that are classified into different families have proven equally difficult to resolve. This problem is exasperated by the fact that viruses are likely the most common type of nucleic acid on earth and with the greatest genetic diversity, but where only a tiny fraction of the virosphere has been sampled. In addition, it is likely that lateral (horizontal) gene transfer events have occurred between diverse RNA viruses so that aspects of viral evolution may be better represented by an interconnected network of genes than a bifurcating phylogenetic tree (Liu *et al.*, 2012).

Many studies in this area have relied on phylogenetic analyses of the sequences that encode the RNA-dependent RNA polymerase (RdRp), the most conserved RNA virus protein. However, RdRp phylogenies are often highly uncertain, especially at the inter-family level, where there is

sometimes no more sequence similarity across the protein as a whole than expected by chance alone (Zanotto *et al.*, 1996). Despite this, different RNA virus families still share a number of short amino acid motifs in the RdRp, some of which are also found in the RT used by retroviruses, again arguing for the ancient common ancestry of these replicase proteins (Koonin, 1991).

RdRp phylogenies, combined with information on genome structure, have also been used to construct higher level classification schemes, manifest as 'orders' of viruses that represent assemblages of multiple viral families. At the time of writing, four such orders exist: the *Mononegavirales*, comprising multiple families of unsegmented negative-sense RNA viruses, and three orders of positive-sense RNA viruses – *Picornavirales*, *Nidovirales*, and *Tymovirales*. Despite the apparent limits to phylogenetic analysis, it is likely that more orders will be identified in the future, and that apparent 'gaps' in the RNA virus phylogeny will be filled by more in-depth sampling of the virosphere.

RNA Virus Emergence

There are two ways in which RNA viruses become associated with their hosts: co-divergence in which viruses diverge (speciate) along with their hosts, perhaps over many millions of years (as in the case of the foamy viruses described above), and cross-species transmission, in which viruses jump between host species and which can happen on very short timescales. Cross-species transmission is the major process driving viral emergence, and hence is essential in understanding the origins of infectious disease. However, most 'emerging viruses' only cause sporadic (dead-end) infections with no onward transmission in the new host, such that ongoing viral evolution is needed to ensure full host adaptation. A simple rule of thumb in this context is that the closer the phylogenetic relationship among the host species, the more likely viruses will be able to successfully jump between them, and which is supported by large-scale comparative studies (Kitchen *et al.*, 2011). Indeed, as the ability to recognize, infect, and be released from host cells is central to cross-species transmission (Parrish *et al.*, 2008), then viruses should be better able to jump between closely related host species as these will usually harbor similar cell receptors. Other key adaptive barriers that need to be breached to ensure successful host adaptation include the evasion of host immunity and successful transmission to other individuals in the population.

The principal evidence for co-divergence is a match (congruence) between the tree topologies of host and virus, and which may be relatively commonplace in the case of persistent viral infections (Sharp and Simmonds, 2011). Although co-divergence may occur across long-evolutionary timescales, and has been relatively commonly documented in some DNA viruses, it is possible that some of the match between virus and host phylogenies may in fact be due to preferential host switching in which viruses jump to related hosts (Hoyal Cuthill and Charleston, 2013). More cases of host jumping will doubtless be discovered when our sampling of the viral world increases. Conversely, host and virus phylogenies may appear to be more different than they are because of incomplete lineage sorting (Sharp and Simmonds, 2011).

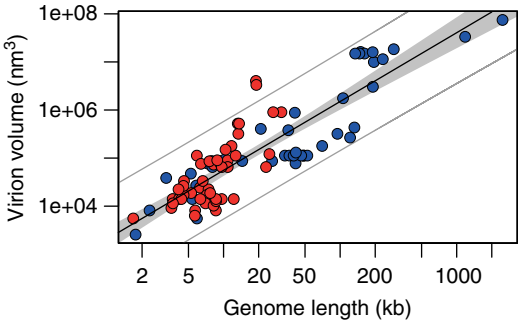

Figure 2 The relationship between the genome and virion sizes of viruses. RNA viruses are shown in red and DNA viruses in blue. Virion sizes, displayed as volume (nm^3) on a log scale, are shown on the *y*-axis, while genome length (kb) on a log scale is shown on the *x*-axis. The black line depicts the linear regression between log–log transformed data. The gray area shows the 95% confidence interval for the linear regression, while the outer gray lines represent the 95% prediction interval. Reproduced from Cui, J., Schlub, T., Holmes, E.C., 2014. An allometric relationship between the genome length and virion volume of viruses. Journal of Virology 88, 6403–6410.

Genome-Scale Evolution

The Evolution of Genome Structure and Size

RNA viruses exhibit a huge diversity of genome structures, including those with positive- and negative-sense orientations, those with single or double strands of RNA, and those with single or multiple segments. Despite such diversity, it is possible that all these forms of genome organization have evolved as ways of controlling gene expression (Holmes, 2009a). Interestingly, recent studies have revealed important phylogenetic links between segmented and unsegmented RNA viruses (Qin *et al.*, 2014), and shown that genome segmentation may allow RNA viruses to explore a greater proportion of sequence space than when only a single segment is present (Moreno *et al.*, 2014).

In contrast, the genome sizes of known RNA viruses exhibit relatively little variation, from less 2500 nucleotides (nt) in the case of some mitoviruses to approximately 32 Kb in the *Nidovirales*, with a mean value of approximately 10 000 nt. One theory is that genome sizes are constrained by the maximum size of the genomic material that can be contained within a virion. However, there is a remarkably strong (allometric) relationship between viral genome and virion sizes that covers viruses of all sizes and types (including RNA and DNA), indicating that viral genomes expand in size along with their virions (Cui *et al.*, 2014; **Figure 2**). A more plausible explanation is therefore that genome sizes are set by (high) background mutation rates, particularly as there is a strong relationship between mutation rate and genome size (see below; Gago *et al.*, 2009).

Mechanisms of Genome Evolution

Whatever the reasons for the genomic diversity in RNA viruses, a number of major evolutionary processes have been at play, although at uncertain frequencies and with complex patterns.

For example, while gene duplication must be responsible for some of the genomic and functional diversity seen in RNA viruses, particularly in the creation of different viral families, it has been relatively rarely documented (Simon-Loriere and Holmes, 2013). Importantly, more refined homology searching has revealed additional examples (Kuchibhatla *et al.*, 2014). It is possible that the relatively low frequency of gene duplication may reflect the major fitness costs (i.e., deleterious mutation load) associated with increases in genome size (see below). Lateral gene transfer (LGT) also appears to be a relatively rare event in RNA viruses (Song *et al.*, 2013), perhaps because it will similarly result in larger genomes. However, LGT has now been shown to have played a key role in the genesis of some virus groups (Liu *et al.*, 2012), and so may have generated important biodiversity. In addition, LGT can occasionally occur among between viruses and hosts with, for example, reports of RNA viruses incorporating host genome sequences (Megens *et al.*, 2014). A related theory is that of 'modular evolution,' in which viral genomes are proposed to comprise functional modules that can be exchanged through recombination to create novel viruses (Botstein, 1980), although its role in RNA virus evolution remains uncertain.

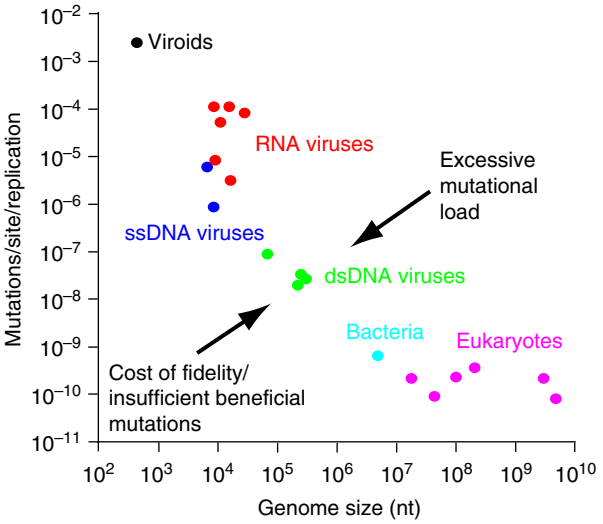

Figure 3 The fundamental relationship between log mutation rate (mutations/site/replication) and log genome size in diverse organisms, including RNA viruses and viroids. The evolutionary forces that might be responsible for the observed range of genome sizes and mutation rates are shown. Modified from Holmes, E.C., 2011. What does virus evolution tell us about virus origins? Journal of Virology 85, 5247–5251 and Gago, S., Elena, S.F., Flores, R., *et al.*, 2009. Extremely high mutation rate of a hammerhead viroid. Science 323, 1308.

Processes of RNA Virus Evolution

Rates of Viral Evolution

One of the most important facets of RNA virus biology is the rapidity with which these infectious agents evolve. Indeed, such is the pace of viral evolution that it can often be measured within the time-frame of human observation, providing the foundation for the science of phylodynamics (Holmes and Grenfell, 2009). This rapid evolution dictates much of the life history of RNA viruses and, in turn, evolutionary rates are a useful metric by which to compare RNA viruses.

Although complex to measure, current estimates of mutation rates in RNA viruses range from 10^{-3} to 10^{-6} mutations/nucleotide/cell infection (Sanjuán *et al.*, 2010). Lower rates, ranging from 10^{-8} to 10^{-6} mutations/nucleotide/cell infection, are observed in DNA viruses and reflect the higher fidelity of DNA polymerases, although ssDNA viruses mutate more rapidly than dsDNA viruses for reasons that are currently unclear (Holmes, 2009a). Importantly, these mutation rate estimates are compatible with the idea that there is an inverse relationship between mutation rate and genome size that applies to all living systems (Gago *et al.*, 2009; Holmes, 2011), such that mutation rates that are either too high or too low are selected against (**Figure 3**). Accordingly, mutation rates in RNA viruses are likely to be close to their maximum possible values (about one per genome replication; Drake *et al.*, 1998), with higher rates causing an excess of deleterious mutations, but where lower rates are also associated with significant fitness costs (Vignuzzi *et al.*, 2005).

Rates of nucleotide substitution show similar levels of variation (Duffy *et al.*, 2008), reflecting differences in both mutation and replication rates, including those associated with the host cell type infected as these turnover at different rates (Hicks and Duffy, 2014). Despite this variation, most substitution rates in RNA viruses fall between 10^{-3} and 10^{-4} nucleotide substitutions per site, per year (Duffy *et al.*, 2008). These rates are orders of magnitude higher than those seen in dsDNA viruses and cellular organisms (Holmes, 2009a), and are a major reason why RNA viruses are often able to adapt to new hosts and evade our attempts to control them through vaccines and antivirals.

Processes of Molecular Evolution in RNA Viruses

As with other organisms, understanding the major processes of evolutionary change in RNA viruses is of central importance. Because of the intimate evolutionary 'arms race' with their hosts, RNA viruses provide some of the very best examples of natural selection in action, with examples involving immune evasion, antiviral resistance, and the adaptation to new host species (Holmes, 2009b; Stapleford *et al.*, 2014). In turn, RNA viruses have exerted some of the strongest selection pressures on host genes (Daugherty and Malik, 2012).

Because the genomes of RNA viruses are small, with frequent epistasis (see below) and multifunctionality, it may be that very few mutations are strictly neutral. One study of mutational fitness in an experimental viral population revealed that nearly 40% of random mutations were lethal, 29% deleterious, a further 27% neutral, and only 4% beneficial (Sanjuán *et al.*, 2004). Broadly similar results have obtained using next-generation sequencing with, as expected, more synonymous than nonsynonymous mutations falling into the neutral class (although some were also advantageous), and with more lethal mutations nonsynonymous falling in nonstructural than structural genes (Acevedo *et al.*, 2014; **Figure 4**). Importantly, however, these measures are performed within a single cell, and many more deleterious

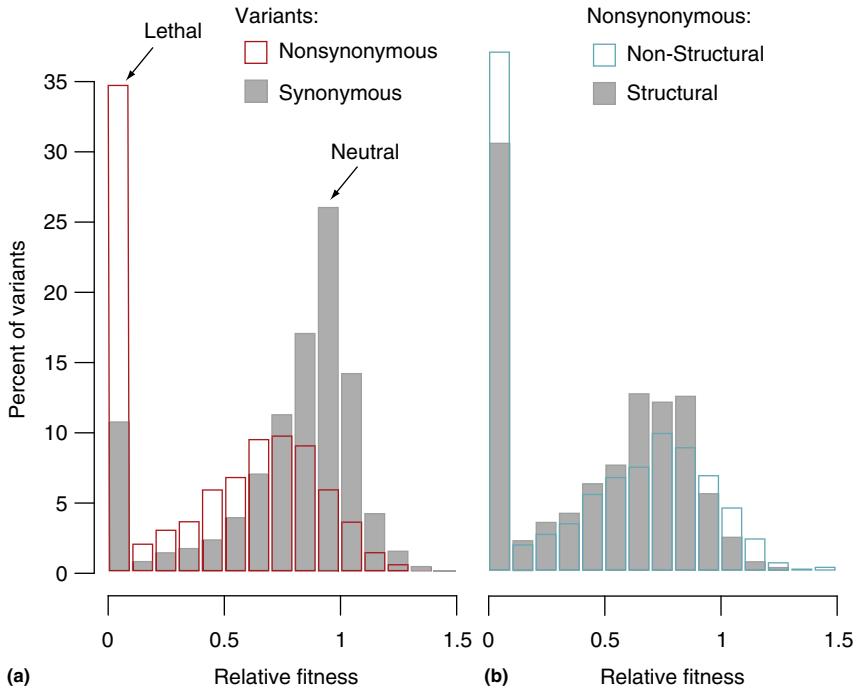

Figure 4 The fitness distributions of mutations in poliovirus as revealed by a next-generation sequencing approach. (a) The fitness of synonymous (gray) and nonsynonymous (red) mutations; (b) The fitness of nonsynonymous mutations in structural (gray) and nonstructural (blue) genes. Adapted from Acevedo, A., Brodsky, L., Andino, R., 2014. Mutational and fitness landscapes of an RNA virus revealed through population sequencing. Nature 505, 686–690.

mutations (and few neutral ones) are expected to accumulate across the entire virus life cycle, including inter-host transmission. Indeed, true measures of viral fitness must include the propensity for onward transmission within a population.

Similarly, RNA viruses possess very few, if any, pieces of functionless noncoding sequence, such as pseudogenes, and the common occurrence of overlapping reading frames (also known as 'overprinting') means that synonymous sites in one reading frame will often be nonsynonymous in another. As well as changing selective pressures, overprinting may lead to the creation of new viral proteins (Sabath *et al.*, 2012). The likelihood of neutral evolution will also be reduced by the presence of the RNA secondary structures that are a common feature of many RNA viruses, and which may contain control signals for such processes as replication and translation.

Codon usage may likewise have a profound effect on fitness. For example, there is growing evidence that the alteration of synonymous codon usage results in major fitness losses (Coleman *et al.*, 2008). This is supported by observations that synonymous sites can contain the signals for transcription and encapsidation (Marsh *et al.*, 2008). Many RNA viruses utilize synonymous codons that tend to match the nucleotide composition of the viral genome as a whole, suggesting that codon choice is either set by background mutational pressure, or that there is selection on overall nucleotide composition (Jenkins and Holmes, 2003). In other cases, there is evidence for direct selection on codon choice (Aragonès *et al.*, 2010), and codon choice has been suggested to influence the trajectory of virus evolution (Lauring *et al.*, 2012). Despite the mounting evidence that synonymous mutations can have important fitness consequences, it is also the case that levels of synonymous variation at the genomic scale are consistently higher than those at nonsynonymous sites, indicating that the fitness effects of synonymous mutations are usually less than those of nonsynonymous mutations (Acevedo *et al.*, 2014).

Another factor that influences the relative potency of natural selection is the virus effective population size (N_e), which is itself linked to the occurrence and strength of population bottlenecks. Large values of N_e are likely to occur within individual hosts following multiple rounds of replication and at the epidemiological (inter-host) scale if many hosts are infected. Natural selection is expected to be an efficient force in these cases. Conversely, low values of N_e, when genetic drift will be strongest, will occur during inter-host transmission and which may involve a major population bottleneck (Stapleford *et al.*, 2014). In some cases, such as certain forms of HIV-1 transmission, it is possible that infections are even initiated by a single virus particle (Keele *et al.*, 2008), although looser bottlenecks are seen in other viruses (Ghedin *et al.*, 2009).

Interactions between Virus Mutations and Genomes

Virus genomes and the mutations therein may interact in a variety of ways. Epistasis appears to be a common occurrence in RNA viruses, and most of the epistatic interactions determined to date are antagonistic rather than synergistic (Sanjuán *et al.*, 2004). Importantly, epistatic effects also vary between hosts, and therefore may be central to virus emergence (Lalić and Elena, 2013). The type of epistasis also impacts the level of genetic robustness seen in RNA viruses, itself a key aspect of adaptability. The high error rates of RNA viruses, combined with their highly compact genomes with little

redundancy, mean that mutations will repeatedly damage the same functions, resulting in antagonistic epistasis (Elena *et al.*, 2006). To overcome this, RNA viruses have evolved various mechanisms that provide them with some genetic robustness (Elena, 2012), and are in part protected by large population sizes.

Virus genomes can interact through the presence of defective interfering (DI) particles and complementation, both of which require a high multiplicity of infection. DI particles are a common observation in RNA viruses (Saira *et al.*, 2013), often harbor large genomic deletions, and have interesting evolutionary consequences. The shorter genomes of DI particles mean that they can replicate more rapidly than full-length viral genomes, and hence may theoretically inhibit the spread of the advantageous mutations. DI particles are likely to be maintained in virus populations through complementation, which is predicted to be a common occurrence when multiple viruses are able to infect a single cell (García-Arriaza *et al.*, 2004). Complementation is of evolutionary significance because it allows deleterious mutations to persist for extended periods (Aaskov *et al.*, 2006), but may also increase robustness (Gao and Feldman, 2009).

RNA Virus Recombination

Although RNA viruses are in some sense defined by their high mutation rates, recombination may also be a major contributor to their evolution. Recombination rates vary from effectively clonal (i.e., asexual) to cases in which the recombination rate per nucleotide exceeds that of mutation. There are two main forms of recombination in RNA viruses (**Figure 5**). 'True' RNA recombination occurs when two viruses co-infect a single host cell and a hybrid molecule is produced, most likely through 'copy-choice replication' in which the RNA polymerase jumps templates during replication (Simon-Loriere and Holmes, 2011). In contrast, reassortment occurs in segmented RNA viruses, and takes place when a virus packages segments with different ancestries.

The importance of recombination in viral evolution reflect its association with such key traits as host range, host immunity, antiviral resistance, and creating new viruses (Holmes, 2009a). For this reason it is commonly thought that recombination functions as a form of sexual reproduction to create advantageous genetic configurations. In addition, recombination may disassociate advantageous from linked deleterious mutations, and prevents clonal interference. Similarly, recombination facilitates the efficient removal of deleterious mutations. A related theory is that of Muller's ratchet, which has been observed in some laboratory viral populations (Chao, 1990). Although the rate of deleterious mutations (approximately one per genome replication) may be sufficient to provide a selective benefit to recombination in RNA viruses, this theory also requires that mutations interact through synergistic epistasis, for which there is currently little good evidence (Sanjuán *et al.*, 2004). In addition, because high rates of recombination are relatively infrequent in RNA viruses they are unlikely to be universally advantageous. It is also apparent that recombination rates vary across viral genomes, with distinct hot-spots and cold-spots, which may in part reflect patterns of RNA secondary structure (Runckel *et al.*, 2013).

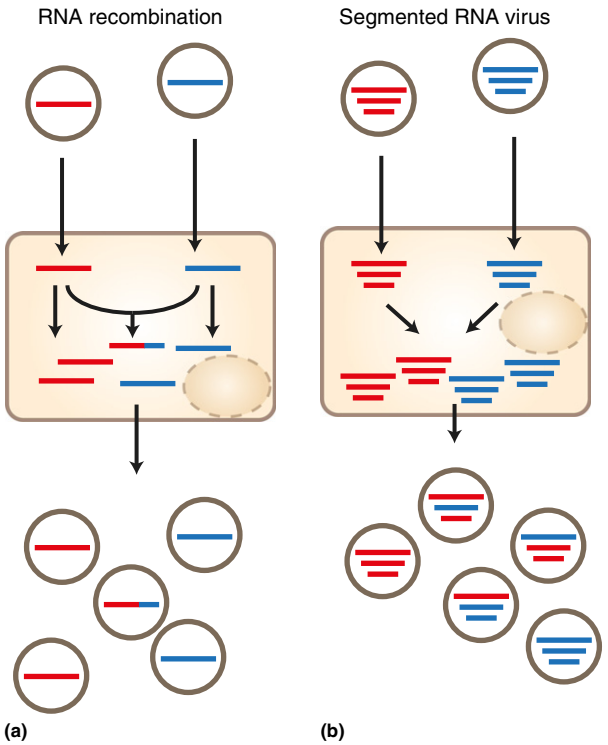

Figure 5 RNA recombination and reassortment in RNA viruses. (a) RNA recombination. This occurs following co-infection of a cell by different virus strains and can lead to the generation of recombinant viruses through copy-choice replication. RNA recombination can occur in both unsegmented viruses (as shown) or within the segment of a segmented virus. (b) Reassortment. This occurs following co-infection of a cell by different strains of a segmented virus, and can generate different combinations of reassortant progeny. Adapted from Simon-Loriere, E., Holmes, E.C., 2011. Why do RNA viruses recombine? Nature Reviews Microbiology 9, 617–626.

An alternative theory is that recombination rates simply reflect differing types of genome architecture and replication strategy (Simon-Loriere and Holmes, 2011), particularly because there is a general association between recombination frequency and virus genome structure. For example, the low recombination rates in (unsegmented) negative-sense RNA viruses may reflect the fact that their genomic and anti-genomic RNA molecules are bound to proteins to form ribonucleoprotein complexes that reduces the frequency of template switching.

See also: Endogenous Retroviruses and Coevolution. Species Concepts: Viral Quasispecies

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Robustness and Evolvability in Molecular Evolution

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Glossary

Evolvability The ability of organisms to bring forth novel, beneficial phenotypes through DNA mutations.
Folding The process by which the linear amino acid strings of proteins and the linear nucleotide strings of RNA molecules adopt a planar or three-dimensional shape that helps them perform their function.
Genotype network, neutral network A set of genotypes with the same broadly defined phenotype, represented as a network where vertices correspond to genotypes and edges connect vertices if their genotypes are separated by a single small mutation.
Genotype space A collection of genotypes that is usually large. For example, the genotype space of proteins with 500 amino acids comprises all possible 20^{500} amino acid strings of this length.
Mutational distance The number of mutations required to transform one genotype into another.
Neighborhood Comprises all those genotypes that differ from a given genotype through a small genetic change, for example, all neighbors of a protein that are derived from it through a single amino acid change.
Neighbors Two genotypes that differ in a small genetic change, such as a single amino acid change in a protein.
RNA secondary structure A planar shape that linear RNA molecules form through internal base pairing of their nucleotides.
Robustness The persistence of a phenotype in the presence of genetic mutation (the focus of this article) or environmental change.

Life is tough. Environments change, nutrients fluctuate, and mutations perturb. Yet life persists. What is more, it innovates. From the photoreceptors and lens crystallins that facilitate vision to the eyespots of butterfly wings that protect against predation, the natural world brims with examples of evolutionary innovation. Understanding how life balances its robustness, the ability to withstand perturbations and especially DNA mutations, with its capacity to exploit these mutations to create new and beneficial phenotypes – evolvability – is a central theme of biological research (Masel and Trotter, 2010) and is the subject of this article.

Any evolving population can be viewed as exploring a large space of possible genotypes, where each member of the population occupies one location in this space and moves through this space via random DNA mutation. Natural selection facilitates the survival or reproduction of those genotypes (individuals) that are best adapted to the environment they live in. Several decades of research have shown that genotypes with the same phenotype often form large and far-reaching networks in this space (**Figure 1**). These networks are a consequence of mutational robustness, and have been called neutral networks or genotype networks (Lipman and Wilbur, 1991; Schuster *et al.*, 1994; Ciliberti *et al.*, 2007a,b; Rodrigues and Wagner, 2009; Wagner, 2011a,b).

We here discuss evidence from the evolution of proteins, RNA, and gene regulatory circuits (**Figure 2**), which suggests that robustness can facilitate evolvability. The reason is that a genotype network allows an evolving population to explore genotype space while preserving well-adapted phenotypes, and in doing so, permits the exploration of novel phenotypes in different regions of this space. Some of these phenotypes may become evolutionary adaptations and innovations. We also discuss circumstances where the organization of a genotype network may reduce evolvability.

Proteins

In a 1970 article in the journal *Nature*, evolutionary biologist John Maynard Smith speculated on the existence of networks of proteins in the space of all possible proteins (Maynard Smith, 1970). In 1991, a computational analysis of a simple

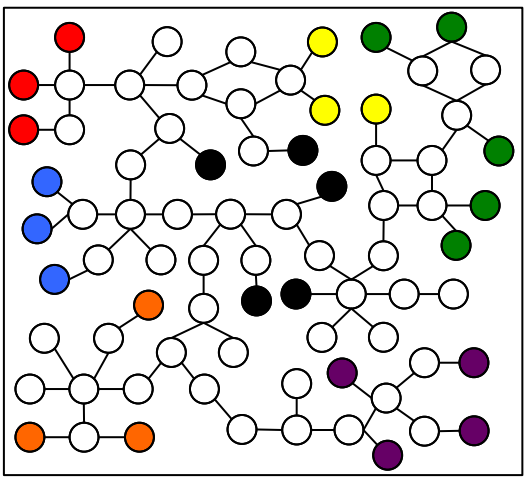

Figure 1 Mutational robustness facilitates evolvability via the formation of large genotype networks. This schematic shows how genotypes (open circles) in genotype space (rectangle) can be organized as a genotype network, where edges connect vertices if their corresponding genotypes differ in a single small mutation. Since most of these genotypes have more than one neighbor in the genotype network, they are to some extent robust to mutation. This schematic also illustrates how, by extending throughout genotype space, a genotype network provides access to a variety of phenotypes (filled circles; color indicates phenotype).

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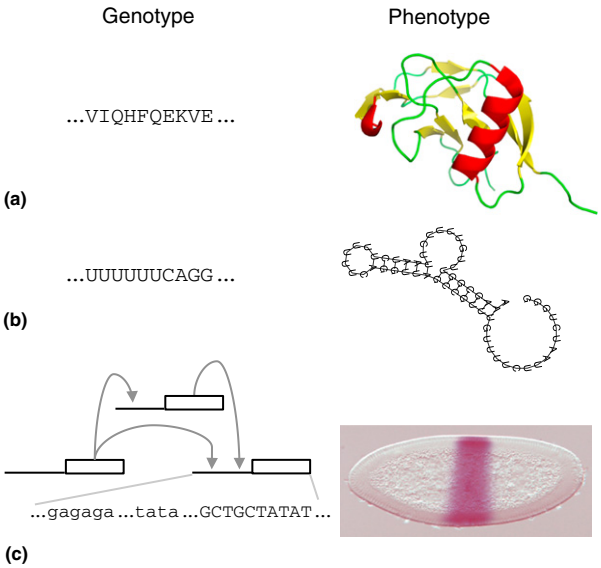

Figure 2 The genotype and phenotype of proteins, RNA, and regulatory circuits. (a) A protein genotype is its amino acid sequence and a protein phenotype is its tertiary structure and associated function. (b) An RNA genotype is its nucleotide sequence and an RNA phenotype is its spatial fold, of which the planar secondary structure is shown. (c) A gene regulatory circuit genotype is the DNA sequence encoding the circuit's constituent transcription factors and regulatory regions. For illustration, we show a hypothetical three-gene circuit and the DNA sequence of the transcription factor binding sites (lower case) and a part of the protein-coding region (upper case) of one of the genes in the circuit. The phenotype of a gene regulatory circuit is a spatiotemporal gene expression pattern. As an example, we show the expression pattern of *Krüppel* – one of the gap genes – in the developing embryo of *Drosophila melanogaster*.

model of protein folding first demonstrated the existence of such networks (Lipman and Wilbur, 1991). This model used a simplified amino acid chain consisting of two different kinds of amino acids (hydrophobic or hydrophilic) that fold on a two-dimensional lattice, such that the number of energetically favored interactions among amino acids is maximized. Each vertex in such a network corresponds to a single amino acid sequence, and two vertices are adjacent if they differ in a single amino acid. More specifically, this article demonstrated that proteins that fold into the same structure form connected networks in genotype space (Lipman and Wilbur, 1991). Each member of such a genotype network has one or more neighbors with the same phenotype, which means that each protein is to some extent robust to mutations that cause single amino acid changes. Mathematical work has shown that genotype networks are a consequence of robustness to mutations (Reidys *et al.*, 1997; Wagner, 2011a,b).

Genotype networks and robustness do not just exist in model proteins. For example, phylogenetic analyses of proteins such as oxygen-binding globins have demonstrated that proteins with the same tertiary structure and function can diverge in the vast majority of their amino acids (Aronson *et al.*, 1994). Proteins with highly diverged amino acid sequence yet preserved structure or function are the rule rather than the exception in biology (Rost, 2002; Todd *et al.*, 1999). In addition, random mutagenesis studies that alter a single or multiple amino acids in proteins such as lysozyme, or β-lactamase (Huang *et al.*, 1996; Rennell *et al.*, 1991), have shown that most single amino acid changes do not affect protein function detectably.

Such robustness can also facilitate the origin of proteins with novel functions. For example, laboratory experiments that engineer proteins such as cytochrome P450 toward increased robustness have demonstrated that robustness can facilitate the origin of novel enzymatic functions through point mutations. The reason is that mutations that create novel enzymatic functions often destabilize a protein. Increased robustness can counteract this destabilization effect, and thus help preserve mutations that create new functions (Bloom *et al.*, 2006). Another way of increasing protein robustness involves chaperones, proteins that help other proteins fold, and that can mitigate the detrimental effects of mutations on a protein's stability. By increasing the expression of a chaperone, mutations that would otherwise have destabilizing effects on proteins can become neutral, and this buffering of mutational effects can help new catalytic activities emerge. For example, in an *Escherichia coli* laboratory evolution experiment, overexpression of a chaperone enhanced the ability of a phosphotriesterase to cleave the novel substrate 2-naphtylhexanoate (Tokuriki and Tawfik, 2009). This link between robustness and the emergence of novel enzymatic functions also exists on evolutionary timescales. Specifically, the more robust a protein fold's tertiary structure is, the greater is the number of novel enzymatic functions that this fold has acquired in its evolutionary history (Ferrada and Wagner, 2010).

RNA

As in proteins, the earliest analyses of the genotype–phenotype relationship in RNA used a simplified computational model of phenotype formation. Specifically, they focused on RNA secondary structure, because it can be computationally predicted, and because proper RNA secondary structure is usually a necessary prerequisite for RNA function. Pioneering computational work identified extended networks that RNA genotypes with the same minimum free energy secondary structure form in RNA genotype space (Schuster *et al.*, 1994). These networks – which have also been called neutral networks – exist because individual RNA molecules typically have many neighbors in sequence space that have the same secondary structure phenotype. Large neutral networks harbor more RNA molecules, which are also on average more robust to mutations than RNA molecules from smaller neutral networks. Although experimental work shows that not all RNA molecules form neutral networks that are as extended in genotype space as computational work suggests (Jiménez *et al.*, 2013), biological RNA molecules often diverge greatly in sequence while preserving structure and function (Parsch *et al.*, 2000), suggesting that they do indeed show substantial robustness to genetic change. Large neutral networks can facilitate the discovery of novel RNA phenotypes by populations that evolve on them. The reason is that such populations experience fewer deleterious mutations, and can thus spread more rapidly through genotype space, which increases their chances to find novel

phenotypes (Wagner, 2008). The fact that these chances vary amongst genotypes raises the intriguing possibility that evolvability is itself evolvable. Because there is at present very little experimental data to support this notion, we do not discuss it further here.

Experimental work on ribozymes showed that genotype networks of RNA molecules can play an important role in the creation of RNA molecules with novel functions (Hayden *et al.*, 2011; Schultes and Bartel, 2000). One pertinent study designed a 'walk' in sequence space to interconvert the hepatitis delta virus self-cleaving ribozyme and a synthetic ligase ribozyme, which have different catalytic activities, folds, and sequences. During most of the individual steps (nucleotide changes) along this walk, ribozyme function did not change. At about half the distance between the two ribozymes in genotype space, a small number of key mutations were sufficient to change one evolving ribozyme's catalytic functions into that of the other ribozyme (Schultes and Bartel, 2000). This suggests that genotype networks and their proximity in genotype space can facilitate evolutionary transitions between ribozymes with different functions. Another experiment showed that genotype networks can facilitate the origin of new adaptations in an evolving population (Hayden *et al.*, 2011). To this end, it compared two evolving populations of the Azoarcus ribozyme, a self-splicing intron from proteobacteria of the genus *Azoarcus*. The first population was concentrated in a small region of genotype space, whereas the second population was spread out on the genotype network of the ribozyme's ancestral phenotype. The second population adapted faster to a new and challenging chemical environment, in which the ribozyme's native RNA substrate for a self-ligation reaction was replaced by a chemically modified substrate that required the population to adapt evolutionarily (Hayden *et al.*, 2011). In sum, these experiments demonstrate that genotype networks in RNA molecules can facilitate the origin of novel enzymatic phenotypes.

Gene Regulation and Gene Regulation Circuits

Gene regulatory circuits drive fundamental physiological, developmental, and behavioral processes in organisms across the tree of life (Carroll *et al.*, 2001). Examples include chemotaxis in bacteria (Alon *et al.*, 1999), mating behavior in yeast (Tsong *et al.*, 2006), and developmental patterning in the fruit fly (Lawrence, 1992). Such circuits comprise a set of genes – typically encoding DNA-binding proteins known as transcription factors – that regulate the expression of other genes in the circuit. The genotype of a regulatory circuit comprises the DNA encoding transcription factor genes, as well as DNA-binding sites for these factors near circuit genes. It encodes two aspects of circuit behavior, namely the interactions between genes (i.e., 'who-regulates-whom') and the signal-integration logic used by each gene to interpret the signals provided by its regulating gene products. The former aspect is encoded by the presence or absence of transcription factor binding sites near a gene, whereas the latter is encoded by the number, spacing, and binding affinity of these sites (Sharon *et al.*, 2012; Smith *et al.*, 2013). The phenotype of a regulatory circuit is its spatiotemporal gene expression pattern, which specifies when, where, and to what extent each gene in the circuit is expressed. A classical example of such a circuit is that formed by the gap genes of *Drosophila melanogaster*, which interprets a maternally deposited morphogen gradient along the anterior–posterior axis of the developing embryo to create precise expression bands, which are fundamental to defining the segmented body plan of the fly and constitute the phenotype of this gene circuit (Lawrence, 1992).

Each circuit genotype with a given expression phenotype can be viewed as a member of a genotype network. Vertices in such a network represent entire circuits and edges connect vertices if their corresponding circuits differ in a single regulatory interaction, or in the regulatory logic of a single gene. Most of what we know about the genotype networks of regulatory circuits come from computational models. For example, Ciliberti *et al.* (2007a,b) used such a model to demonstrate that for any given gene expression phenotype, the vast majority of genotypes form a single, connected genotype network. Similar observations were made using model regulatory circuits inspired by *Drosophila* development, in which a morphogen gradient is interpreted along a spatial domain to form a single, centralized band of gene expression (Cotterell and Sharpe, 2010). There too, stripe-forming circuits form genotype networks. In both models, individual genotypes typically have many neighbors with the same phenotype. Such genotypes are thus to some extent robust to mutations that cause small genetic changes. Moreover, such networks extend far throughout the space of possible genotypes. For instance, two circuits from the same genotype network can be as different from one another as are two circuits chosen at random from genotype space (Ciliberti *et al.*, 2007a). Empirical evidence that circuits with very different genotypes can have the same phenotype exists for circuits that regulate galactose metabolism, mating type, and ribosomal protein expression in fungi (Martchenko *et al.*, 2007; Tanay *et al.*, 2005; Tsong *et al.*, 2006).

Genotype networks not only confer mutational robustness to the expression phenotypes of gene regulatory circuits, they also facilitate evolvability. Ciliberti *et al.* (2007a) demonstrated this by sampling pairs of circuits from genotype networks and determining the sets of novel expression phenotypes that could be realized via regulatory mutations to each circuit in the pair. They found that these sets became increasingly distinct as the difference between the sampled circuits increases. In other words, because genotype networks extend far throughout the genotype space of regulatory circuits, they permit access to a large diversity of novel gene expression phenotypes and thus facilitate evolvability.

While computational analyses have allowed for the characterization of entire spaces of regulatory circuits, experimental data from protein-binding microarrays (Berger *et al.*, 2006) have permitted characterizing the smallest units of circuit organization, transcription factor binding sites, and the spaces they form. These short DNA sequences define the regulatory interactions of a circuit, and mutations to these sequences can affect a circuit's gene expression phenotype (Wray, 2007; Prud'homme *et al.*, 2007), either by altering binding affinity or by abolishing binding. Understanding the robustness of transcription factor binding sites is therefore important to understanding the robustness of regulatory circuits. A recent study

using protein-binding microarray data from 89 yeast and 104 mouse transcription factors analyzed the genotype networks of each of these factors' binding sites (Payne and Wagner, 2014). For 99% of the 193 factors, the majority of sequences bound by the factor are part of a single genotype network. Moreover, these networks are densely connected, implying that individual binding sites are to some extent mutationally robust. Some networks are larger than others – they comprise more binding sites – and individual binding sites in larger networks are more robust than binding sites in smaller networks.

For each of the 193 transcription factors that Payne and Wagner (2014) examined, they also sampled pairs of sites from the same genotype network and determined the sets of transcription factors that bind sites that neighbor those in the pair. As the mutational distance between sites increases, so does the diversity of transcription factors that bind neighboring sites. Moreover, the larger a genotype network is (and the more robust its binding sites are on average), the greater the number of unique transcription factors that bind sites adjacent to the genotype network. In sum, these observations suggest that robustness and evolvability exhibit a synergistic relationship in gene regulatory circuits and their transcription factor binding sites, made possible by the existence of large genotype networks that spread throughout genotype space.

Caveats

Although robustness facilitates evolvability in general, exceptions to this rule may exist. First, theoretical arguments suggest that certain topological properties of genotype networks, together with a population's location in a genotype network, can hinder evolvability. For example, if a genotype network has a dense interconnected core where many neighbors preferentially connect with one another, a population may become entrapped in this core such that it is no longer able to explore many novel phenotypes (Manrubia and Cuesta, 2014). Empirical evidence for this phenomenon can be found in the genotype network formed by four key amino acids of the PhoQ protein interface of *E. coli* (Podgornaia and Laub, 2015), in which each vertex represents a combination of amino acids capable of binding the protein PhoP. This PhoQ genotype network is expansive and includes 13 residue combinations found in natural PhoQ orthologs. All of these orthologs are contained in the genotype network's dense interconnected core, indicating that the structure of the genotype network may have constrained the evolutionary exploration of alternative PhoQ variants.

A second caveat is that the genotype networks of different phenotypes may be biased in their connectivity to one another, such that a random mutation may be more likely to allow evolving genotypes to enter a larger genotype network than a smaller genotype network. Computational work has shown that this connectivity bias may lead to an 'ascent of the abundant,' where populations evolve phenotypes with large genotype networks, even if these phenotypes do not provide adaptive advantages (Cowperthwaite *et al.*, 2008). The secondary structure phenotypes of multiple short noncoding RNA molecules have genotype networks much larger than expected by chance alone, i.e., based on randomly sampled phenotypes, suggesting that this ascent of the abundant is more than a theoretical possibility (Jorg *et al.*, 2008).

A third exception exists in situations where a genotype network is fragmented into multiple components or subnetworks (Jiménez *et al.*, 2015). For example, in stripe-forming regulatory circuits, there are several dynamical mechanisms by which a single, centralized band of gene expression may form (Cotterell and Sharpe, 2010). Even though each mechanism yields the same phenotype, the genotypes that encode these mechanisms form distinct genotype networks that are isolated from one another in genotype space. These genotype networks differ in their evolvability, because each provides mutational access to the genotype networks of different sets of novel gene expression phenotypes (Jiménez *et al.*, 2015). Here, individual circuits may be to some extent robust to genetic change, but because their genotype networks are fragmented, each circuit can access only a limited repertoire of novel phenotypes. Similar fragmentation has been observed for the secondary structure of RNA genotypes (Aguirre *et al.*, 2011; Schaper *et al.*, 2012). The extent to which such fragmentation hinders evolvability depends on multiple factors. For example, if population sizes are sufficiently high or mutation rates sufficiently large, populations can 'tunnel' between different components of fragmented genotype networks (Weinreich and Chao, 2005).

In sum, with possible exceptions, robustness can facilitate evolvability by virtue of genotype networks that allow evolving populations to preserve well-adapted phenotypes, while exploring distant regions of genotype space and the novel and potentially beneficial phenotypes they contain. As high-throughput technologies for phenotyping macromolecules and regulatory circuits continue to advance, it will become possible to experimentally probe the genotype networks of biological systems at ever-greater resolution, further elucidating when, how, and to what extent robustness and evolvability exhibit a synergistic relationship.

See also: Adaptive Landscapes. Adaptive Mutation Controversy. Developmental Biases on Morphological Evolvability. Gene Networks Driving Development, Conservation and Evolution of. Genotype to Phenotype: Insights from Evo-Devo. Neutral Evolution, Population Genetic Tests of

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Further Reading

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Rooting Trees, Methods for

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Rooted versus Unrooted

Phylogenetic trees are either rooted or unrooted, depending on the research questions being addressed. The root of the phylogenetic tree is inferred to be the oldest point in the tree and corresponds to the theoretical last common ancestor of all taxonomic units included in the tree. The root gives directionality to evolution within the tree (Baldauf, 2003). Accurate rooting of a phylogenetic tree is important for directionality of evolution and increases the power of interpreting genetic changes between sequences (Pearson *et al.*, 2013).

Many techniques such as molecular clock, Bayesian molecular clock, outgroup rooting, or midpoint rooting methods tend to estimate the root of a tree using data and assumptions (Boykin *et al.*, 2010). However, Steel (2012) discusses root location in random trees and points out that information in the prior distribution of the topology alone can convey the location of the root of the tree. These results show that the tree models that treat all taxa equally and are sampling consistently convey information about the location of the ancestral root in unrooted trees (Steel, 2012).

Why Do We Need a Rooted Tree?

We are interested in rooting a phylogenetic tree in order to show the path of evolution of biological species. Therefore most users of phylogenetic trees want rooted trees because they give an indication of the directionality of evolutionary change. The root of phylogenetic tree is crucial in evolutionary interpretation of the tree (Williams, 2014), because an unrooted tree species shows only the relationships among the taxa and does not define the evolutionary path (**Figure 1(a)**).

When Do We Need an Unrooted Tree?

An unrooted tree is desired when we do not have a distantly related group (sequence) for comparison or when primary interest is focused only on relationships among the taxa rather than on the directionality of evolutionary change. Unrooted trees are beneficial in depicting clusters of related sequences. Unrooted gene trees have also become more prevalent within the multispecies coalescent phylogenetic framework, leading

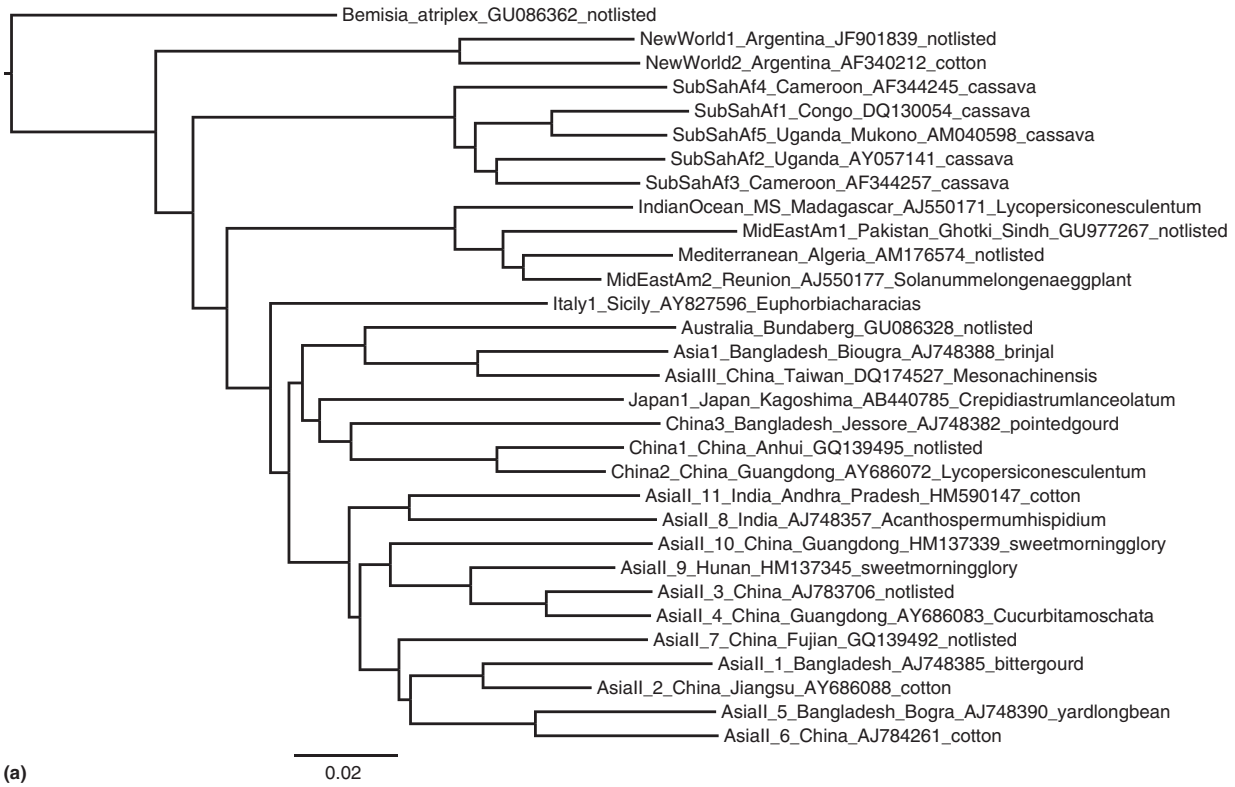


Figure 1 (a) Outgroup rooted phylogenetic tree of the *Bemisia tabaci* species complex (whiteflies) from a modified dataset (Boykin *et al.*, 2013). Tip labels correspond to geographic location_sublocation_GenBank accession number_host. (b) Unrooted phylogenetic tree of the *Bemisia tabaci* species complex (whiteflies) from a modified dataset (Boykin *et al.*, 2013). Tip labels correspond to geographic location_sublocation_GenBank accession number_host. (c) Unrooted star phylogenetic tree of the *Bemisia tabaci* species complex (whiteflies) from a modified dataset (Boykin *et al.*, 2013). Tip labels correspond to geographic location_sublocation_GenBank accession number_host.

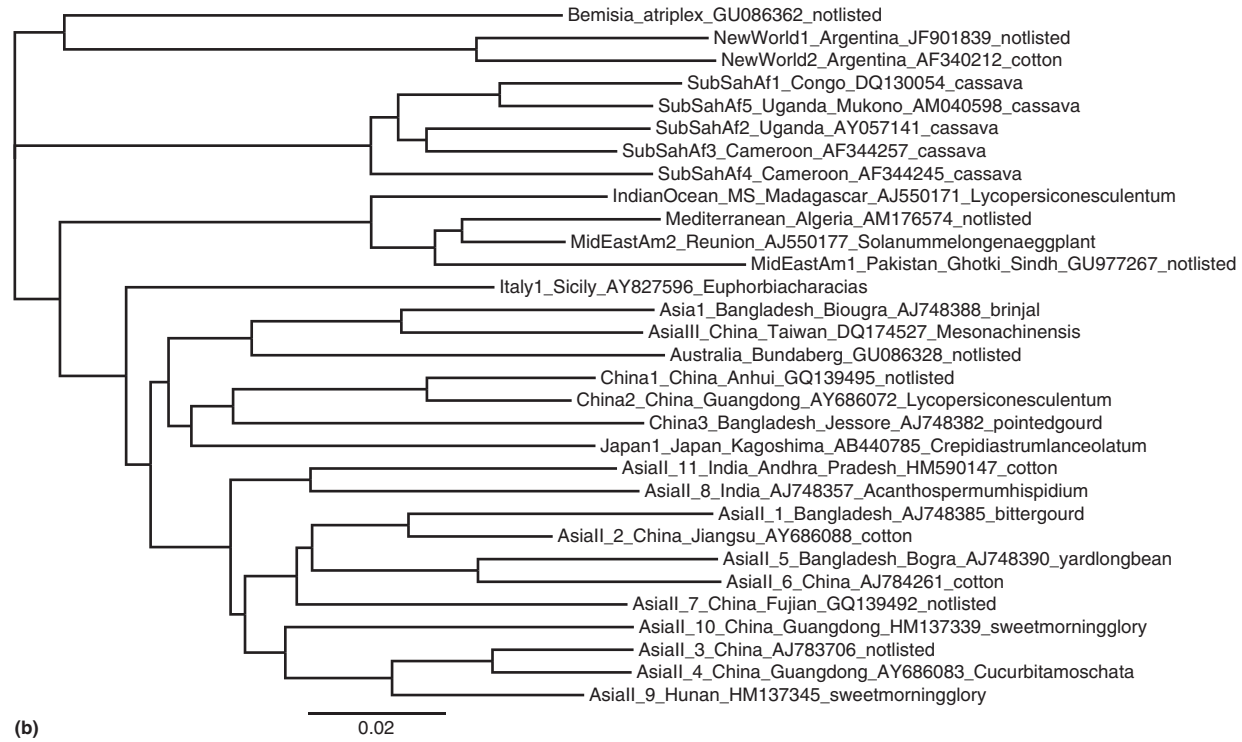


Figure 1 Continued.

to systematic approaches for inferring unrooted species trees from unrooted gene tree topologies (Liu and Yu, 2011). Gene trees and species trees can have similar topologies but often there is considerable discordance between gene tree and species trees (Degnan, 2013). For this reason, understanding how to root gene trees will have implications for accurate species tree inference (**Figures 1(b)** and **1(c)**).

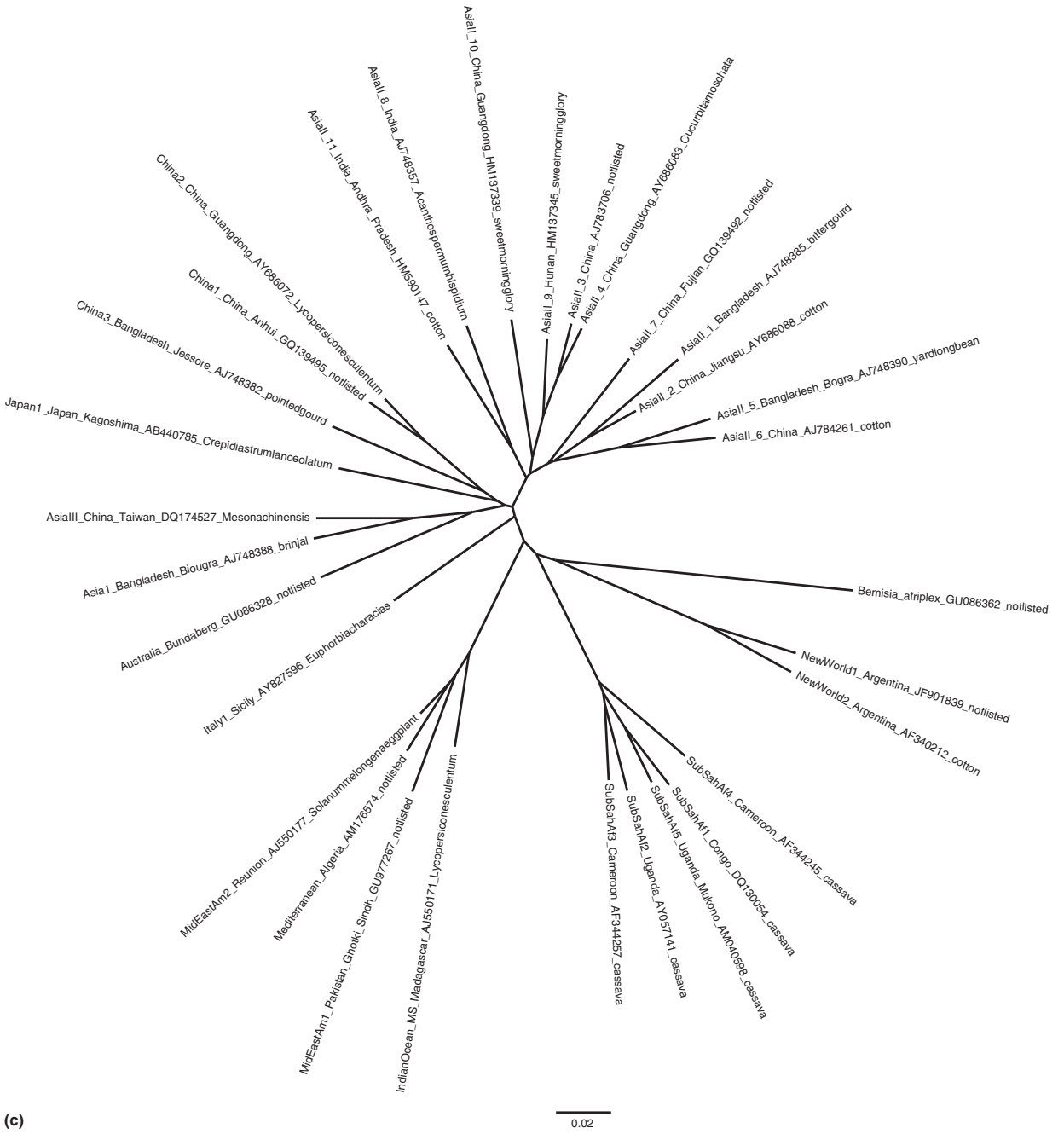
How Do You Root a Phylogenetic Tree?

Outgroup Rooting

There are several rooting methods (**Table 1**), however the most popular and widely used is the outgroup method (Wheeler, 1990; Tarrío *et al.*, 2000; Hess and De Moraes Russo, 2007; Boykin *et al.*, 2010). Outgroup method assumes that one or more of the taxa are divergent from the rest of the taxa (ingroup). The branch linking the ingroup and outgroup becomes the starting point, and defines all subsequent evolutionary events within the tree (Brady *et al.*, 2011; Williams, 2014). In addition to providing evolutionary information of the ingroup, the outgroup has other additional functions. It allows the identification of distinct features within ingroup sequences (Wheeler, 1990). An important aspect of outgroup method is the need for a priori knowledge on the appropriate outgroup to use for the set of sequences (Wheeler, 1990; Hess and De Moraes Russo, 2007). However, this is also the main bottleneck for this method, especially within higher taxonomic groups such as angiosperms, birds, and mammals where a consensus outgroup is lacking (Qiu *et al.*, 2001). As a consequence, many authors are forced to choose between different sorts of outgroups that are either phylogenetically close or phylogenetically distant (Rota-Stabelli and Telford, 2008).

Lack of an appropriate outgroup results in drawbacks such as the long branch attraction (LBA). LBA occurs mainly when the outgroup taxa are distantly related to the ingroup due to either large divergence time and/or increased rate of evolution (Tarrío *et al.*, 2000). This results in homoplastic changes occurring at rapidly evolving sites thus resulting in artifactual rooting (random rooting) (Wheeler, 1990; Hendy and Penny, 2011; Maddison *et al.*, 1984). Several criteria have been proposed to prevent LBA within phylogenetic trees, through a multistep process as proposed by Rota-Stabelli and Telford (2008) to assist in outgroup selection especially in the case of arthropod classes. They include: (1) low substitution rate; (2) ingroup like G + C composition; (3) new strand bias estimators 'skew index'; (4) the tendency of the outgroup to avoid 'random branding effect'; and (5) phylogenetic proximity to the arthropod.

An alternative approach to assess the importance of an outgroup in rooting the tree is explored by Graham *et al.* (2002); this is by establishing whether the outgroup provides sufficient signal in response to root location, indicative of historic linkage or due to LBA. Using Pontederiaceae, an aquatic monocot, as the case study they assessed how the nearest outgroup provides for rooting Pontederiaceae compared to those less closely related relatives and further investigate the role of LBA when determining the optimal rooting of Pontederiaceae. However, they concluded that LBA may influence rooting, and may be supporting the wrong outgroup. To further reduce LBA and to ensure robustness of the outgroup rooting method they recommend multiple sampling of outgroups within the sister group rather than sampling within less closely related taxa.



(c)

Figure 1 Continued.

Midpoint Rooting

Midpoint rooting calculates tip to tip distances and then places the root halfway between the two longest tips (Swofford *et al.*, 1996). The ancestral point will be identified if the tree has constant rates of evolution. The method is exclusively dependent on the branch length of the phylogenetic tree and the assumption of the molecular clock (Holland *et al.*, 2003). Homogeneity is assumed across the branch and that the two most divergent taxa evolved at equal rates (Holland *et al.*, 2003; Swofford *et al.*, 1996). If the tree is balanced, midpoint rooting works well. However, a major limitation of midpoint rooting is the dependence on having clocklike data and a balanced topology.

The midpoint rooting method is often applied to viral genetic datasets because in many cases outgroups are unknown. For example, Stavrinides and Guttman (2004) utilize midpoint rooting to establish the evolutionary relationship of severe acute respiratory syndrome (SARS) coronaviruses. They carried out phylogenetic analysis of the viral genes encoding for viral structural proteins specifically, envelope matrix (M) and nucleocapsid (N) proteins. The midpoint rooting of the

Table 1 Four methods for rooting phylogentic trees

Rooting method	*Pros*	*Cons*	*Software*
Outgroup	Accurate	Must have an outgroup Long branch attraction	PAUP Figtree R package: ape (http://www.inside-r.org/packages/cran/ape/docs/unroot)
Midpoint	Fast No outgroup needed	Dependent on clocklike data Not good with unbalanced trees	PAUP R package: phangorn (https://cran.r-project.org/web/packages/phangorn/phangorn.pdf)
Molecular clock	No outgroup needed Robust to violations of the clock	Computationally intense	PAUP PAML
Bayesian molecular clock	Alternative rootings uncovered No outgroup needed	Must customize the prior	Post Root (http://www.stat.osu.edu/~lkubatko/software/phyl_util.html) Root annotator (http://sourceforge.net/projects/rootannotator/)

trees generated using the M protein data shows two groups, one that consisted of porcine, feline, and canine and one that contained bovine and murine coronaviruses (*Coronaviridae*). On the other hand, the N protein tree is midpoint rooted on the branch leading to the group 1 coronaviruses. Moreover, the appropriateness of midpoint rooting is supported by the results of Tajima's relative rate test (Tajima, 1993), indicating no rate heterogeneity among the coronavirus groups (Stavrinides and Guttman, 2004).

This method should be used as an alternative to the outgroup rooting method and could be adopted as the default method when the outgroup method is difficult to apply either due to problems with available outgroups, such as LBA or lack of a priori knowledge of the outgroup (Hess and De Moraes Russo, 2007).

Molecular Clock Rooting

The molecular clock rooting method has one assumption: the rate of evolution is constant for the sequences of interest (Yang and Rannala, 2012). The rate is typically expressed in substitutions per site per year or substitutions per site per million years (Brown and Yang, 2011). The strict clock is often used in analyses of sequences sampled at the intraspecific level, for which usually there is an exceptionally low rate of variation (Brown and Yang, 2011; Ho and Duchêne, 2014). The molecular clock assumption becomes problematic for distantly related species because there is a linear relationship between the genetic distances and approximate divergence. The slope of the line directly corresponds to the evolutionary rate variation among species especially among divergent taxa (Welch and Bromham, 2005). Before utilizing the molecular clock method for rooting a phylogenetic tree users should test if a molecular clock is appropriate to describe the data. Testing for the molecular clock entails generating two maximum likelihood trees, one computed with the molecular clock enforced and one without the molecular clock enforced and then utilizing the likelihood ratio test (Felsenstein, 1983; Holder and Lewis, 2003).

Bayesian Molecular Clock Rooting

Huelsenbeck *et al.* (2002) proposed the use of Bayesian inference under the molecular clock assumption to infer the root of a phylogenetic tree. After obtaining the posterior distribution of trees under Bayesian inference, the root of the tree is inferred to be the root position with the highest posterior probability. This method also provides the posterior probability that the root lies on any branch of the ingroup topology. Another advantage of the Bayesian method is that it allows the user to evaluate alternative rootings. Other rooting methods only return one rooting for a particular dataset, without any numerical assessment of confidence in that rooting. A Bayesian molecular clock analysis successfully identified the root of Orcuttieae (Poaceae) (Boykin *et al.*, 2010) when all other methods failed. Post_root was developed to analyze the output from MrBayes (Ronquist *et al.*, 2012) or ExaBayes (Aberer *et al.*, 2014) runs. The output from Post_Root will give the number of unique roots and also the most probable root position.

Most recently, Calvignac-Spencer *et al.* (2014) have further developed Post_Root to a web-based interface in their quest to identify the branch root posterior probability (RPP) of the most recent Ebola outbreak in West Africa. They were forced to rely on Bayesian molecular clock rooting because there is no known outgroup for Ebola. It is often the case when analyzing viral sequences that no outgroup is known; therefore the Bayesian molecular clock rooting is a very useful alternative, especially when rooting is crucial for viral outbreaks.

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See also: Bayesian Phylogenetic Methods. Phylogenetic Tree

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S

Schools of Classification

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Glossary

Floras Enumerations of the plants of particular areas.

Keys Sequence of questions about features of an organism which when answered lead the user to its correct name.

Monophyletic A group containing all and only the descendants of a common ancestor.

The history of what today we could call taxonomy, the description of organisms and the construction of a hierarchical classification, and systematics, the study of the relationships between organisms, is complex. Understanding the connections between 'botany,' 'natural history,' 'zoology,' and 'biology' is central to this history and to that of the history of biology as a whole. It should also be remembered that most of the authors mentioned below distinguished rather simply between plants and animals, algae and fungi being considered to be plants (Box 1). In the middle of the seventeenth century, many, like Aristotle 2000 years before, believed in a nature that could be represented as some version of the scala naturae, a linear sequence of organisms arranged according to ideas of 'highness' and 'lowness,' in which man was above all organisms (and often not part of nature), and angels and ultimately god might be above him. There were many other ways of representing nature, and as the geologist Francis Bather observed in 1927, "not a single naturalist had a clear idea of what he meant by 'natural'. All he knew was that the other fellow's classification was unnatural" (Bather, 1927). In early usage, natural history itself for the most part had no historical element; 'history' meant 'story' or 'description.' In the nineteenth century in particular, men and women and amateurs and professionals studied different parts of natural history. Below authors may be referred to as being 'naturalists.' This is not always true, but it is the only general term that can be used. I carry the story through to the late 1980s, but do not attempt to other than outline some of the major issues, and these may play out in different ways in different countries as well as in different parts of natural history.

Box 1 Ambiguous names

The relationships between botany, natural history, zoology, and biology after Linnaeus published his *Systema naturae* in 1735 have been little studied. Indeed, throughout the nineteenth century, particular words that are in common use today like system, method, and organization were used with sometimes very different connotations than those they have today. 'Nature' and 'natural' are particularly ambiguous (e.g., Atran, 2008).

Classifications before Linnaeus

Members of cultures worldwide name the organisms that they commonly encounter, and detailed studies have been made of these folk taxonomies (Atran, 1999). They tend to include relatively few members, often under 500, perhaps reflecting limitations of human memory. Here the hierarchy is shallow (there are few ranks), and the taxonomies are partly shaped by the relationships between organisms and society. These may be other than simply utilitarian – hence the title of a paper, 'Why the cassowary is not a bird,' which explains why cassowaries are not included with other birds in some New Guinea local classifications.

Aristotle (384–322 BCE), Plato's student, is noted for his *History of Animals*, *Parts of Animals*, and *Generation of Animals*. Importantly, the *History of Animals* starts off with a discussion of the different relationships – excess, defect, and analogy – between similar structures in different organisms. These books include many quite accurate observations, and Aristotle is known to have dissected animals (but not humans). The botanical works of Theophrastus (ca. 381–287 BCE), also Plato's student and Aristotle's successor, include information about exotic organisms encountered by Alexander the Great's armies. Rather later Dioscorides in his *De materia medica* of ca. 60 CE discussed the uses of plants in medicine. His work, in particular, remained well known and the close association of plants/botany and medicine persisted into the nineteenth century.

In Europe, in the sixteenth to eighteenth centuries in particular, cabinets of natural curiosities, or natural history cabinets, were very popular. These might include everything from

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geological and anthropological artifacts such as crystals and Eskimo canoes to animals and plants, skeletons, fibers, and the like. At the same time naturalists like Leonhart Fuchs in his much-reprinted *The Great Herbal* (1542) and Conrad Gesner and his five-volume *Historiae animalium* (1551–1558) produced very popular profusely illustrated volumes that in part integrated classical literature with European knowledge of plants and animals. Subsequently, organisms unknown to classical authors, particularly those newly encountered by Europeans in the course of their travels, were described and illustrated.

Particularly important in the questioning of classical knowledge was the anatomical work of Andreas Vesalius, whose *De humani corporis fabrica* (1543) consisted of superb dissections of the human body. Prior to this Galenic ideas of human anatomy predominated; Galen, born in 120 CE, seems to have based his ideas of human anatomy on dissections of Barbary apes and other animals. In 1555, Pierre Belon illustrated human and bird skeletons side by side, similar bones in the two being indicated by the use of the same letter, an early example of comparative anatomy. Microscopes became popular with the work of Robert Hooke and his *Micrographia* of 1665, while in the 1670s, Antoine van Leeuwenhoek discovered the teeming world of minute animals and plants in water.

Linnaeus

There had been many classifications before Linnaeus. For example, John Ray in his *Historia plantarum* (1686–1704) drew important distinctions between major groups of plants. Ray's friend Francis Willughby wrote the important *Ornithologia libri tres* of 1675. However, there was no generally accepted classification for organisms. This is what Linnaeus provided in successive editions of his *Systema naturae*, *Species plantarum*, and *Genera plantarum*. The first edition of the *Systema naturae* (1735) includes inventories of the three kingdoms of nature, plants, animals, and minerals, and is a linear listing of all natural phenomena according to the number, shape, position, and proportion of their parts (Koerner, 1999). Linnaeus brought order to previous work, bringing together different citations of the same species by earlier naturalists. In the first edition of the *Species plantarum* (1753) he introduced binomials. Binomials like *Canis familiaris* are noun–adjective combinations, as is the domesticated dog, to which that name refers, the only difference being that the first is in Latin, the *lingua franca* of the time. Naturalists worldwide soon found binomials indispensable. They were unambiguous and much shorter and more convenient than the descriptive phrase or polynomial that had previously been used. The 10th edition of *Systema naturae* of 1758 is the starting point of most zoological nomenclature, and that of plant (and bacterial) nomenclature is the *Species plantarum* of 1753.

Linnaeus's hierarchy had five main ranks, and he acknowledged that his higher groupings were for the most part artificial, and he recognized groups like 'worms,' 'fish' (which initially included whales), and so on. However, Linnaeus thought his genera and species were more or less natural and represented ranks in nature. There are also elements of the *scala naturae*, or chain of being, in how he arranged animals and plants in his classification. Species at the end of one genus might be more similar to a species at the beginning of the next than to some of the other species in its own genus; Aristotle had called such organisms dualizing, showing relationships in two directions.

Linnaeus had many students, and in some of their theses, which for the most part Linnaeus himself wrote, he did outline one or two more 'natural' groups of plants.

Although Linnaeus classified both plants and animals, he was more interested in the former, and most naturalists since have worked largely on plants or animals, rarely on both. Indeed, the histories of botanical and zoological systematics are rather different, partly because of the apparent simplicity of many plants and greater complexity of many animals.

Developments Immediately after Linnaeus

In botany, names such as J.E. Smith, Antoine-Laurent de Jussieu, and Augustin-Pyramus de Candolle were prominent in the late eighteenth and early nineteenth centuries, the last two being members of dynasties of scientists/botanists. J.E. Smith early acquired most of Linnaeus's collections and library and the prestige associated with them. Smith and de Candolle in particular distinguished between the various parts of natural history. Thus the 'philosophical' botany of Smith included physiology, while de Candolle distinguished between plant physics, which included Smith's philosophical botany, applied botany, the use of plants by humans, and botany 'strictly speaking,' botany as classification, i.e., taxonomy and systematics. Indeed, botany became synonymous with classification and naming.

Jussieu and Candolle are both noted for constructing natural systems, and this became common in the nineteenth century. Jussieu recognized precisely 100 families, surely hardly by chance, and if his system is examined in detail, it represents a modified chain of being. Candolle's revisions of plant families also showed continuity in relationships, genera being arranged in circles, these circles touching adjacent circles. In both cases the plant features used were largely those of external form, but they included features of the plant embryo and Candolle in particular was interested in anatomical information (Stevens, 1994).

As the century wore on, flora-writing became ever more important in botanical work, and these floras might cover large areas of the world – usually colonial posessions – and could take half a century or more to complete. Keys allowed the plants to be identified. Thus, Carl Friedrich Philipp von Martius's *Flora brasiliense* came out in 130 fascicles between 1840 and 1896 (Martius was then long dead). Martius also wrote the great *Historia naturalis palmarum* (3 vols, 1823–1850), which included remarkable anatomical studies by Hugo von Mohl.

Hugo von Mohl was basically an anatomist-physiologist, and the name of Robert Brown, 'botanicorum facile princeps,' who was active in the first half of the nineteenth century, is also prominent here (Mabberley, 1985). Initially Brown's work was more floristic and taxonomic, but he also carried out detailed microscopic work on pollen, plant cells, etc., naming

the nucleus and noticing what is now called Brownian motion. At this time, 1820–1850, a number of botanists took up physiological and anatomical studies, a shift particularly evident in Germany. However, anatomical work was not commonly carried out by taxonomic botanists, but not because of any limitation of early microscopes. The remarkable *Traité d'organogénie comparée de la fleur* (1854–1857) by Jean-Baptiste Payer includes engravings that show as much detail as early electron micrographs.

Naturalists working on animals had taken up anatomical studies earlier. George-Louis Leclerc, Conte du Buffon, wrote a 36-volume *Histoire naturelle, générale et particulière* (1749–1788) in which the accounts of quadrupeds were accompanied by drawings of dissections by Louis-Jean-Marie Daubenton. In the first part of the nineteenth century, Georges Cuvier in France and Richard Owen in England, both also paleontologists, were the foremost vertebrate anatomists. Buffon developed evolutionary ideas, while Cuvier was a catastrophist, organisms appearing anew after life had been wiped out. Although Owen is often remembered for being anti-evolutionary, his anatomical work led him to make clear distinctions between homology, largely determined by the relative position and connection of parts, and analogy, largely functional similarity. From his detailed comparative studies he abstracted an archetype, which, although it looks like a primitive fish, depended not on evolutionary ideas but more to German Romantic thought.

Darwin and Ideas of Relationships

A goal of many naturalists was to discover the relationships between organisms, and many diagrams showing relationships – some tree-like – were published before 1859 (Winsor, 1991).

The publication of Darwin's *On the Origin of Species* (1859) did not much affect the practice of systematics, since it did not suggest any obvious way to detect phylogenies or promote new ways of describing species, although it did explain why there might be uncertainty over species limits. However, after 1859, diagrams showing relationships became more common.

In these diagrams there was often an attempt to convey both ideas of relationships and of evolutionary advancement. Ernst Haeckel's thought that ontogeny recapitulated phylogeny (oversimplified!), and embryology was considered to provide the most important evidence about relationships. The default arrangement in many early evolutionary works was for organisms to be arranged in a series from simple to more complex, and there was much talk of lowness (simple) versus highness (more complex), primitive versus advanced.

Comparative anatomy also became very popular among some botanists in the latter part of the nineteenth century, and subsequently features like chromosome numbers and plant chemistry were all at least briefly popular. Karl Mez and Hermann Ziegenspeck studied the serological reactions of plant proteins, publishing the very controversial 'Konigsberg Stammbaum' in 1926. Rather later, and also quite controversial, was the technique of DNA hybridization used by Charles Sibley and Jon Ahlquist in the early 1980s to determine the relationships of birds.

But how were naturalists to evaluate differing ideas of relationships? How does – or even can – the mind analyze all the observations made on a group when deciding on its limits, the limits of the species it contained, and its relationships? What characters were important in evolution and so important in determining relationships? Naturalists as far back as Aristotle had claimed to use many characters in their classifications, but close analysis of earlier classifications suggests that often only one or two conspicuous features were used, while the reason particular characters were thought to be evolutionarily important often seemed to be based on circular arguments. Some classifications were structured in such a way as to make them easy to memorize.

The 1960s saw the short-lived but important flowering of phenetics or numerical taxonomy, in which Peter Sneath and Robert Sokal were prominent names. Sneath and Sokal developed methods to enable the analysis of many characters simultaneously to produce estimates of overall similarities between organisms, methods facilitated by the use of early computers. The emphasis was on objectivity; subjective opinions as to which features were important took second place to the analysis of large numbers of carefully defined characters. However, it was unclear how the branching diagrams produced in these analyses related to phylogeny, and understanding phylogeny remained important for most systematists. Nevertheless, techniques that began to be developed at this time are used when describing the shapes of organisms, analyzing data to determine species limits, as well as in ecology and elsewhere.

When it came to constructing evolutionary trees, the philosophy of the entomologist Willi Hennig, popular in the 1970s and afterwards, had a profound effect. Methods were developed to determine what character states (e.g., scales versus feathers) are plesiomorphic ('primitive': scales) and what are apomorphic ('derived': feathers). The phylogenetic trees that were constructed minimized the number of evolutionary steps between these character states, the principle of parsimony, and this led to quite different ways of reconstructing relationships. Classifications also changed, only monophyletic groups being given names, and groups like reptiles and dicotyledonous plants began to disappear from the vocabulary of biologists. As always, claims were made that the groups recognized were 'natural.'

Species

However, the way species were described changed only little. The usual procedure was to assemble specimens and write a description, in consultation with the earlier literature. No more, no less. It is hardly surprising that Darwin in the *Origin* said that species were what competent naturalists said were species, largely because taxonomic practice was so opaque to the observer.

This 'authoritarian' approach persisted in much of botany, for example. However, in the latter half of the nineteenth century Spencer Baird of the Smithsonian Institution listed specimens and measurements taken from those specimens. The ornithologist Robert Ridgway wrote 8 of the 11 volumes of the monumental *The Birds of North and Middle America* (1901–1950). There we find maxima, minima, and means of

measurements, analyses of variation broken down geographically, etc. Indeed, absent such studies, one could argue taxonomy, often described as being a cumulative science, integrating and building on the work of naturalists since the time of Linnaeus, accumulates very little other than names and specimens.

Of course, what species represent in the mind of the taxonomist certainly changed. For Linnaeus, species were divinely created; for Darwin, they were the result of evolution. With the advent of evolutionary ideas and still more the rediscovery of the rules of inheritance at the end of the nineteenth century, the study of variation became more important. One issue was how evolution occurred, gradually, or in fits and starts, and another was whether natural selection drove evolution, or not. Biometricians, who primarily studied continuous variation at the infraspecific level and rejected mutation, took the first position on these two issues, while Mendelians took the second position.

At about this time Karl Jordan, a curator of Walter Rothschild's collections in Tring, England, began working on various insect groups (Johnson, 2012). He developed a species concept similar to the biological species concept later championed by Ernst Mayr, that is, a species is a population of group of populations reproductively isolated from other such populations. Indeed, the Mendelian and biometrician approaches were reconciled in the evolutionary synthesis of the middle of the last century (Mayr, 1982). But the biological species concept signals a shift away from describing species to thinking about their origin, and Mayr, although he described species early in his career, thought of himself as an evolutionary biologist. The emphasis in ornithology had long been changing, from descriptive work ('scientific ornithology' for Ridgway) to behavioral studies and the like ('popular ornithology'), a major shift.

Collecting and Collections

Much collecting in the nineteenth and early twentieth century in particular was on expeditions that had geopolitical or colonial implications. Thus Darwin circumnavigated the world in H.M.S. *Beagle* (1831–1836) while Hooker sailed on H.M.S. *Erebus* (1839–1843), both were surveying trips that collected information about geography, ocean currents, etc., important for British Empire and its commerce.

In the early days of natural history possession of a personal collection was the mark of a serious naturalist. Perhaps the largest collection was accumulated by Baron Walter Rothschild; at its peak it contained approaching three million specimens, of which about two-thirds were insects. Much of his collection is now in the Natural History Museum, London, funded by the government, and his birds are largely in the American Museum of Natural History, a private institution; most natural history collections are now in institutions. Many Universities had collections, although these have tended to be dispersed as priorities shifted within biology.

Biology, Natural History, and Botany

The term biology had come into use intermittently by the end of the eighteenth century. Both Lamarck and Gottfried Reinhold Treviranus, early proponents of biology, thought that biology had to do with the understanding of the 'organization' of organisms, how they were put together and what made them function; classification was not central to this endeavor, particularly for Lamarck. How they and others saw biology, botany, and natural history proved very important in shaping what became the subdisciplines of biology.

By late in the eighteenth century, women and botany had become associated; Linnaean botany was a subject fit for study by women, and could also be helpful in the education of their children. As translated, Jean-Jacques Rousseau's *Elementary Letters on Botany* (1782) conveyed a gendered idea of botany that emphasized an already dated Linnaean botany. Indeed, throughout the nineteenth century there was a botany that was a science of names, something suitable for study by women and children, healthy but not involving undue exertion, and that could be carried out in safety. In 1887 J.FA. Adams wrote a paper in *Science* protesting that the study of botany was indeed suitable for young men – although mainly because it was healthy.

Natural history was also gendered. An exchange in William MacGillivray's *History of British Birds* (1837) makes this clear: "It is a barbarous practice, this practical ornithology of yours" (it involved shooting and dissection), … "Ladies indeed cannot become practical ornithologists…". Natural history outside of museums involved strenuous outdoor activities and was the provenance of boys and men.

Thus, botanists and naturalists studied different parts of nature in different ways; furthermore, botanists who described species were very different kinds of botanists than those who botanized around their houses with their children. Botany did remain part of the medical curriculum, but its value there was often seen as being decidedly ambiguous.

With the advent of the tellingly named 'wissenschaftliche Botanik' or the New Botany, a more physiological, laboratory-based, and experimental botany was introduced from Germany to other parts of Europe and America by the end of the century. Early proponents were Julius Sachs, a physiologist, and Mathias Schleiden, a father of the cell theory. As the *Encyclopedia Britannica* of 1911 noted, Schleiden's work, "did much to shake the tyranny of the purely systematic Linnean school, whose accumulations he was accustomed irreverently to describe as 'hay.'" Proponents of the New Botany in North America like Charles Bessey and John Merle Coulter used a caricature of a gendered and discredited 'old' botany to support the New Botany, Coulter noting that botany had been "[r]ecommended especially to the ladies as a harmless pastime, not overtaxing to the mind" (Coulter, 1895).

The new-style biologist studied internal characters and used a compound microscope. However, in North America the nineteenth century closed with a vitriolic debate about 'Sham biology' – should biology include botany, particularly classificatory botany? Zoological natural history with its history of comparative anatomical studies fared better in such debates. Classificatory botany was close to being marginalized, and through the next century there were fears that botany might be replaced by a zoology wearing the mask of biology, that botany departments would disappear from universities (many did), and that taxonomists would disappear (not yet, at least).

See also: Darwin–Wallace Theory of Evolution. Species Concepts and Speciation

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Searching Tree Space, Methods for

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Glossary

Approximation algorithm An algorithm for an NP-hard problem that has a guaranteed worst case performance.

Heuristic search An ad hoc algorithm for an NP-hard problem that has no guaranteed worst case performance.

NP-hard Term from theoretical computer science, referring to a combinatorial optimization problem for which no efficient algorithm is known, that is, all combinations need to be scored to find the best one.

Topological alteration mechanism An operation for changing a tree topology.

Introduction

The reason why a substantial amount of research effort has been invested into designing tree search algorithms is that the problem of finding the optimal tree according to criteria such as parsimony or likelihood (see 'See also' section on these topics) that indicate how well the sequence data (e.g., a multiple sequence alignment) fits a given tree is NP-hard. Consider, for instance, that one intends to find the optimal tree under parsimony for a set of 50 species (taxa). For 50 taxa there exist approximately 2.83×10^{74} different binary unrooted tree topologies (see, for instance, Yang (2014) for a derivation of the number of possible trees); the exact number is:

28380632508077991283772917269612815092062858799810511441573766775415039062

Now, consider that calculating the parsimony score on one of those trees requires 1 s. To find the best tree, one would need to evaluate all 2.83×10^{74} tree topologies which will require a total of about 9×10^{65} years, which is in turn roughly 9 times the age of the universe. Hence, finding the best tree for just 50 taxa is simply not feasible, which means that a workaround is required.

Heuristic search strategies that traverse the tree space in a 'clever' way to find a tree with a 'good' score are therefore typically used to address this problem. Note that, most of these heuristics are ad hoc heuristics, that is, they do not offer any guarantees of how close or far away (with respect to the score) the trees they will find are from the globally optimal tree. To this end, the inexact terminology that is often used in evolutionary biology papers should be avoided. Authors often refer to 'the Maximum Likelihood tree,' whereas this is simply the best tree that could be found with the ad hoc heuristic search strategy that was used.

In this context a digression on how Bayesian methods (see 'See also' section) are affected by the enormous size of the tree space is in place. When introducing Bayesian methods to students the equation for the posterior probability can be written as follows:

$$P(T|D) = P(T) \times P(D|T)/\text{ouch}$$

where T is the tree, D the data, $P(T)$ is the prior probability, and $P(D|T)$ the standard phylogenetic likelihood (as used for maximum likelihood-based inferences; see 'See also' section) score of the tree. The term 'ouch' represents the marginal probability. Note that, for calculating this probability exactly one needs to evaluate the likelihood scores of all possible trees which, as explained before, is extremely painful.

Hence, it is impossible to compute the posterior probability $P(T|D)$ exactly, and thus it needs to be approximated via Markov-Chain Monte-Carlo (MCMC) methods (see 'See also' section). So-called MCMC chains are guaranteed to converge to the true posterior distribution if run for infinity. Since this is typically not feasible, the convergence of the chains is assessed using a plethora of methods (e.g., Nylander *et al.* (2008)). However, such convergence diagnostic methods can never ever tell the user that the chains have converged; they will merely indicate that they have not converged or that they might have converged. Therefore, in analogy to parsimony and maximum likelihood and the respective best-found trees, the inexact terminology authors frequently use when referring to converged MCMC chains should be avoided. One may state that one believes that they have converged or that they have reached apparent convergence.

In the following tree search algorithms will be introduced in a top-down manner. Initially, it will be outlined how search algorithms work in principle, that is what they have in common. Then the standard and most widely used tree alteration mechanisms will be discussed. Thereafter, it will be described how these standard alteration mechanisms are used in current tree search tools. Then, some advanced issues pertaining to the optimization of tree moves and terraces in tree space will be outlined. This part concludes with a short discussion and summary of the most important issues users should be aware of when using such algorithms. The main focus is on search algorithms under the parsimony and ML criteria, but commonalities with Bayesian inference methods will be emphasized where appropriate.

Top-Level View of Search Algorithms

Here, the design of most search algorithms will be outlined, before describing some strategies in further detail. In general, search algorithms comprise two steps:

1. Build a starting tree containing all taxa.
2. Change the topology of that starting tree to find a tree with a better score.

Building Starting Trees

To build a starting tree (often also called comprehensive tree), there exist three main options. One can either build a random starting tree, build a tree using a simple, that is, fast-to-compute tree building method (e.g., Neighbour Joining: NJ (see 'See also' section)), or build a randomized stepwise addition order starting tree.

Building a random tree or a tree using an existing NJ implementation is straightforward. Below, the construction of a randomized stepwise addition tree, given n taxa (see Figure 1) is outlined:

1. Select three taxa at random and build the one and only possible unrooted binary tree containing these three taxa.
2. Select the next taxon i at random.
3. Insert that taxon into every branch of the tree with $i-1$ taxa, calculate and store the score (using parsimony or likelihood) and remove the taxon from the tree again.
4. Insert taxon i into the branch that yielded the best score.
5. If $i<n$, increment i by 1 and go to step 2, otherwise the algorithm terminates and can return the comprehensive starting tree.

There are two things worth noting here: Firstly, this algorithm already uses a criterion to select among trees when adding taxa, that is, it will typically have a better score than a pure random starting tree. Secondly, depending on the randomized order by which taxa are added, one might obtain topologically distinct starting trees.

The fact that a randomized stepwise addition order starting tree already has a 'good' score helps to reduce the inference times for optimizations of the tree topology because the search already starts in a 'good' or reasonable region of the search space.

The fact that different random addition orders might produce distinct starting trees allows to start topological optimizations for finding the best-known tree from different good points in the tree space and might thus help to avoid some local optima (see below and Figure 2). However, under parsimony and for large phylogenomic datasets (e.g., in the analyses conducted for Misof *et al.* (2014)) with strong signal it has been repeatedly observed that different random addition orders can yield exactly the same starting tree. Hence, users should not implicitly assume that randomized addition order starting trees will be distinct, but perform appropriate tests (e.g., using the Robinson–Foulds distance, see 'See also' section) to verify that this is the case.

Note that, Bayesian inference programs usually start from a random tree, but some implementations also offer the option to start the MCMC procedure on a randomized stepwise addition order parsimony tree.

Changing Topologies

Given a comprehensive starting tree, one can then try to further improve its score by changing the tree topology and subsequently re-evaluating the score of the new tree using likelihood or parsimony. Here, one needs to distinguish between topological alteration mechanisms, that is, operations to change the tree topology and search strategies, that is, how and in which sequence those mechanisms are applied. Initially, the three basic tree search mechanisms that are used in every parsimony or ML search algorithm and also in every Bayesian MCMC implementation are introduced. Examples of search strategies are provided in the next section.

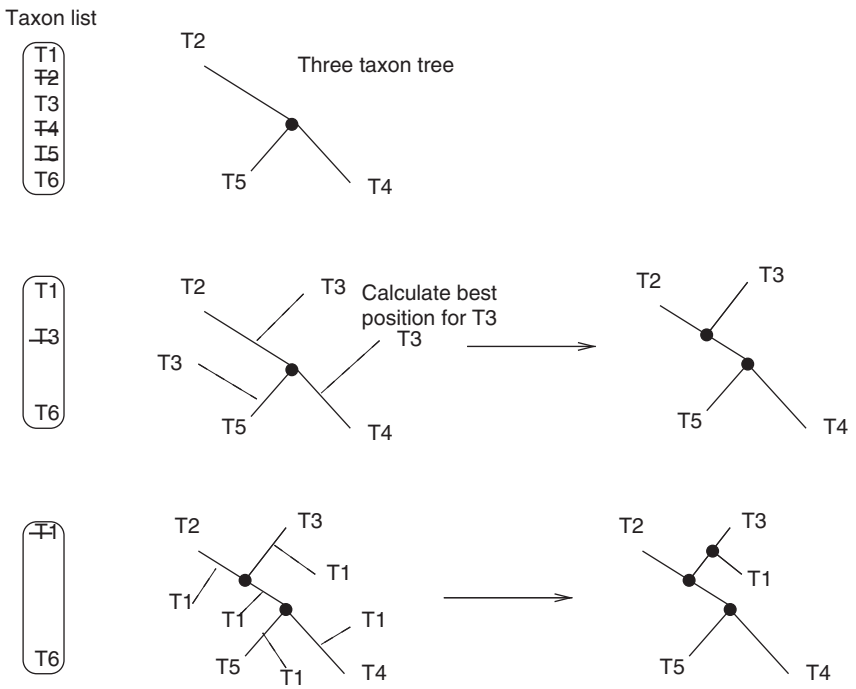

Figure 1 Schematic outline of randomized stepwise addition order algorithm for constructing starting trees.

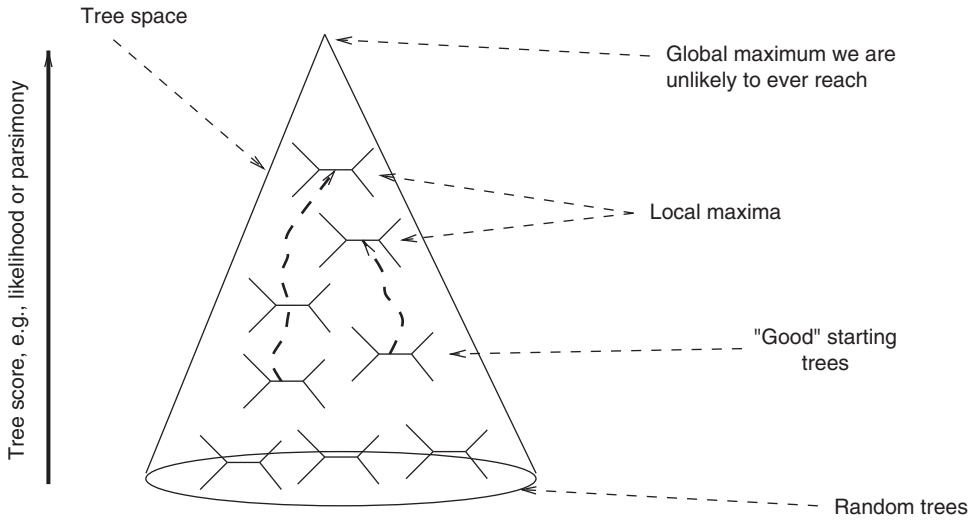

Figure 2 Schematic outline of the maximum likelihood tree search space.

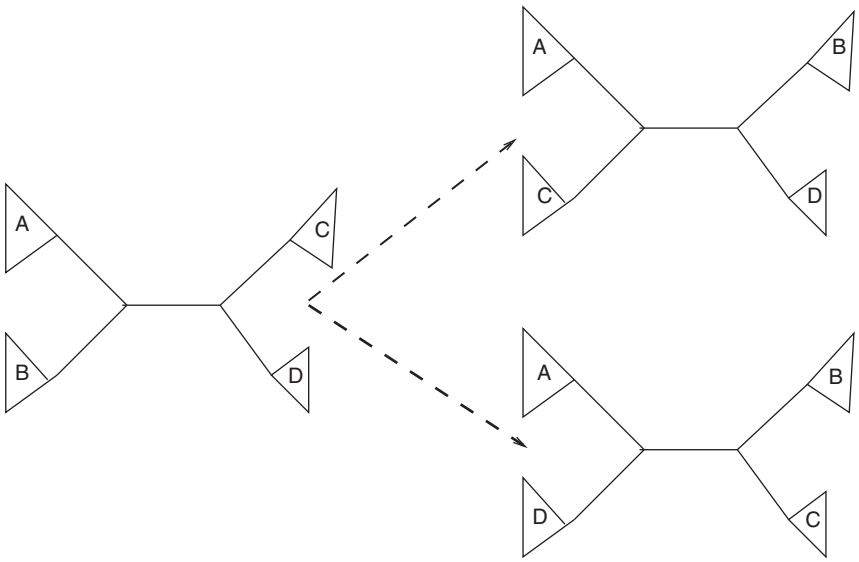

Figure 3 Schematic outline of NNI moves.

There exist three basic topological alteration mechanisms:

1. NNI: Nearest Neighbour Interchange (see **Figure 3**).
2. SPR: Subtree Pruning and Re-grafting (see **Figure 4**).
3. TBR: Tree Bisection and Reconnection (see **Figure 5**).

The most simple move is the NNI move. To execute an NNI move one selects an inner branch of the tree that defines four subtrees *A*, *B*, *C*, and *D*. Assume that *A* and *B* are located on one side of the branch and *C* and *D* on the other side of the branch (often denoted by *AB*|*CD*). One can then flip subtrees over that branch to construct two alternative topologies (*AC*|*BD* and *AD*|*BC*) and evaluate their respective scores.

A slightly more complex move is the SPR move. Here, one initially selects a subtree root in the comprehensive tree. Next, the selected subtree is removed (pruned) from the branch (called pruning branch) in the comprehensive tree to which it was attached. Thereafter, one can insert (and remove again) the subtree successively into all branches of the remaining tree and calculate the score for each subtree attachment. A frequently used variant of the SPR move is to limit the size of the region where the subtree will be reinserted into the remaining tree instead of reinserting it into all branches. Usually, a so-called rearrangement radius is used that specifies up to how many nodes away from the pruning branch the subtree shall be inserted.

The most complex or powerful move is the TBR move. Here, one initially chooses a branch and removes it from the comprehensive tree such that it is bisected, that is, two subtrees are obtained. These subtrees are then reconnected by using a new branch to connect any pair of branches in the two disjoint

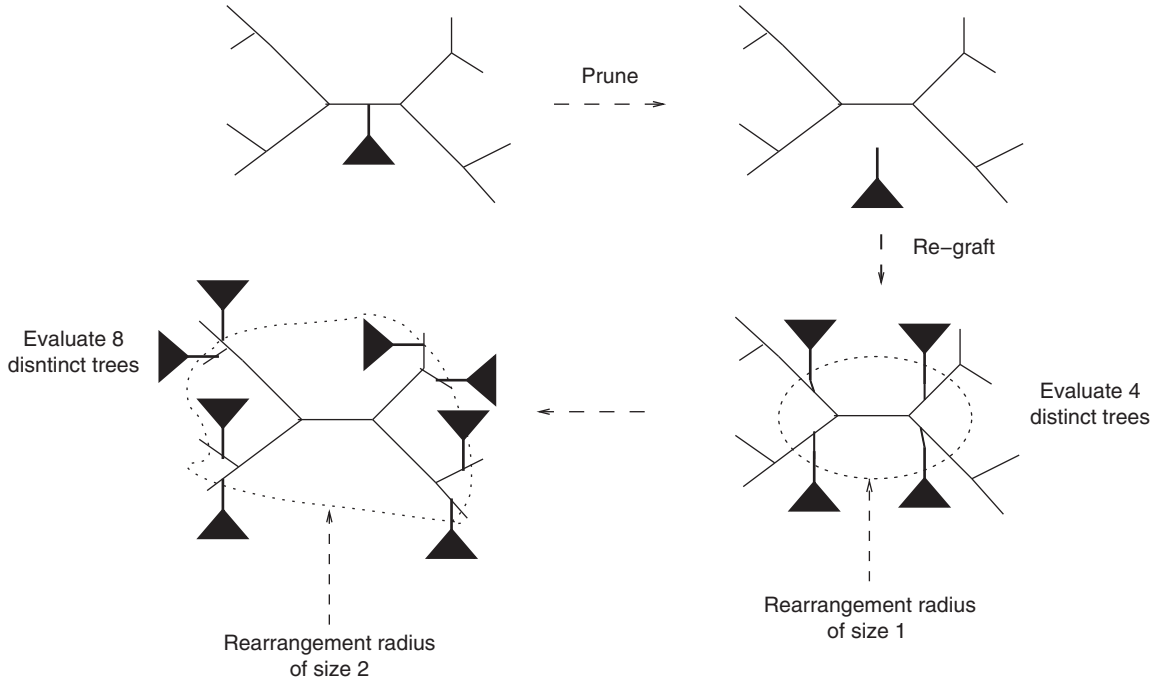

Figure 4 Schematic outline of SPR moves.

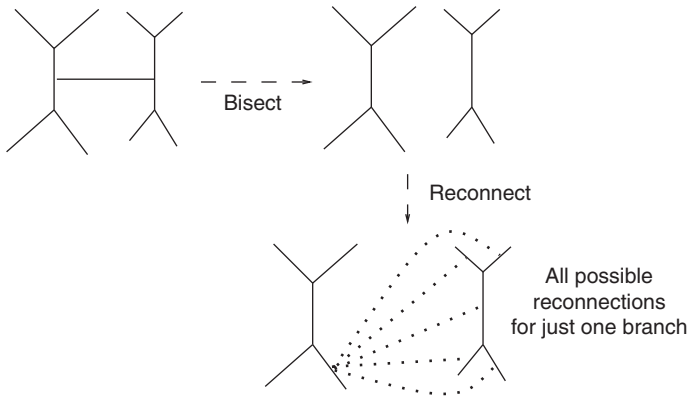

Figure 5 Schematic outline of TBR moves.

subtrees. In analogy to SPR moves, one can once again limit the size of the regions (number of branches) in the two disjoint subtrees that are considered for reconnecting the subtrees.

Search Strategies

Given these topology alteration mechanisms, they can be used to devise tree search algorithms or strategies. Most algorithms apply such topological moves until there is no move that yields a tree with a better score. In this case one says that the search algorithm has converged and the tree can be returned.

A simple example algorithm might initially build an NJ starting tree and subsequently apply NNI moves. For applying NNI moves it can repeatedly visit all inner branches of the tree. At each branch it will evaluate the two alternative NNI topologies and apply an NNI move if one of the two alternative topologies yields a better score. It will terminate if for none of the inner branches there is an improving NNI move.

The main problem one faces with such approaches is that, upon convergence, the search algorithm is most likely stuck in a local maximum (see **Figure 6**) that it cannot leave again via an NNI, SPR, or TBR move. There exist different approaches to alleviate this problem.

As mentioned before, one can initiate tree searches on distinct starting trees (e.g., RAxML, Stamatakis (2014)) in the hope of getting stuck in a more optimal local maximum. An extension of this approach is to use so-called genetic algorithms (e.g., GARLI, Zwickl (2006)) that also conduct searches on a set (population) of trees, but at some given points exchange information between trees to include, for instance, specific subtrees with good scores in most trees (e.g., tree fusion option in TNT, Goloboff *et al.* (2008)).

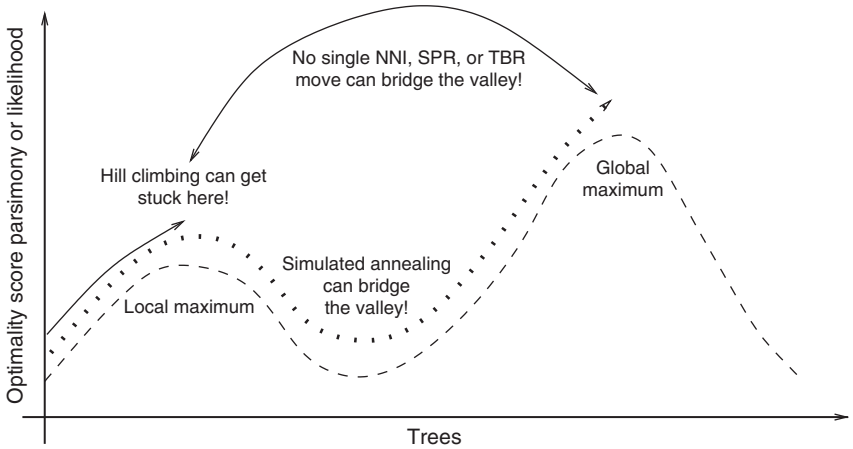

Figure 6 Outline of local maxima in tree searches.

The recently introduced IQ-Tree (Nguyen *et al.* (2015)) program tries to quickly navigate out of local optima by randomly perturbing the current locally optimal tree and then re-optimizing it. A similar idea for quickly navigating out of local optima by means of random perturbation is the ratchet method that has been applied to parsimony and ML searches (Nixon (1999) and Vos (2003)).

Another option is to use simulated annealing algorithms that sometimes, randomly, but not arbitrarily allow for backward steps, that is, moving to trees with lower scores which might in turn allow to navigate out of local maxima (e.g., Barker (2004) or Stamatakis (2005)). Note the connection between simulated annealing methods and Bayesian MCMC sampling methods that also sometimes allow for randomly but not arbitrarily accepting downhill steps toward trees with lower posterior probability. In addition, MCMC-based implementations typically also deploy Metropolis-Coupled chains to facilitate moving between and sampling of different local maxima (e.g., MrBayes by Ronquist *et al.* (2012) or ExaBayes by Aberer *et al.* (2014)).

Finally, a rather distinct approach for designing tree search heuristics relies on the quartet puzzling idea as implemented in Tree-Puzzle (Schmidt *et al.* (2002), see 'See also' section).

Advanced Topics

Computational Costs of Tree Moves

All tree search algorithms spend the largest fraction of overall run-time in evaluating scores on trees. Note that, as ML methods, Bayesian methods also spend between 90% and 95% of their time in evaluating likelihoods on changed trees or for altered model parameters. Thus, from an engineering point of view, ML and Bayesian inference face the same underlying computational problems.

Despite the fact that search heuristics are already being used, some moves and in particular SPR as well as TBR moves are still computationally very expensive such that further shortcuts need to be taken. In the following, the main focus will be on likelihood-based methods (Bayesian and ML) that require substantially more computational resources than parsimony. For instance, a parsimony search using a standard laptop computer on a DNA dataset with 125 taxa and around 30 000 sites takes 2.25 s under parsimony and 4464 s under maximum likelihood.

One common approach in ML-based inference is to deploy so-called lazy NNI or SPR moves. To calculate the ML score of an altered tree one normally needs to re-optimize all branch lengths of the changed tree which is a highly compute-intensive step. To avoid this, one may choose to pre-score and sort alternative SPR moves using parsimony which is substantially faster and subsequently simple to evaluate the most promising candidate moves under ML as done in PHYML (Guindon *et al.* (2010)). Alternatively, one can choose to only optimize a small set of branches around the new subtree insertion point of an SPR move as done in GARLI (Zwickl (2006)) and RAxML (Stamatakis (2014)).

MrBayes and ExaBayes use similar parsimony-based pre-scoring techniques to conduct topological SPR and TBR proposals in order to achieve a good proposal acceptance rate. Note that the implementation of such biased (i.e., non-random) proposals in the Bayesian framework is non-trivial because the reverse move (e.g., undoing a TBR) must have the same probability to be carried out. For an in-depth introduction to Bayesian phylogenetic inference, see 'See also' section.

Terraces in Tree Space

An issue whose existence for likelihood-based methods was only recently discovered and that yields tree searches even more challenging is that of terraces in tree space. For parsimony, the issue was already known by design of the criterion since researchers were aware of the existence of equally parsimonious trees, that is, trees with different tree topologies that have the same parsimony score. Such a set of topologically distinct trees with identical optimal scores is called a terrace in tree space.

For likelihood-based methods, terraces may exist for partitioned phylogenomic alignments with missing data and unlinked branch lengths. Branch lengths are unlinked when a set of completely independent branch lengths is estimated for each partition (e.g., each gene) of the input dataset. Whether a

dataset gives rise to terraces depends on the structure of the missing data (Sanderson *et al.* (2011)). The two main problems one faces with terraces are the following: Firstly, one should avoid conducting tree moves that will just take the search to another tree located on the same terrace. Secondly, the presence of terraces can severely bias bootstrap and Bayesian support values (Sanderson *et al.* (2014), see 'See also section).

Discussion

Readers should keep in mind that most search heuristics are ad hoc methods that work well on the empirical and simulated datasets on which they have been tested and tuned. In contrast to so-called approximation algorithms that guarantee to always yield results that are, for instance, at most 50% worse than the optimal solution, current phylogenetic inference tools do not guarantee for anything whatsoever. This also holds for Bayesian inference, albeit there is a more solid theoretical underpinning that guarantees that the chains will converge to the true posterior distribution if run for an infinite amount of time, regardless of the quality (acceptance rate) of the proposal mechanisms that are being used.

Thus, development of tree search methods generally represents an engineering task. Engineering always entails tradeoff decisions. For instance, when designing a search algorithm, one needs to decide how much additional computing time one is willing to sacrifice in order to obtain trees with potentially only slightly better scores. In addition, program performance can be dataset-specific. Thus, users should try to run two or three ML and Bayesian inference codes to identify the search strategy that works best on their dataset.

See also: Bayesian Phylogenetic Methods. Distance-Based Phylogenetic Inference. Maximum Likelihood Phylogenetic Inference. Parsimony Methods in Phylogenetics. Phylogenetic Tree Distances. Support Measures, Phylogenetic Tree

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EDITOR IN CHIEF

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EDITOR IN CHIEF

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Kliman's research interests center on questions in molecular evolution, including the evolution of codon usage bias in a variety of organisms; speciation and natural history; and ecology and conservation. Much of this work has relied on population genetics/genomics and bioinformatics approaches. He has also collaborated with Cedar Crest colleague John Cigliano on an Earthwatch-supported "before-after-control-impact" study on the effects of a new marine reserve in Belize on queen conch populations. His research in evolutionary and ecological genetics has been supported by the US National Institutes of Health and by Conservation International.

Kliman has served on the editorial boards of *Genetica* and *The Journal of Molecular Evolution*. He has been deeply involved in evolution education, helping to coordinate "Undergraduate Diversity at SSE/SSB," an NSF-supported program to bring a diverse group of undergraduates to the annual Evolution research conference. He was a lead editor of population/quantitative genetics and evolutionary genetics for *Nature Education/Scitable* at its inception. He is a member of the Education and Outreach Committee of the Society for the Study of Evolution, and editor of the society's peer-reviewed educational resource, the *EvoEd Digital Library*.

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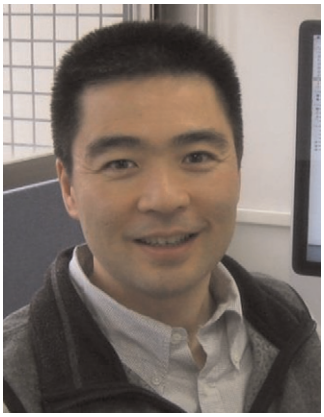

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Tim Coulson's primary interest is in creating better links between the fields of ecology and evolution. He does this by developing theory, parameterising models for field and laboratory systems, making predictions from these models, and, where possible, testing these predictions with experiments. He works on a range of systems, from bulb mites within the laboratory, to guppies living in streams in Trinidad, to wolves in Yellowstone. His motivation to do this comes from observations that ecological and evolutionary change can be observed occurring on similar time scales, yet ecological theory typically ignores evolutionary processes and vice versa.

Tim was awarded his PhD in plant ecology from Imperial College, London, in 1994. He moved on to research genotype-by-environment interactions as Natural Environment Research Council (NERC)-funded post-doc at the Institute of Zoology in London. He remained at the Institute on a fellowship where he developed models to investigate the economic and life history consequences of a range of population management strategies. In 2000 he moved to the University of Cambridge, where he briefly lecturered in the Zoology department. In 2004 he moved back to Imperial College London as a senior lecturer where he started developing models that allow the simultaneous investigation of the dynamics of life history, populations, and quantitative characters. In 2007 he became Professor of Population Biology at Imperial College London. He left Imperial in 2013 to take up his current position as Professor of Zoology at the University of Oxford. He is also a Professorial fellow of Jesus College, Oxford.

Andrew Forbes

Rosemary Gillespie is a Professor at the University of California, Berkeley, where she also holds the Schlinger Chair in Systematics. She is Past President of the *International Biogeography Society* and Trustee and Fellow of the *California Academy of Sciences*, and serves as Associate Editor for *Molecular Ecology*. Gillespie was born and educated in Scotland, receiving her BSc in Zoology from Edinburgh University in 1980. She came to the US to conduct graduate work on the behavioral ecology of spiders at the University of Tennessee. After her PhD she spent several months at the University of South in Tennessee, and then started work at the University of Hawaii in 1987, initially as a postdoc, and then in 1992 as Assistant Professor in Zoology and Researcher in the Hawaiian Evolutionary Biology Program. It was during her first year in Hawaii that she discovered an adaptive radiation of *Tetragnatha* spiders. She left Hawaii in 1999 to join the faculty at the University of California in Berkeley, where she continues her research focus on the islands of the Pacific, Hawaii in particular, using islands of known age and isolation to assess the combined temporal and spatial dimension of biogeography and determine patterns of diversification, adaptive radiation, and associated community assembly.

David Guttman received his PhD from Stony Brook University in 1994 working with Daniel Dykhuizen on questions related to the role and importance of recombination in structuring genetic diversity in bacterial populations. He followed this with a postdoc in molecular evolution with Brian and Deborah Charlesworth at the University of Chicago, and a second postdoc at the University of Chicago with Jean Greenberg to gain experience in the fields of molecular plant pathology and plant-microbe interactions. He started his faculty position at the University of Toronto in 2000, and is currently a Professor in the Department of Cell & Systems Biology (CSB). He is also the Associate Chair for Research in CSB, founder and Director of the University of Toronto Centre for the Analysis of Genome Evolution & Function, and Canada Research Chair in Comparative Genomics. He has served as the Chair of the American Society for Microbiology, Division R (Evolutionary and Genomic Microbiology), and was the *PLoS Pathogens* Section Editor for Bacterial Evolution & Genomics.

Dr. Guttman runs a highly diverse research program generally focused on bacterial evolutionary genomics, with three major foci: (1) the evolution of host specificity and virulence in plant pathogenic bacteria; (2) microbial comparative genomics; and (3) studies of the human and plant-associated microbiome. He is best known for elucidating and linking evolutionary and mechanistic processes that determine the course and fate of bacterial infections, and characterizing the impact of genetic variation on the balance between disease and immunity.

Norman A. Johnson, the section editor for Applied Evolution, is an evolutionary geneticist and author. He received his PhD from the University of Rochester in 1992 and did postdoctoral research at the University of Chicago. His research interests have generally focused on aspects of speciation, specifically those related to the genetics and evolution of hybrid incompatibility: sterility, inviability, or other reduction of fitness in hybrids between species. Dr. Johnson, an adjunct professor in the Biology Department at the University of Massachusetts at Amherst, has taught classes there, as well as at Hampshire College, the University of Texas at Arlington, and the University of Chicago.

Dr. Johnson also has a long-standing commitment toward improving the communication of science in general and evolutionary biology in particular to other scientists, educators, and the public at large. He is the author of *Darwinian Detectives: Revealing the Natural History of Genes and Genomes* (Oxford University Press: 2007), a book geared to general audiences that shows how biologists use DNA sequence data to make inferences about evolutionary processes. He also was the lead organizer for a working group on communicating human evolution at the National Evolutionary Synthesis Center (NESCent).

Laura Kubatko received a PhD in Biostatistics from The Ohio State University (OSU) in 1999. After seven years on the faculty at the University of New Mexico, she returned to OSU in the Fall of 2006, and is now Professor of Statistics and of Evolution, Ecology, and Organismal Biology at OSU. Laura served as an Associate Director of the Mathematical Biosciences Institute at OSU from 2013–2015. At OSU, she is a Faculty Affiliate of the Initiative in Population Research, and a Faculty Affiliate in Translational Data Analytics (TDA@OSU). She holds appointments as an Affiliate Faculty Member at the Battelle Center for Mathematical Medicine at Nationwide Children's Hospital in Columbus and as an Adjunct Research Scientist at Lovelace Respiratory Research Institute in Albuquerque, NM. Laura's research interests are in statistical genetics, with a focus on the development of statistical methods for inferring phylogenies from molecular data. Her recent work in this area concentrates on bridging the gap between traditional phylogenetic techniques and methodology used in population genetics analyses, primarily through the application of coalescent theory to species-level phylogenetic inference. She develops and distributes several software packages for phylogenetic inference, and has been an active member of the *Society of Systematic Biologists*. She has served as an Associate Editor for the journal *Systematic Biology* since 2007.

Amy Litt has been studying plant evolution and diversity since her PhD on floral structure and evolution in the neotropical plant family Vochysiaceae, known for its beautiful but unusual flowers many of which have only one petal and one stamen. While completing her PhD in plant systematics and morphology in the joint City University of New York/New York Botanical Garden Plant Sciences program under Scott Mori and Dennis Stevenson, she became interested in the molecular basis of plant diversity. She did her post-doc in the developmental genetics lab of Vivian Irish at Yale University on the evolution of a family of transcription factors involved in flower development, and she continues to study the functional evolution of this gene family currently. After one year on the faculty of University of Alabama, she moved back to The New York Botanical Garden as Director of Plant Genomics, where she developed her research program studying the evolution of plant form along two paths: studying evolutionary changes in genes to see how those changes affected flower and fruit form; and identifying the genes that underlie differences in form among closely related species. Dr. Litt also served as a program director in Plant, Fungal, and Microbial Development and Evolutionary Development at the National Science Foundation. She recently moved to the University of California at Riverside, where she continues to study the genetic basis of plant diversity.

Maria E. Orive is a professor of evolutionary genetics in the Department of Ecology and Evolutionary Biology at the University of Kansas. Her research in theoretical population genetics aims to develop mathematical models that provide a conceptual framework for exploring important questions in evolutionary biology and analytical tools for demographic and genetic data. Her work has considered levels of selection and mutation in organisms that reproduce both sexually and asexually, the relationship of population structure and life-history attributes to gene flow and genetic diversity, and models of within- and between-host pathogen and symbiont population dynamics. Orive received her BS from Stanford University and her PhD from the University of California at Berkeley. After spending two years as a postdoctoral researcher in genetics at the University of Georgia, she was an NSF-NATO Postdoctoral Fellow at the University of Edinburgh. Her research has been funded by multiple grants from NSF and NIH. In 2007–2008, she was the Carl and Lily Pforzheimer Foundation Fellow at the Radcliffe Institute for Advanced Study (Harvard University), and has served as the University Faculty Ombudsman for the University of Kansas since 2007.

Daniel Ortiz-Barrientos is an Associate Professor in evolutionary genetics in the School of Biological Sciences at The University of Queensland, Brisbane, Australia. During his scientific career he has investigated the ecological and genetic basis of speciation both in plants and animals. His current research program explores the early stages of speciation, the molecular basis of parallel speciation, and the interplay between recombination and natural selection during the origin of new species. His research funds come from The Australian Research Council. He is married to Antonia Posada, and is the father of three energetic and beautiful kids.

Claudia Russo was born in Leeds, England, but has lived in Rio de Janeiro, Brazil since she was two years old.

Claudia has an academic major in Ecology from Universidade Federal do Rio de Janeiro completed in 1989, and finished her Master's thesis in 1991 on population genetics of two actiniid species of sea anemones with different reproductive strategies, under the supervision of Associate Professor Antonio Mateo Sole-Cava. Her PhD dissertation was on the diversification of drosophilids and on the use of a known phylogenetic tree to estimate the reliability of tree building methods. The dissertation was completed in 1995 under the supervision of the Evan Pugh Professor Masatoshi Nei who recently received the prestigious Thomas Hunt Morgan Medal. Her graduate degrees were obtained as a student at the Genetics Program from the Universidade Federal do Rio de Janeiro and as a visiting scholar at the Pennsylvania State University (1992–1995).

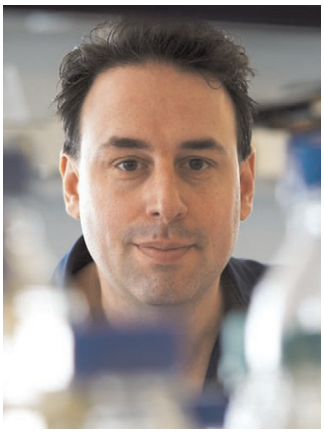
Claudia is currently the Head of the Genetics Department at the Federal University of Rio de Janeiro, having been a member since 1997. Claudia has supervised 13 Master's dissertations, eight PhD theses and seven post-docs, of which eight are now Assistant Professors at universities in Brazil and abroad. She has published 42 academic papers that have been cited over 1,200 times. Her *h-index* is 14. Since 2012, Claudia has been a member of the editorial board, and an associate editor of the *Molecular Biology and Evolution* journal. Since 2012 she has been a council member for the Pan American Association of Computational Interdisciplinary Sciences and since 2009 for the Brazilian Association for the Advancement of Science.

Claudia's general academic interests are on key aspects of animal phylogenetics, including their diversification patterns in time and space. She has worked with various metazoans groups but more prominently on marine sponges, sea anemones, arthropods, passerine birds, and mammals. Claudia has also published on the use of known phylogenetic trees to estimate the efficiency of phylogenetic methods in recovering and rooting those trees. More recently, she has developed some interesting *hands-on* educational tools for evolutionary biology practices in the classroom.

Karen E. Sears is an evolutionary developmental biologist whose primary research goal is to determine how developmental variation within a species produces congenital malformations in humans, and among species generates new evolutionary forms in mammals. Dr. Sears earned her PhD from the University of Chicago, did postdoctoral research at in the Howard Hughes Medical Institute (HHMI) lab of Dr. Lee Niswander, and joined the faculty of the University of Illinois at Urbana-Champaign. At Illinois she holds positions as an Associate Professor in the Department of Animal Biology, a Faculty Member in the Institute of Genomic Biology, and an Affiliate of the Program in Ecology, Evolution and Conservation Biology and the Department of Cell and Developmental Biology. She is also the President of the Pan American Society for Evolutionary Developmental Biology. She has authored or co-authored over 35 publications including first-authored publications in *Nature*, *Proceedings of the National Academy of Sciences*, and *Evolution*. She has served as a principal investigator on multiple, nationally-funded research projects, and presented invited seminars at more than 30 institutions and symposia. She is routinely ranked among the top 10% of Illinois professors for her teaching, and was a featured scientist in the PBS/HHMI documentary "*Your Inner Fish*."

Vassiliki "Betty" Smocovitis is Professor of the History of Science in the Department of Biology and in the Department of History at the University of Florida. Her areas of expertise include the history of evolutionary biology, genetics and systematics and the history of botany. She is best known for her contributions to understanding the historical event known as the "evolutionary synthesis" and in gaining greater understanding of the origins of the discipline of evolutionary biology. She has published extensively on both the intellectual and social aspects of the history of evolutionary biology including a history of the Society for the Study of Evolution, a history of the Darwin Centennial of 1959, and the integration of botany, genetics, and anthropology into the evolutionary synthesis. She was the contributor to the *Oxford Bibliographies* entry on Charles Darwin at over 25,000 words and the entry on the modern synthesis. She is the author of *Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology* (Princeton: Princeton University Press, 1996).

Nina Wedell is a professor of evolutionary biology with research interests focused on the evolutionary ecology of sex. She has worked extensively on various aspects of sexual selection and sexual conflict, in particular on the role of selfish genetic elements in reproductive biology. Nina is the Academic lead for the Behaviour research group at the University of Exeter.



Jason Wolf is Professor of Evolutionary Genetics in the Department of Biology & Biochemistry and The Milner Centre for Evolution at the University of Bath. His research is unified with a special focus given to understanding the influence that frequently ignored or under-appreciated sources of genetic variation have on the genotype-phenotype relationship and how this, in turn, influences evolutionary processes. He integrates theoretical, computational and empirical quantitative and population genetic techniques to achieve this goal. He is particularly interested in understanding the evolutionary consequences of various types of interactions, including gene interactions (epistasis), parent-offspring interactions and social interactions. He received a PhD from the University of Kentucky, after which he was a postdoctoral researcher at Indiana University and a US National Science Foundation Postdoctoral Fellow at Washington University School of Medicine. Prior to moving to the University of Bath he held positions at the University of Tennessee and the University of Manchester. He won the Dobzhansky Prize from the Society for the Study of Evolution, a Young Investigator's Prize from the American Society of Naturalists and the Scientific Medal from the Zoological Society of London.

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Alphabetical Contents List

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

All articles within the encyclopedia have an extensive list of cross-references which appear at the end of each article, for example:

Adaptive Mutation Controversy

> *See also*: Directional Selection and Adaptation. Microbial Experimental Evolution. Mutation, Population Genetic Models of. Natural Selection, Introduction to. Recombination in Bacterial Populations

3. Subject Classification List

A subject classification list has been developed by the editors to group articles under broad topics. This index is useful for making broad connections between articles and locating the relevant article for a topic that is covered in more than one article.

4. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

5. Contributors

A full list of contributors appears in the front mater of each volume.

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Evolution and Agriculture I. The Evolution of Domestication
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Evolutionary Medicine: I. An Overview and Applications to Cancer
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Plant/Fungus Diversification

Population Genetics

Quantitative Genetics

Sex, Recombination, and Mating Systems

Speciation and Hybridization

PREFACE

The *Encyclopedia of Evolutionary Biology* was developed to provide an authoritative overview of the current state of evolutionary biology. It was an ambitious goal, especially given that the field did not pause for the two and a half years needed to complete the project. The encyclopedia's 15 section editors collaborated to ensure that content gaps were kept to a minimum, and their efforts show. When the project was completed, we had compiled 256 entries, covering a broad range of topics selected by the editors to ensure a comprehensive resource. It was a privilege to read every one of these entries, and I was truly humbled by the collective efforts of hundreds of authors to communicate the excitement and sophistication of a field of study that touches on every conceivable topic in biology today.

There are many ways to envision an encyclopedia of evolution, and we had to choose an approach that would lead to a cohesive resource. Readers will note that, in the more organismal-focused entries (edited by David Guttman, Amy Litt, and Claudia Russo), there is an emphasis on *diversification* of life. We did not set out to provide an overview of the diversity of life, as such a goal would be untenable; rather, we focused on the evolutionary processes and key events responsible for diversity. Numerous entries deal with speciation, life history evolution, evolutionary biogeography, and coevolution. These entries (edited by Daniel Ortiz-Barrientos, Tim Coulson, Rosemary Gillespie, and Andrew Forbes) bring to light how the evolution and diversification of life is intimately entwined with ecology. Of course, there is extensive coverage of population genetics, quantitative genetics, evolutionary developmental biology, the evolution of sex and mating systems, molecular/genome evolution, and phylogenetic analysis (edited by Maria Orive, Jason Wolf, Karen Sears, Nina Wedell, Hiroshi Akashi, and Laura Kubatko), all fundamental to our understanding of evolutionary processes. And as thematic bookends, several entries (edited by Betty Smocovitis and Norman Johnson) cover the history of evolutionary biology and applications of evolutionary biology.

Readers of the encyclopedia will find that entries are generally pitched at a somewhat advanced level, although with great effort by authors to make entries as accessible as possible to a broad audience. Encyclopedias, like living organisms, are compromises. If all entries could be readily understood in their entirety by first-year university students, this encyclopedia would be of limited value to experts. At the other extreme, if entries were extremely technical – and our authors were undoubtedly capable of producing such entries – the encyclopedia might be inaccessible to students. While there is, by necessity, variation among entries in this regard, we settled on a general target: the majority of an entry should be accessible to a motivated, advanced undergraduate. Readers are, of course, directed to additional resources, with authors providing bibliographies and lists of further reading.

As with any undertaking of this scale, there are many individuals who should be recognized for their roles in the development of this encyclopedia. Special thanks go to Norman Johnson for early discussions that helped us develop the general structure of the encyclopedia. The dedicated and distinguished team of section editors deserves the credit for drafting the table of contents, recruiting authors, and working extensively with authors to ensure the highest quality product. It should go without saying that the high quality of this encyclopedia ultimately reflects the efforts of the editors and authors. Finally, the project management and development teams at Academic Press were always ready to assist, and while it is not possible to name everyone who contributed to the effort, I am particularly indebted to Simon Holt, Will Bowden-Green, Paula Davies, and Justin Taylor.

Richard Kliman
Editor in Chief

FOREWORD

What is life, how did it originate, and what accounts for its great diversity? These are fundamental scientific questions that have and will always be the source of endless fascination and wonderment. Charles Darwin and Alfred Russel Wallace provided an answer to the latter question through the grand idea of evolution and the process of natural selection. Darwin also speculated on the where question of the origin of life by hypothesizing it originated long ago in a warm lagoon. Most importantly, however, Darwin shattered the notion that the natural world is static and replaced it with a biology that is dynamic and continually changing. Species are not fixed, typological entities. Rather, they are related by common descent in a great tree of life. Analogous to tracing one's ancestors back in time in a pedigree, one can climb down the tree along its branches and boughs that connect species in a hierarchy of phylogenic relationships until reaching the base of its trunk and the common ancestor of us all. One can also climb up the tree and quickly realize that evolution produces a seemingly endless array of new forms (and sometimes extinction). Thus, as the tree of life grows, populations are continuously evolving and diverging from one another, creating novel varieties and races (showing slight differences) that eventually evolve into new species (separated by distinct gaps). And natural selection – the differential survival of individuals in populations possessing heritable traits favorable for their survival and reproduction – is the primary materialistic process causing evolutionary change and the origin of new species.

The *Encyclopedia of Evolutionary Biology* chronicles our current state of understanding of the dynamics of evolution and its product, Darwin's great tree of life. A diversity of seminal topics are covered including overviews of the history of the field, the origin of life, the history of life (including the phylogentic methods used to reconstruct life's history), the myriad ways and means (including mechanisms other than natural selection) that evolution is affected, and the important roles that conflict versus cooperation, and mergers and acquisitions, occurring within across varying levels of biological organization, play in the narrative of life. In so doing, the *Encyclopedia* highlights the grandeur in Darwin's view of life. We are not separate, but rather a twig along a branch of life, a twig that has evolved the ability to comprehend the existence of and our connectedness to the tree, and climb around its branches to see what has been and think about what may come. It is a wonder of life that it can look at and understand the meaning of itself.

But Darwin's grand view has even larger ramifications, going beyond providing a materialist basis for organismal change and putting us in our place. The reality of evolution also answers the question of what life is. If pressed to define life, most of us would reply with a list of the things that living organisms do. For example, living organisms metabolize, grow, develop, move, behave, mutate (are variable), and reproduce with inheritance. One can investigate these different characteristics of life separately and discern the mechanistic basis for the different processes that constitute life – the "how" of life. And such studies represent the basis for many fields of the life sciences. However, these are only the components of life and, in isolation, produce a static view of the natural world. Rather, the seminal insight is that populations of living beings possessing these characteristics have the emergent property that they evolve. Darwin's "*On the Origin of Species*" therefore not only describes how populations evolve, and as a logical extension how new species form, but also conveys the essence of what life itself is – evolution. Thus, as Theodosius Dobzhansky famously stated "nothing makes sense except in the light of evolution." The Encyclopedia wonderfully brings this view of life to light, providing the reader with the breadth of knowledge and overview of the current state of the field of evolution needed to appreciate and participate in the next major ongoing synthesis in our understanding of life, the so-called "Omics Revolution."

The study of evolution is in an accelerated phase of discovery brought about by major technical advances in our ability to DNA sequence whole genomes (genomics), and to generate profiles of mRNA transcription (transcriptomics), protein levels and enzymatic activity (proteomics), and metabolic products (metabolomics) at varying stages in the life cycle and development of organisms. This "Omics Revolution" may not change foundational evolutionary principals, per se. Our understanding of evolution has been heightened by a series of such advances in the past, including the "Modern Synthesis" when Mendelian genetics was wedded to Darwinian thinking and the "Molecular Revolution" in which genetic technology increasingly allowed allele frequencies in natural populations to be analyzed. The Omics Revolution is an extension of these pervious advances, but one in which the workings of whole organismal systems and the composition of entire communities can be gleaned at once.

Perhaps, the most important discoveries in Omics will come from linking an understanding of the process occurring at the cellular and microevolutionary level with large scale patterns and trends at the macroevolutionary scale. Previously, processes occurring within and interactions occurring among cells could be studied in at least some detail. Omics is providing an opportunity to fully understand how all of these processes interact simultaneously to result in the development and functioning of integrated, multicellular systems of life. At the other end of the spectrum, fossils attest to the evolution of new life forms through time and the creation of great and observable morphological diversity. Genomic sequencing is providing a powerful means to help accurately place these fossils within the framework of a fully resolved molecular phylogenic tree to better understand the history of life, including major trends, themes, and variation in the tempo and mode of evolutionary change. But it is the middle of the micro and macro at the branching points in the tree of life that Omics may prove most insightful. Now it is possible to not only DNA sequence large numbers of individuals within populations, but to equate these genetic differences within and between populations to morphological, physiological, and behavioral phenotypes, and discern the developmental

and physiological mechanisms by which these genetic changes produce organismal variation, diversity, and reproductive isolation – the stuff of evolution and speciation itself. Thus, we will be able to not only understand the everyday processes responsible for how the tree of life grows, but be able to translate this into a mechanistic appreciation of how these processes result in new branches on the tree forming and others dying out, giving shape to the history of being on our planet.

The field of evolution is currently inundated with a mass of Omics data and the bottleneck is the development of bioinformatic analytical tools to edit, analyze, and interpret the results. However, it is clear that many new insights are on the horizon, and even if they do not affect the root principles of evolution, soon a deep connection of the how and why of life will emerge to help forge a truly integrative evolutionary biology: The *Encyclopedia of Evolutionary Biology* is an excellent guide to prepare readers to assimilate these new findings, keeping bioinformatics grounded in the bio and providing a valuable source for seeing the tree through the forest to understanding the grand synthesis of life that is flowering.

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S

Secondary Metabolites, the Role in Plant Diversification of

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Glossary

Allelopathy SM produced by one plant which inhibit germination and growth of other plants.
Aposematic coloration Animals with warning colors are termed aposematic.
Callose A polysaccharide used by plants during a variety of processes in their development and/or in response to multiple biotic and abiotic stresses.
Ectomycorrhiza Symbiotic relationship between a fungal symbiont and the roots of various plants.
Elicitors Compound which can induce a defense reaction in plants.
Endomycorrhiza Symbiotic relationship between an internal fungal symbiont of various plants.
Endophyte Bacteria or fungi, living endosymbiotically within a plant.
Lignin Polymer from phenylpropanoids used to support tissues and structures of vascular plants and some algae.
Phytoalexins Antimicrobial substances synthesized *de novo* by plants at areas of a microbial infection.
Tonoplast Membrane surrounding the vacuole of a plant cell.

What are Secondary Metabolites?

Diversity of Secondary Metabolites

Whereas primary metabolites occur in all plants and are essential for survival, secondary metabolites (SM) have a more restricted distribution. Plants can live without them, as can be seen from some of our cultivated crop plants (Kadereit *et al.*, 2014) from which they have been removed by breeding. Nevertheless, SMs, which occur in great structural diversity, count as typical traits of plants (Seigler, 1998; Wink, 2010a,b; Kadereit *et al.*, 2014). More than 200 000 chemical structures have been isolated and described so far, and these can be divided into SM with and without nitrogen in their structures (**Table 1**). Among nitrogen containing SM, the class of alkaloids is the largest with over 27 000 structures (Roberts and Wink, 1998), followed by 700 non-protein amino acids (NPAA). Cyanogenic glucosides, glucosinolates, and amines are less diverse, with fewer than 100 structures in each class. The largest group of SM do not contain nitrogen in their structures; among them terpenoids (mono-, sesqui-, di-, tri-, and tetraterpenes, saponins, iridoid glucosides) are most numerous, followed by the widely distributed phenolics (phenylpropanoids, flavonoids, catechins, tannins, lignans, coumarins, furanocoumarins, anthraquinones) (**Table 1**). It has been estimated that only 20% of all plants have been studied phytochemically using mass spectrometry and NMR; therefore, it is likely that the real number of SM will exceed 200 000 by far when all plant taxa have been analyzed one day.

Biosynthesis and Storage of Secondary Metabolites

The more than 200 000 SM structures might irritate a student of botany, but the underlying biosynthetic pathways are less diverse (**Table 2**; Dewick, 2002; Wink, 2010b). SM derive from precursors of primary metabolism. Certain amino acids (especially lysine, arginine, ornithine, phenylalanine, tyrosine, tryptophan) are precursors for alkaloids, cyanogenic glucosides, glucosinolates, and amines. Precursors of phenolics are the aromatic amino acids phenylalanine and tyrosine, the latter being synthesized in the shikimate pathway. Acetyl-CoA is a universal precursor for terpenoids, fatty acids, waxes, and polyketides. The polyketide pathway leads to quinones and partially to flavonoids, catechins, and anthocyanins (details in Wink, 2010b; Krauss and Nies, 2014). The main skeletons, which are generated by these key pathways, are further modified by oxidations or reductions, and by introduction of functional groups, such as carboxyl, aldehyde, hydroxyl, and SH-groups. Furthermore, via the functional groups, SM can be esterified, glycosylated, or polymerized. Each SM with its unique 3D structure represents a unique biologically active compound that has been shaped during evolution by natural selection, which can interfere with molecular targets in herbivores, microbes, and even other plants. SM show a

Table 1 Estimated number of described secondary metabolites and their main functions for the plants producing them[a]

Class	*Numbers of structures*	*Toxic or repellent for herbivores*	*Antimicrobial activity*	*Attraction of pollinators or fruit dispersers*
With nitrogen				
Alkaloids	27 000	++++	++	−
Non-protein amino acids (NPAA)	700	++++	+++	−
Cyanogenic Glucosides/HCN	60	++++	+	−
Mustard oils (Glucosinolates)	150	++++	++++	+/−
Amines	100	+++	+	+++
Lectins, Peptides, AMPs	2 000	+++	+++	−
Without nitrogen				
Terpenes				
Monoterpenes (including iridoid glucosides)	3 000	++	+++	+++
Sesquiterpenes	5 000	+++	+++	++
Diterpenes	2 500	+++	+++	−
Triterpenes, steroids, saponins (including cardiac glycosides)	5 000	+++	+++	−
Tetraterpenes	500	+	+	+++
Phenolics				
Phenylpropanoids, coumarins, lignans	2 000	+++	+++	++
Flavonoids, anthocyanins, tannins	4 000	+++	+++	++
Polyketides (anthraquinones)	800	++++	+++	−
Others				
Polyacetylenes	1 500	++++	++++	−
Carbohydrates, organic acids	600	+	++	+

[a]Activity: − = no SM active; +/− very few SM active; + = few SM active; ++ = many SM active; +++ = most SM active; ++++ = all SM active.

considerable turnover during normal metabolism, after wounding or after an infection. Whereas the enzymes of SM formation are highly substrate specific, the degrading enzymes (glucosidases, esterases, phenolase) exhibit a much broader substrate spectrum.

The genes which encode key enzymes of several pathways have been identified, so that the SM of plants can be manipulated by gene technology (Kutchan, 1995; Facchini, 2001; Memelink, 2005; Schäfer and Wink, 2009; Wink, 2010b). This technology offers the opportunity to knock out genes and analyze how this event changes the phenotype and more importantly the survival of a plant in nature. SM-free mutants produced by this technology can help to elucidate the functions of the SM. Furthermore, it is now possible to breed crop plants resistant to microbial pathogens or herbivores, as SM are important contributors to these traits (Wink, 1988). In the future, it will probably be possible to produce valuable plant SM (morphine, vinblastine) in recombinant microorganisms (Memelink, 2005; Schäfer and Wink, 2009; Wink, 2010b).

The cytoplasm is usually the site for SM synthesis. Hydroxylations via cytochrome oxidases are carried out at the smooth endoplasmic reticulum. A few biosynthetic pathways or parts of them can take place in chloroplasts (some terpenoids via the pyruvate – glyceraldehyde pathway; a few alkaloids, such as quinolizidine and steroid alkaloids), mitochondria (some amines, the alkaloid coniine), or vesicles (some isoquinoline alkaloids) (Kutchan, 2005; Zenk and Jünger, 2007; Wink, 2010b; Krauss and Nies, 2014).

SM are not only synthesized but they need to be stored to fulfill their ecological functions (see Section Function of Secondary Metabolites as Defense and Signal Compounds). This is a challenge for a plant because most of the SM constitute active agents which may also disturb the metabolism of the plant producing them. The large central vacuole of most plant cells is not a site for biosynthesis but rather for the sequestration of defense, signal, and storage compounds (Boller and Wiemken, 1986; Kutchan, 2005; Wink, 1997). The vacuole is a storage compartment for many of the hydrophilic SM, such as alkaloids, cyanogenic glucosides, glucosinolates, betalains, flavonoids, anthocyanins, saponins, cardiac glycosides, anthraquinone glycosides, NPAAs, and organic acids (**Table 1**). Lipophilic SM (especially terpenoids, phenylpropanoids, polyacetylenes) (**Table 1**) are not stored in the vacuole, but in dead cells or compartments, such as resin ducts, oil cells, glandular scales, trichomes, or on the cuticle. In Papaveraceae (poppy family), some Asteraceae (aster family), Euphorbiaceae (spurge family), and some Apocynaceae (dogbane family) we find latificers (latex ducts) filled with milky juice containing toxic alkaloids, sesquiterpenes, or diterpenes (Wink, 2008a,b, 2010b; Krauss and Nies, 2014).

Vacuoles often contain high concentrations of an SM. In order to reach the vacuole, the mostly polar and hydrophilic SM have to pass the tonoplast (a biomembrane around the vacuole) against a concentration gradient. A few SM can diffuse across the tonoplast and are later trapped in the vacuole as charged molecules (e.g., nicotine and some other alkaloids)

Table 2 Biosynthetic origin of secondary metabolites: overview of the main biosynthetic pathways

Precursor in primary metabolism	*Class*	*Group of SM*
Acetyl-CoA (cytoplasm); Alternatively in chloroplast: Pyruvate + 3-phosphoglyceraldehyde	Terpenes	(C10) Monoterpenes, iridoid glucosides (C15) Sesquiterpenes (C20) Diterpenes and phorbolesters (C30) Triterpenes and saponins (C27) Steroids and steroid saponins (C40) Tetraterpenes Polyterpenes
Acetyl-CoA/malonyl-CoA	Quinones (polyketides)	Anthraquinones Naphthoquinones (plumbagin)
	Fatty acids	Polyacetylenes Thiophenes
	Conium alkaloids	Coniine
Phenylalanine/tyrosine	Phenols	Phenylpropanoids, phenolcarboxylic acids, lignin Coumarins and furanocoumarins Lignans
	Alkaloids	Isoquinoline alkaloids (papaverine) Morphinane alkaloids (morphine) Protoberberine alkaloids (berberine)
	Glucosinolates	Benzylisothiocyanates
	NPAA	Mimosine
	Cyanogenic glucosides	Prunasin, amygdalin
Phenylalanine + polyketide units	Phenols	Flavonoids, isoflavones Anthocyanins Catechins and catechin tannins
Tryptophan	Alkaloids	Tryptamine Simple indole alkaloids Ergot alkaloids
	Glucosinolates	Glucobrassicin
Tryptophan + secologanin	Alkaloids	Monoterpene indole alkaloids
Ornithine	Alkaloids	Tropane alkaloids (atropine) Pyrrolizidine alkaloids Pyrrolidine alkaloids (nicotine)
Lysine	Alkaloids	Quinolizidine alkaloids Piperidine alkaloids
Glucose	Glucosides	
Quinic acid	Gallic acid	Gallotannins Ellagitannins

(so-called ion-trap mechanism). In most other cases, active transporters are required, which can be energy-dependent ABC transporters or proton-dependent antiporters (Wink, 1997; Kutchan, 2005; Yazaki, 2006; Rea, 2007). ABC transporters are better known from cancer cells, parasites, or drug-resistant bacteria where they pump out any lipophilic substance that has entered a cell via free diffusion (Wink *et al.*, 2012). Genomic studies have revealed that plants also have several ABC transporter genes and that some of them apparently govern the import of polar SM into the vacuole (Yazaki, 2006; Rea, 2007).

The site of synthesis of an SM is not always the site of its accumulation. Some SM, such as nicotine or tropane alkaloids, are made in the root but are stored in all other plant tissues. Lupines and other related legumes (Fabaceae) produce quinolizidine alkaloids in photosynthetically active tissues (mostly leaves) but store them in other tissues, especially seeds (Wink, 1992, 2013). While nicotine and tropane alkaloids are transported via the xylem (which transports water) to the other plant organs, quinolizidine, pyrrolizidine alkaloids, and some glucosinolates follow the route of other assimilates (amino acids, sugars) in the phloem (Wink, 2003, 2010b).

Plants always produce and store mixtures of SM, which are usually produced by differing pathways. For example, an alkaloid-producing plant often produces phenolics and terpenoids at the same time. The composition of such mixtures differs between organs and developmental stages: the SM profile usually differs among the root, leaves, flowers, and seeds. Furthermore, a seedling differs from a young, mature

Table 3 Examples of protoxins, which are activated after an attack by an herbivore or microbe

SM in intact tissues (occurrence)	*Enzyme*	*Active metabolite*
Cyanogenic glucosides (many Rosaceae)	β-Glucosidase	HCN
Glucosinolates (Brassicaceae)	Myrosinase	Isothiocyanates
Alliin (a NPAA from *Allium*)	Alliinase	Allicin
Arbutin (Ericaceae)	β-Glucosidase	Hydroquinone
Gein (*Geum urbanum*)	β-Glucosidase	Eugenol
Ranunculin (many Ranunculaceae)	β-Glucosidase	Protoanemonin
Bidesmosidisic saponins (widely distributed)	β-Glucosidase or Esterase	Monodesmosidic saponins
Coumaroyl glucoside (Apiaceae, Fabaceae)	β-Glucosidase	Coumarins
Iridoid glucosides (Lamiaceae, Plantaginaceae)	β-Glucosidase	Active dialdehydes

and flowering, or old senescent plant. In addition, SM patterns differ among individual plants and populations in nature. This apparent variability has hardly been addressed by phytochemists or plant physiologists but is probably important as a strategy against adaptation of herbivores and microbial pathogens (Wink, 2008b).

SM is dynamic and several SM undergo a regular turnover. Because some SM may be inactivated by a spontaneous racemization or polymerization process, the degradation of these SM and continuous new synthesis ensures that SM are always present in an active form. Furthermore, plants can actively react when challenged by an herbivore or microbe, by either activating a preexisting SM (**Table 3**) or by stimulating the synthesis of existing SM or new types of SM (called phytoalexins). Many SM are stored as inactive prodrugs which become activated in emergency by hydrolysis with glucosidases or esterases which come in contact with their substrate after tissue decompartmentation (**Table 3**). Well-known examples are cyanogenic glucosides, which release the toxic hydrogen cyanide, or glucosinolates that produce the pungent isothiocyanates (Wink, 2003, 2008a, 2010b; Krauss and Nies, 2014).

Production, transport, and storage of SM are energetically costly. Costs occur for the synthesis of precursors and subsequently the SM themselves (in the form of ATP and/or NADPH), but also for the synthesis of proteins, genes, and morphological structures that are involved with SM formation (Wink, 2010b).

Function of Secondary Metabolites as Defense and Signal Compounds

Secondary metabolites as defense compounds

Plants survive in an environment full of enemies. Plants cannot run away from herbivores (roughly 60% of animals are herbivores) nor do they have a powerful immune system with antibodies against infection from bacteria, fungi, and viruses, as used by animals with high success. Similar to the situation of other sessile or slowly moving organisms (such as nudibranchs, jelly fish, corals, sponges) or toxic organisms such as many amphibians, plants began early in their evolution to produce and store a diversity of toxic, deterring or repellent SM (**Table 1**; Levin, 1976; Harborne, 1993; Hartmann, 2007; Wink, 1988, 1993, 2003, 2010a). Using deterrent, pungent, bitter, or toxic SM, plants can defend themselves against most herbivores, such as arthropods, worms, and vertebrates. Plants use some of their SM not only against herbivores and microbes but also against other plants, competing for light, water, and nutrients (termed allelopathy) (**Figure 1**).

In some instances, plants have developed structural specialties, which help to deter herbivores. They include stinging hairs of nettles (filled with formic acid, acetylcholine, and histamine) which hurt after contact and cause an inflammation. Thorns, spines, thick cell walls, leaf hairs, and inert bark also serve as barriers against herbivores and microbes.

If a plant becomes wounded or infected, pathways are activated in which jasmonic acid (made from α-linolenic acid) and salicylic acid are important signaling molecules. Both substances regulate the expression of genes of SM metabolism or proteinase inhibitors. In addition, the volatile ethylene also stimulates the expression of defense genes. Plant cells carry receptors on their surfaces that can detect elicitors, which derive from cell walls of bacteria and fungi. The elicitors are released when plants undergo an infection by microbes, and prompt the plant to initiate its defense strategies (Harborne, 1993; Kadereit *et al.*, 2014; Krauss and Nies, 2014).

The structures of SM have been shaped during evolution, enabling plants to cope with a multitude of herbivores and microbes. In analogy to the 'molecular modeling' of pharmaceutical chemists we could term the process for the selection of SM as 'evolutionary molecular modeling' (Wink, 1988, 2008a,b). SM have been optimized to interact with a multitude of molecular targets in animals and microbes (**Tables 1** and **4**). Many SM attack more than a single target; we can consider them as multi-target agents. As a consequence, many of the anti-herbivore SM (such as many phenolics, terpenoids, and some alkaloids) can also attack microbes (Wink, 2007b, 2008b). On the other hand, some SM appear to have been selected solely as antimicrobials (some saponins, antimicrobial peptides) and contribute to the innate immune system of plants. After an infection, plants not only use their antimicrobial SM, they also produce enzymes which degrade bacterial or fungal cell walls, accumulate callose or stabilize their walls through incorporation of lignin (Kadereit *et al.*, 2014).

Evolution apparently tried to attack several enemies with one stone when developing SM, because in addition to defense some SM serve for UV protection (some phenolics), antioxidants (many phenolics, some terpenoids) and as mobile but toxic nitrogen storage compounds (some NPAAs, alkaloids, peptides, and lectins) (Wink, 2008a, 2010a; **Figure 1**).

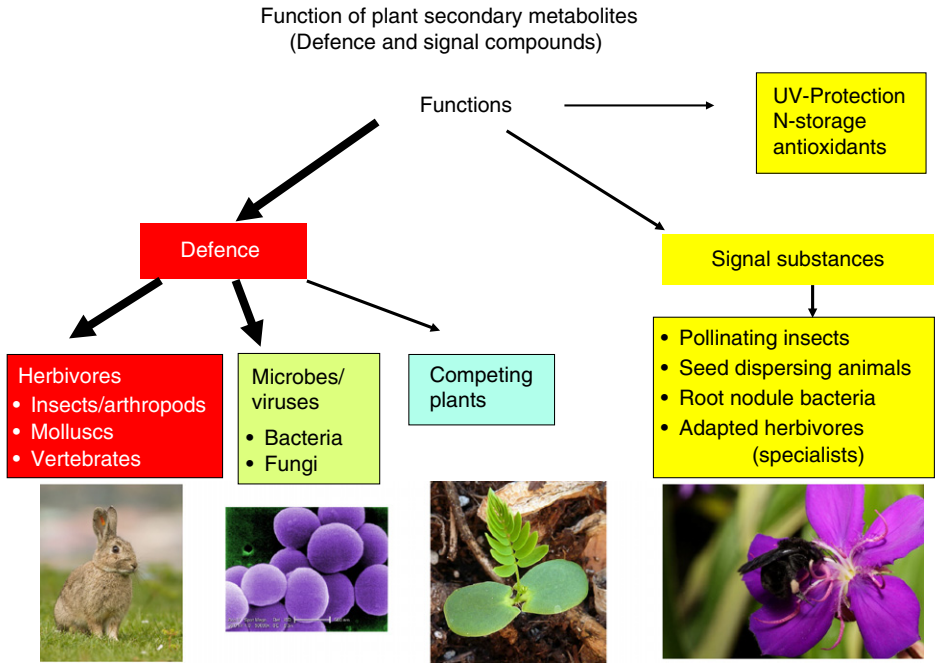

Figure 1 Functions of plant secondary metabolisms (pictures from WikiCommons).

Table 4 Targets of SM and their modes of action

Main target	*SM class*	*Mode of action*
Proteins	Quinone	Reaction with amino-, hydroxyl-, and SH-groups
	Phenolic	Reaction phenolic OH groups with amino groups of proteins; formation of hydrogen and ionic bonds
	Sesquiterpenes, polyacetylenes	Reaction with SH-groups of proteins and glutathione
	Epoxides	Reaction with amino-, hydroxyl-, and SH-groups
	Aldehydes	Reaction with amino- and imino groups
	Alkaloids	Neurotoxins:
		– Binding to neuroreceptors
		– inhibition of acetylcholine-esterase, MAO
		– Modulation ion channels
		– Modulation of ion pumps
		– Modulation of signaling pathways
	Vinblastine, colchicine, podophyllotoxin	Inhibition of microtubule assembly
	Paclitaxel	Inhibition of microtubule disassembly
	Cardiac glycosides	Inhibition of Na^+/K^+ ATPase
	Cyanogenic glucosides/HCN	Inhibition of cytochrome oxidase in respiratory chain
	NPAA	Disturbance of protein synthesis; 3D structure of proteins
	Isoflavones	Modulation of hormone receptors and tyrosine kinases
Biomembrane	Monodesmosidic saponins	Complexation of cholesterol; disturbance of membrane fluidity and permeability
	Terpenes, isothiocyanates	disturbance of membrane fluidity and permeability, modulation of ion channels
	AMPs	Pore formation in biomembranes
DNA/RNA	Alkaloids	DNA-intercalation: Sanguinarine, berberine, Harman alkaloids; DNA-alkylation: Pyrrolizidine alkaloids, aristolochic acids, cycasine
		Inhibition of topo-isomerase: camptothecin
	Furanocoumarins	DNA-intercalation, DNA-alkylation
	Epoxides, aldehydes	DNA-alkylation

Secondary metabolites as signaling compounds

Although defense is the most important function of SM for plants, it should not be overlooked that many plants sometimes use the same SM as signaling compounds to attract animals for pollination (especially insects, sometimes small rodents, fruit bats, humming birds, sunbirds) or seed dispersal (many frugivorous birds, fruit bats, primates) (Harborne, 1993; Wink, 2010b; Kadereit *et al.*, 2014; **Figure 1**). The attraction is mediated by the color of some SM in flowers (anthocyanins, chalcones, aurones, betalains, and carotenoids) and/or volatile and aromatic terpenoids, alcohols, aldehydes, ketones, amines, and phenylpropanoids. However, pollinators should only be attracted to flowers, and not eat them. Therefore, SM serve as signal compounds from a distance and as a deterrent when an animal has settled on a flower, where it is rewarded by nectar, oils, or energy-rich pollen.

Fruits store a number of SM and are therefore often unpalatable or even toxic when they are not mature. Upon maturation, they incorporate attractive colors (anthocyanins, carotenoids, aurones), perfumes (many volatile mono- and sesquiterpenes), and sweet sugars (glucose) (Harborne, 1993; Wink, 2010b; Kadereit *et al.*, 2014). Plants follow the strategy to attract frugivores, which eat the pulp, but leave the seeds unharmed. After passage through the gastrointestinal system, the seeds are later deposited in the feces at sites generally distant from the mother plant. Using this strategy frugivores employ some SM for seed dispersal. In the complex interaction of symbiotic nitrogen-fixing rhizobial bacteria and their host plants, SM (especially flavonoids) serve as signal substances (Harborne, 1993; Krauss and Nies, 2014).

SM can only serve as defense and signal compounds if they are present at the right time and in the right location. Many SM are stored in epidermal cells, hair cells, or the bark. This makes sense, considering the role as defense compounds, as these cells and tissues have to ward off intruders in the first place (Roberts and Wink, 1998; Wink 1988, 2003). As discussed above, SM are biologically active agents which may also interfere with the metabolism of the plants producing them. The production of SM as inactive prodrugs (**Table 3**) or of SM which affect targets that are only present in animals (e.g., alkaloids which modulate neuroreceptors) (Roberts and Wink, 1998; Wink *et al.*, 1998; Wink, 2000, 2007b) has probably been stimulated by this selection pressure. In addition, the sequestration of SM in cellular compartments, by which SM do not interfere with the cell's metabolism, appears to be another relevant strategy. The question of how plants protect themselves against their own toxins is not fully understood although interesting adaptations can be expected.

Exceptions to the rule

If you walk with open eyes in the field you will certainly find plants which are affected by herbivores or microbes. Very often these are crop plants that have been domesticated by humans during the last 10 000 years. With regard to SM, crop plants (except spices) usually do not contain toxic, bitter tasting or pungent SM; these traits have often been selected away (Wink, 1988). As a consequence, most crop plants produce only few SM and are more susceptible to herbivores and microbial pathogens than their wild ancestors. Modern agriculture employs synthetic pesticides instead, which replace the originally present defense compounds. Because agriculture often uses single substances (instead of mixtures as the plants do) and grows plants in large monocultures, resistant herbivores and pathogens quickly develop, resulting in the need for new pesticides. An alternative approach would be to cultivate crops with high concentrations of defensive SM and to remove the compounds later during food processing.

It should be remembered that for defense, plants use complex mixtures of SM, the composition of which varies in a time-, site-, and development-specific fashion. This phenomenon can be regarded as a strategy to reduce the risk that herbivores and microbes develop resistance or tolerance against the toxic SM. As a mixture contains dozens of bioactive SM which affect different targets, it is more difficult for a herbivore or microbe to develop resistance than if a single compound were present (Wink, 2008a,b). However, even highly protected plants have their adapted enemies. A close analysis of these herbivores or pathogens will reveal that they often represent specialists which attack a single or few often phylogenetically related host plants (Harborne, 1993; Bernays and Chapman, 1994; **Figure 1**). What allows a specialist to attack these highly protected plants? With the tools of genetics and chemical ecology, a number of these adaptations have been explored.

Larvae of the monarch butterfly (*Danaus plexippus*) feed on *Asclepias* (milkweed) plants, which produce cardiac glycosides (CG) which are highly toxic for most herbivores (Harborne, 1993). We demonstrated that the target of cardiac glycosides, the Na^+/K^+ ATPase in *D. plexippus*, has been inactivated by a point mutation, so that CG are no longer toxic to them (Holzinger and Wink, 1996). As the larvae sequester CG and pass them on to the adult butterflies, both of them become toxic to avian and other predators. Monarch butterflies are a good example of adapted insect herbivores, which can not only tolerate a plant toxin but use it for their own defense. Similar cases are known for insects which sequester pyrrolizidine, quinolizidine alkaloids, and aconitine, but also for iridoid glucosides and glucosinolates (Harborne, 1993; Wink, 1993, 2010a,b; Brown and Trigo, 1995; Krauss and Nies, 2014; Wink, 2010a). These insects often bear aposematic warning colors.

Furthermore, herbivores and microbes have developed a series of general adaptations and detoxification strategies against plant SM. These include ABC transporters, which can export a wide variety of lipophilic SM that enter cells via free diffusion. If a food is rich in such toxins, the expression of ABC transporter genes is often upregulated (Wink *et al.*, 2012). Animals have mechanisms by which lipophilic SM become conjugated with a hydrophilic component (often glucuronic acid, sulphate, or amino acids) which facilitates their elimination via the urine (Krauss and Nies, 2014). Many herbivores cultivate microbes in their digestive system which can inactivate SM. These adaptations enable herbivore generalists to feed on SM containing plants.

When Did the Secondary Metabolism Evolve?

The first land plants developed in the Silurian/Devonian about 420 million years ago from green algae. These algae had survived in a world full of animals (mostly arthropods) and

microbes (bacteria, fungi) that were ready to attack these aquatic plants (Kadereit *et al.*, 2014; Wink, 2003, 2008b). It is therefore highly likely that the early land plants already used SM for chemical defense, especially against microbes. The SM profiles of extant mosses, horsetails, lycopods (club mosses) and ferns – all plants that reproduce by spores and that had their prime time in the Carboniferous about 320 million years ago – show that they produce a diversity of phenolics and terpenoids that occur with similar chemical structures in seed plants. Only a few spore-bearing plants produce alkaloids (except for some lycopods and horsetails), cyanogenic glucosides or glucosinolates. Whereas phenolics and terpenoids are still dominant in gymnosperms, N-containing SM are more common in modern angiosperms, which started to evolve about 140 million years ago (Roberts and Wink, 1998; Wink, 2010b). Plants with wind pollination (many grasses, trees) often produce terpenoids and phenolics for defense, but plants which actively attract pollinators and frugivores (plants with attractively colored flowers or fruits) often use toxic alkaloids, cyanogens, and isothiocyanates, which affect the nervous system of animals.

How Did Plants Acquire the Genes for Secondary Metabolism?

When we analyze the distribution of SM across the plant kingdom, we see that a number of classes, such as phenolics and terpenoids occur throughout. Other SM occur in restricted genera or families, for example, glucosinolates are common in the order Brassicales (mustards and relatives) but not anywhere else. However, several alkaloids (quinolizidine and pyrrolizidine alkaloids) or terpenoids (iridoids, cardiac glycosides) dominate a few genera or families but also occur in exactly the same chemical structure in nonrelated families (Wink *et al.*, 2010; Wink, 2013, 2014). For example, quinolizidine alkaloids are dominant in a clade of Fabaceae (legumes) but are also found in Ranunculaceae (buttercup family) and Amaranthaceae (amaranth family), and cardiac glycosides are found in several unrelated families including Asparagaceae (asparagus family), Apocynaceae (dogbane family), Brassicaceae (mustard family), Ranunculaceae, and more. This is an argument why SM pattern should not be used naively for reconstructing phylogenies (as is sometimes done in chemosystematics).

Monoterpene indole alkaloids (MIA) occur in many members of the Apocynaceae. Therefore, one would expect that the key enzymes of MIA biosynthesis, such tryptophan-decarboxylase (TDC) and strictosidine synthase (STS) and the corresponding genes would only be present in plants which produce MIA (Wink, 2010a,b). However, genome analyses have revealed that TDC and STS genes are present in most plants (Facchini *et al.*, 2004; Wink *et al.*, 2010); because we find MIA only in the Apocynaceae we assume the corresponding genes are downregulated or inactivated in the other plant families (Wink *et al.*, 2010). When genomes of bacteria, fungi, and animals are included in the search for MIA genes, we find homologues in microbes and even animals. This finding not only applies for TDC and STS but also for a number of key genes of other pathways (overview Wink *et al.*, 2010b; Wink, 2014). One conclusion would be that many genes of plant SM have a ubiquitous distribution but are only locally expressed (Wink, 2003, 2008a,b). This assumption does not rule out that specific plant groups have developed a set of their own SM genes.

How to explain the patchy distribution of SM?

It is well established that bacteria and fungi produce a wide variety of SMs showing identical or similar chemical structures to plant SM. Also some key genes of bacteria, fungi, and plants are closely related, such as polyketide synthetases and terpene cyclases (Krauss and Nies, 2014).

According to the endosymbiont hypothesis, about 1.8 billion years ago early eukaryotic cells acquired protobacteria which later became the mitochondria. It is likely that these protobacteria carried several genes for SM pathways in their genome and that these genes were incorporated into the nuclear genome of the host cell. This was an early event of horizontal gene transfer (HGT) which occurred several times during evolution (**Figure 2**). When photosynthetically active protists and algae evolved, another symbiosis took place by incorporating cyanobacteria, which later became the chloroplast of modern plants. Cyanobacteria are known for extensive synthesis of SM; thus we can assume that they introduced a further set of SM genes into the plant genome. We postulate that early land plants used these microbial genes for the synthesis of phenolics and terpenoids. These genes were later modified to produce the diversity of SM we know today. This scenario would help to explain the occurrence of SM and SM genes described above (Wink, 2008a,b, 2010a,b, 2013, 2014).

About 90% of plants live together with symbiotic fungi, such as ecto- and endomycorrhiza, which help plants to obtain nutrients and water (Kadereit *et al.*, 2014). Fungi also produce a high diversity of toxic and repellent SMs. Do fungi support their host plants with SM? For a long time phytochemists have wondered about the occurrence of ergot alkaloids: Ergot alkaloids are known SM of the fungus *Claviceps purpurea*, which occurs on rye and other grasses. Ergot alkaloids function as neurotoxins, affecting the neuroreceptors of serotonin, noradrenaline, and dopamine (Wink, 2000). In grasses that are infected with *Claviceps*, the alkaloids serve as defense compounds against herbivores (Wink, 2008b). The interaction of *Claviceps* with their host plants is no longer regarded as parasitic but symbiotic. Ergot alkaloids of similar chemical structures have also been detected in some North American species of Convolvulaceae (morning glory family). Formerly, it was assumed that these plants produced these alkaloids themselves. A close analysis of the Convolvulaceae revealed that some of them carry endophytes related to *Claviceps* which produce the ergot alkaloids. The insular distribution of ergot alkaloids in the plant kingdom is thus not a function of a wide distribution of SM genes (as discussed above) but can be explained by symbiotic endophytes.

The example of ergot alkaloids is apparently not an exception, but might represent a widespread phenomenon (**Table 5**). Several instances have been documented in which certain SMs of a plant, which had been considered to be a typical plant SM, are produced by endophytes. Very likely, many more examples of this kind will be discovered in the future. In some instances, apparently endophytes but also the

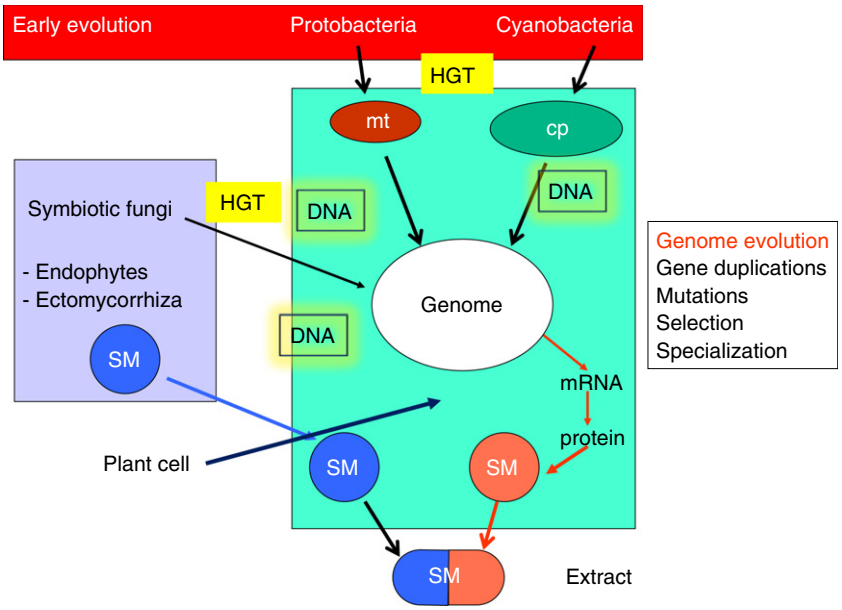

Figure 2 Putative origins of genes encoding biosynthesis enzymes of secondary metabolites and the SM found in a plant.

Table 5 Examples for SM from endophytes

Host plant	Fungal SM, accumulating in host plant
Astragalus spec.; *Oxytropis* spec.	Swainsonine and other indolizidine alkaloids
Hypericum perforatum	Hypercin
Ipomoea tricolor	Ergot alkaloids
Lolium pratense	Loline (PA)
Maytansia	Maytansinoids
Nothapodytes foetida	Camptothecin
Podophyllum peltatum	Podophyllotoxin
Taxus brevifolia	Paclitaxel

plant without endophytes produce identical SM. We may speculate that fungal genes might have been transferred into the genome of their host plants by HGT (**Figure 2**; Wink, 2008a,b, 2010b, 2014).

Considering the diversity of plant SM, we may speculate that a large proportion of genes which govern plant SM have been transferred by HGT from protobacteria, cyanobacteria, and endophytes into the plant genome during evolution (**Figure 2**). Future genome analyses will reveal the dimension of such gene transfers.

Evolutionary Pharmacology and Phytotherapy

As discussed above, plants have evolved a wide set of bioactive SM (Wink and Schimmer, 2010), whose bioactivities were discovered by humans a long time ago. Toxic alkaloids and cardiac glycosides have been used as arrow poisons for hunting and war. Other plants were used to poison wild animals, rivals, or enemies (Mann, 1992; Wink, 1988; Wink and van Wyk, 2008). Aromatic SM (mono- and sesquiterpenes, phenylpropanoids) were employed as perfumes, colored SM for dyeing of clothes or skin. Neurotoxins (mostly alkaloids and amines) were (and still are) widely selected as stimulants, intoxicants, and hallucinogens (Wink, 2000; Wink and van Wyk, 2008). For thousands of years humans have used plants and plant extracts to treat infections, inflammations, pains, and health disorders. About 2000 years ago Dioskorides published a Materia medica in which 400 medicinal plants and their applications were described; several of these plants are still in use in present day phytomedicine (overview in van Wyk and Wink, 2004; van Wyk *et al.*, 2015).

Plants that produce powerful poisons affecting a single or few targets are often still in use today. Mostly, isolated SM, such as morphine, colchicine, vinblastine, paclitaxel, galanthamine, huperzine, or emetine, are applied in medicine as registered drugs (Schmeller and Wink, 1998; van Wyk and Wink, 2004; **Table 3**). In addition, traditional medicines and phytotherapy employ extracts from medicinal plants that contain complex mixtures of phenolics, terpenoids, saponins, and polysaccharides. These extracts affect a multitude of targets and are therefore often prescribed to treat more than one health problem (**Table 3**). Such multi-target drugs also affect molecular targets in diseases, for which the relevant targets have not yet been identified. An advantage of the extracts is the presence of components which potentiate each other in a synergistic matter (Wink, 2008, 2015).

In the time of genome analyses modern medicine more and more discovers the molecular causes of diseases and health disorders. In consequence, the number of disease-relevant molecular targets, towards which the pharmaceutical industries will develop modulators or inhibitors, will increase substantially. From a perspective of evolutionary pharmacology it is obvious that SMs of plants, but also of microbes and sessile marine organisms (Proksch and Ebel, 1998), represent a treasury of potential active agents which only waits to be discovered by bioprospection (Wink, 2007a,b). This treasury of potential drugs is an important aspect of ecosystem services of biodiversity and it is evident, that it is important to preserve the diversity of plants and animals so that future

generations have the chance to find new drugs for medical and other applications.

See also: Antagonistic Interspecific Coevolution. Convergent Evolution, Adaptive Radiation, and Species Diversification in Plants. Parallel and Convergent Molecular Evolution. Predation and Parasitism

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Security, Evolution and

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Key Definitions

Escalation An evolutionary process where enemies are the predominant selective agents.
Evolutionary security (synonyms: natural security, Darwinian security) Field of study that applies the principles of evolutionary biology to problems in human security.
Operational adaptation Research on how to achieve effective adaptation in war.

Introduction

Security affairs in human society are often discussed in the context of geopolitics, religious and ideological conflict, and human history. Conflicts and the resulting security challenges they create also may have environmental drivers, though conclusive evidence is mixed (Homer-Dixon, 1994; Stewart, 2002; Barnett and Adger, 2007; United Nations Environment Programme, 2009; Forsyth and Schomerus, 2013). Although there have been scattered efforts in the past, since the 9/11 attacks, there has been more concerted and interrelated attempts to examine human security within an evolutionary context. These efforts come from a vast range of fields and viewpoints and accordingly this review cannot be exhaustive but attempts to highlight key areas and to provide a general framework to organize the body of evolutionary security works and illustrate how they might be applied to address human security concerns.

Evolution provides a potentially valuable framework for looking at security for several reasons. From a practitioner standpoint, there is a feeling that the old methods and lenses with which to look at security had either failed (e.g., the 9/11 attacks) or were inadequate to address the complexities and nuances of today's security environment (Sagarin and Taylor, 2008). Evolution provides a lens to look at security broadly and across a number of different gradients. For example, evolution provides insights into the relative role of technology (phenotypic trait variation) and tactics (behaviors) in adapting to security threats. Evolution helps us consider the origin and maintenance of offensive weaponry vs. defensive structures or behaviors. Evolution helps us think about the relative role of cooperation vs. conflict. And, a functional and historical approach can help us think about ways to better prevent and respond to security breaches.

Individual scholars from within and outside biology have raised parallels between security in natural and human systems for many years. Most notably Warder Clyde Allee applied his studies of cooperation among organisms to questions about international conflict and cooperation, including the nascent idea of a United Nations, in several works (Allee, 1943, 1951). Ecologist Ed Ricketts (a student of Allee) frequently referred to evolution and ecology in his essays (Rodger, 2006), and he attempted to apply his studies to the intelligence efforts of the US in World War II, with disappointing results (Steinbeck, 1986). Economist Kenneth Boulding has described his 1962 book *Conflict and Defense* as the 'beginning of an evolutionary theory of conflict, seeing conflict as merely one element in the vast process of evolutionary and ecological interaction' (Boulding, 1962 [1988], p. ix). Paleobiologist Geerat Vermeij created a grand synthesis of paleobiological research suggesting that increasing capacities of organisms to obtain resources leads to increasing dangers in the environment, thus driving an escalation of offensive and defensive capacities for all organisms that interact strongly with their environment (Vermeij, 1987). Later, Vermeij applied this synthesis to human military escalation, making a pointed criticism of missile defense programs in noting that the inevitable evolutionary escalation of weaponry obviates the "false claim that weapons provide absolute security in the event they are used" (Vermeij, 2004, p. 300).

These and other individual efforts have been augmented in recent years through interdisciplinary collaborations and working groups dedicated to evolutionary security studies. Sagarin, for example, convened in 2005 a 'Darwinian Security' working group comprised of biologists and security experts at the National Center for Ecological Analysis and Synthesis. Dominic Johnson convened in 2010 an international workshop on 'Operational Adaptation' featuring biologists, anthropologists, civilian and military security experts and informed by evolutionary models, which was sponsored by the US Office of Naval Research Global. These efforts have resulted in books, collaborative papers and briefings to security agencies, although their ultimate effect on security policy is unknown (see below).

Approaches

There are several basic approaches that have been used in applying evolutionary thinking to security issues. Briefly, these include using analogies from nature to illuminate what appear to be similar situations in human security struggles, or the inverse approach in which direct observation of human security situations are contextualized in evolutionary terms.

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An alternative approach is developing models, including but not limited to those previously used in ecological and evolutionary studies, to simulate or elucidate underlying causes of behaviors and patterns in human security. All of these approaches have been used in either an analytic/explanatory sense to shed light on past or ongoing security situations, or in a pro-active/prescriptive sense to make predictions or warnings about potential future security scenarios (examples in Sagarin and Taylor, 2008).

Direct Analogies from Nature

Analogies from nature provide rich ground for examining security in society. While simple 'biomimicry' has turned up numerous 'products' of evolution (e.g., the fusiform shape of a tuna, camouflage mechanisms in cuttlefish) that may be effectively used in security situations (Armstrong and Warner, 2003), there is far richer ground to explore in the 'process' of evolution and the activities of biological organisms, when put into the context of security studies.

Perhaps the most obvious area to look, because it is so apparent in the phenotype of many organisms, is in studies of development and escalation of armaments and defensive structures of conflict. A recent review of this topic, particularly focused on modern fauna and making a number of direct analogies to human weaponry, is Emlen's *Animal Weapons* (Emlen, 2014). Whereas behavioral changes may be an initial, and reversible defense, animals also can add morphological defenses. Thus, the ecological interest in constitutive vs. inducible defenses in plants and animals (Harvell, 1990; Poitrineau *et al.*, 2004) is another potential analogy to human security, regarding to what extent we invest in a baseline level of security vs. maintaining the capacity to quickly ramp up defenses when necessary.

Looking at predator–prey relationships has been used to provide insight into warring sides in conflict. The relative costs of predation can also be examined in societal terms. Applying the 'life-dinner' principle (in which one side is running for its life where the other is merely trying to obtain a single meal) (Dawkins and Krebs, 1979) to conflict can illuminate the relative motivations of terrorists vs. states (Guerra-Pujol, 2012).

These differential benefits and costs relate to a larger and growing field of security studies – that of asymmetric conflict – that have relevant analogies in evolved systems. Parasite–host interactions, for example, resemble many features of asymmetric conflict among human societies where one side at relatively low energetic cost can have enormous effects and even modify the behavior of a typically larger and better-resourced host. The analogy of viruses, in particular, has been used widely to describe the actions of terrorists, the relatively lower cost of terrorist action vs. counter-terrorism by a 'host' country, cybersecurity threats, and surprisingly, the roots of cooperation (Villarreal and DeFilippis, 2000; Stares and Yacoubian, 2005; Lafferty *et al.*, 2008; Villarreal, 2008). Likening crime to an infectious disease and examining spatial and temporal patterns of its spread has also been an effective analogy (Zeoli *et al.*, 2012; NPR, 2015).

Intraspecific competition also has lessons for human security. 'Arms races' among species (particularly due to sexual selection on armaments such as antlers or oversized claws) are apt descriptors of some types of human conflicts and thus can be analyzed for their underlying causes and long-term trends. Fiddler crab males, for example, have been likened to both the posturing of both sides of the cold war, as well as the stability conferred by the concept of 'mutually assured destruction' (Sagarin, 2012; **Figure 1**). Such analogizing is more than descriptive because it allows a mechanistic view of the conditions in which symmetrical conflict can lead to stability or escalation. For example, Emlen (2014) argues that certain conditions, including competition for limited resources, resources that are localized and defensible, and opportunities for face-to-face conflict, make escalation of armaments more likely.

Figure 1 The Cold War arms race between the Soviet Union and the US was similar to male fiddler crabs competing with each other for mates. Crabs wave their oversized claws at each other but actually don't use them in battle, and analogously, superpowers assume that the threat of using strong nuclear weapons against the enemy prevents the enemy's use of those same weapons. https://upload.wikimedia.org/wikipedia/commons/5/5f/Fiddler_crab_4.jpg.

More subtle linkages between evolution and human security have been elucidated within the behaviors and life histories of organisms. Threat detection and alarm calling are particularly apt areas of study. On this question, a concern for the natural and human social realm is how can threats be detected accurately, and alarms about these threats be conveyed clearly and with enough urgency to get a population to react appropriately. The issue of habituation to alarm calls (or ignoring false alarms) is particularly worrisome in the human security realm (Blumstein, 2008), where it has been estimated that 21 million Americans don't keep batteries in their smoke detectors and even residents of areas hard hit by the 2005 Boxing Day tsunami soon dismantled newly installed tsunami warning alarms because of the high rate of false alarms (Sagarin, 2012). Accordingly, analogies from the natural world tend to focus on 'honest signaling,' which is expected to be costly when used (Blumstein *et al.*, 2012). Yet we should expect many situations where honest signaling is rare and individuals are trying to provide mis-information to modify the behavior of another individual. Understanding the value of information is key and to properly interpret information, knowledge about the signaler's reliability is often essential. For instance, Blumstein revealed that some individuals in-groups of marmots make alarm calls only when there is a clear and present danger, whereas others make abundant

alarm calls, even to non-consequential threats (Blumstein and Armitage, 1997; Blumstein, 2008). Marmots use their estimates of caller reliability when making decisions about how to respond to these vocalizations.

Observing human conflict, cooperation, and other security-related behaviors and then reaching back for evolutionary causation represents the inverse approach of using evolutionary analogies. The field of evolutionary psychology attempts to identify ultimate drivers of human behavior (Barkow *et al.*, 1992) and may be useful in helping to explain seemingly irrational or maladaptive behaviors (such as suicide bombing or the inability to properly weigh the relative risk of terrorism relative to other more common threats) which may have been adaptive (or neutral) in humans' ancestral environments (Liddle *et al.*, 2011). Nonetheless, because our own deep evolutionary history is intertwined with a more modern social evolution that itself may feedback on our evolution, seminal works in the areas of evolutionary psychology, 'sociobiology' (Wilson, 1975), and 'biopolitics' (Somit and Peterson, 1997) have generated considerable controversy that continues to be debated (e.g., Wilson, 1998; Corning, 2001; Somit and Peterson, 2001; Goetze and James, 2004).

Much aggressive and cooperative behavior among humans has been linked to human cultural biases, including deep seeded ethnocentrism (Hammond and Axelrod, 2006) and favoring in-group members against out-group members, which are reinforced through cultural norms and religion, as portrayed in a rich evolutionary literature (Henrich and McElreath, 2003; Ehrlich and Levin, 2005; Sosis and Alcorta, 2008). Villarreal examined the deep evolutionary roots of in-group/out-group biases, suggesting they are a human manifestation of self/non-self recognition systems that date back to the earliest bacterial and viral interactions (Villarreal and DeFilippis, 2000; Villarreal, 2008). Hatemi and colleagues (2013) links this out-group bias directly to fear of out-group members and discuss the conflict it creates in a political context. Because many of these biases are expressed through religious identification, global assessments of common features of religion can helpfully point out likely development-related points of entry and exit from radical religious beliefs (e.g., adolescence) (Sosis and Alcorta, 2008).

Peeling back a layer from strong out-group biases in humans reveals that cooperation among in-group members is an essential and somewhat enigmatic process (Henrich and Henrich, 2006) when contrasted to self-interest, and this is particularly debated in the context of altruism. Altruism in humans has been explained through basic evolutionary mechanisms such as kin selection (Davis, 1997; West *et al.*, 2011) as well as more nuanced explanations such as the role of empathy (which likely goes back deeper than the human lineage; de Waal, 2008), costly displays that reinforce group identity (Henrich, 2009), potential for group punishment of defectors (Fehr and Gachter, 2002) and even 'supernatural' punishment (acknowledged in a religious sense) of defectors (Johnson and Kruger, 2004).

Dominance hierarchies are a particular set of behaviors that have been viewed for many years of having direct linkages between human and nonhuman analogs. Allee (1938), for example, carefully considered the 'pecking order' in hen houses as a direct lesson for human cooperation and conflict. Silk (2002) used studies of primates to suggest that random acts of aggression may effectively discourage subordinates from defecting in a hierarchical society. More recently, the work of Robert Sapolsky and colleagues has linked dominance hierarchies in primates to factors that maintain cooperation or foment conflict, with commensurate lessons for humans (Sapolsky, 2006). Individuals (or nations) may have special roles in stabilizing societies. For instance, Flack *et al.* (2006) showed that the removal of especially powerful dominant male pigtail macaques (*Macaca nemestrina*) led to increased conflict and group fragmentation. Especially powerful entities can have a stabilizing effect on groups. Related to dominance hierarchies, with important implications for warfare and peace building, are studies on the evolution of leadership (King *et al.*, 2009).

Emergent Properties

The most intriguing and complex view of evolution in security comes through examining the widespread or in some cases emergent properties of evolutionary systems. Chief among these properties is that of adaptability – the capacity of a biological system to cope with (adapt to) the unexpected disturbances of the environment, internal or external. Especially after 9/11, many security agencies and advisors talked of the need to be more adaptable, and the process and outcomes of adaptable biological systems likewise became a unifying theme of evolutionary security work (Sagarin and Taylor, 2008).

While adaptability is an attractive conceptual framework for security, it needs to be broken down into processes that can be emulated if it is to be useful in an applied security context. One such process is symbiosis, which is both universal and emergent in evolutionary systems (Margulis, 1998). Symbiosis can be seen as an appropriate model for the kinds of partnerships necessary in security practice because in nature it is not a simple *quid pro quo*, and it includes relationships between unlikely partners including partners that once had an antagonistic relationship (Allee, 1951; Margulis, 1998). Corning (2005) suggested that synergistic (cooperative) effects have played an important role in the emergence of more complex natural and social systems and conversely that a global security system might emerge through the selective advantages of cooperative interactions. Analogous symbioses in human affairs have resulted in health provisioning partnerships between Israeli, Palestinian, and Jordanian health practitioners (Gresham *et al.*, 2009), and likely led to a rapid reduction in deaths due to improvised explosive devices in Iraq for US forces (Sagarin, 2012).

Redundancy is another general feature of adaptable systems that appears universal in biology (Vermeij, 2004). At the upper end of biological complexity, redundancy (of functional types) seems to provide resiliency to ecosystems (Levin, 1999). In human terms, redundancy can provide a 'hedge' against the uncertainty of unpredictable security situations. Indeed, individuals often seek to reduce uncertainty about the true risk of predation and using different modalities of stimuli to assess risk is likely favored in noisy, dynamic, and uncertain situations (*sensu* Munoz and Blumstein, 2012).

Finally, adaptable systems often rely on decentralized abilities to sense change in the world (Vermeij, 2008). The adaptive vertebrate immune system is an exemplar, wherein

immune cells search for, identify, and neutralize invading pathogens throughout a body with very little contact with the central nervous system. In the realm of human security, the last decade has seen an explosion of decentralized sensory systems – particularly those facilitated by cell phone networks – aimed at security-related concerns such as mapping disease outbreaks or areas needing immediate assistance following a natural disaster.

Evolutionary and Ecological Models

The descriptive approaches above have been augmented by modeling approaches that capitalize on the enormous body of existing evolutionary and ecological models made for situations analogous to those that are found in human security concerns. Examples of models used in studies of conflict are especially rich in game theory with Axelrod's *The Evolution of Cooperation* being a key early contribution (Axelrod, 1984, 2006). Many games focus particularly on the conditions under which cooperation between people or groups arise. Interpretations of games have relied on both economic and evolutionary models, which may lead to conflicting conclusions about root causes for the observed behaviors (Hagen and Hammerstein, 2006), suggesting that there is still need to reconcile the relative roles of cultural and biological evolution in determining peoples' willingness to cooperate with or punish other people.

Ecological models stemming from various levels of biological complexity have been used to draw light on terrorism and conflict. Turchin and collaborators (2003) have used various spatial ecological models of animal movement and population cycles to draw conclusions about seemingly synchronous events in economics and international relations. Drapeau *et al.* (2008) used heuristics of predator–prey and competition models to outline potential counter-terrorism strategies. Bohorquez and colleagues (2009) developed models that treat insurgent populations as part of an ecology of dynamically evolving, decision-making groups to identify commonalities in the timing and size of violent events within conflicts. Keohane and Zeckhauser (2003) used an ecosystem model of stocks and flows to examine how individuals might respond to terrorism.

More recently, food web and network models have been manipulated for use in security studies. For example, using the medium of TED talks, Eric Berlow applied food web theory to an overly-complex map of the US war strategy in Afghanistan and showed that it could be simplified to just a small number of key actions. Ferenc Jordan used network theory to analyze the July 7, 2005 London subway terrorist attacks and determined that terrorists chose the second-most destructive combination of stops to attack (out of 3.2 million possible combinations) (Jordan, 2008).

Finally, natural selection models can be used to study conflict. Johnson (2009), for example, studied coalition vs. insurgency success across several years of combat in Iraq using the three pillars of natural selection theory (variation, selection, replication) as metrics. He found that insurgent organizations had several characteristics favoring faster rates of adaptation under all three of these components of selection. However, the battlefield creates strong selective forces and the creation of 'hillbilly armor' illustrates that even coalitionary forces can adapt (**Figure 2**).

Figure 2 Operational adaptation on the battlefield. When US forces were caught off-guard by the development of improvised explosive devices (IEDs) in Iraq, active duty personnel up-armored their vehicles with whatever spare parts and scrap metal was available. It took years for the military to develop and deploy better-armored vehicles, but such hillbilly armor provided an interim solution. https://en.wikipedia.org/wiki/Improvised_vehicle_armour#/media/File:HillbillyArmor5tonCargo.jpg.

Targets and Prospects

There is always a concern that analogies from nature can be overstretched or inappropriately applied and most recent works on evolution and security acknowledge this directly. For example, Ehrlich and Levin (2005) explicitly discuss differences between human social evolution and biological evolution *via* natural selection. Likewise, a discussion of the ethics of applying evolutionary models to societal concerns graces most extended works on this topic (e.g., Vermeij, 2004). The misuse of 'social Darwinism' and adoption by proponents of eugenics has left scars on the academic community that are still felt today.

There are numerous fields of security studies and practice that have been examined from a functional and historical perspective, including crime analysis and policing strategies, homeland security, and natural disaster prevention (Felson, 2006; Sagarin, 2012; Roach and Pease, 2013). Cyber and information security, which already commonly uses the biological language of viruses, still suffers from a relatively static, firewall-based approach to security (Wulf and Jones, 2009), a situation that may benefit from an evolutionary treatment (Sagarin and Taylor, 2012; Sagarin, 2013).

Conflict between states might also be examined in an evolutionary context. From a behavioral point of view, Thayer attempted to reexamine various theories in international relations with an evolutionary lens and then applied this viewpoint to warfare and ethnic conflict (Thayer, 2004). Dietl, taking a macro-level approach, used concepts from selection theory to strengthen the idea that structural changes in world politics can be viewed in evolutionary terms (Dietl, 2008). While scholars have debated the role of environmental conditions in leading to conflict or hampering peace building (Crocker *et al.*, 2014), actual applications of evolutionary theory to peace building have been limited (Sagarin, 2014).

Likewise, an area of security that would seem to most naturally lend itself to lessons from adaptable natural systems is the growing field of climate change adaptation, but thus far

this field has primarily focused on social sciences approaches to stakeholder engagement (Willows and Connell, 2003), rather than viewing adaptation as a biological process. One of the key lessons from nature here is to understand whether there is sufficient adaptive flexibility (i.e., phenotypic plasticity) in a particular system or whether evolutionary changes are necessary. While there is extensive work studying the phenotypic plasticity of plants and animals, we need to know much more about human societal plasticity, its drivers, and the adaptive consequences of it.

While we can retrospectively identify the logic of evolution being applied to security policy, it is hard to identify particular cases where evolutionary approaches have been used at the outset to solve a security problem. A renewed focus on identifying both security problems amenable to applying evolutionary logic as well as identifying mechanisms underlying potential evolutionary responses may be helpful in developing concrete case studies of successful natural security.

There is a worry that although evolutionary approaches to security have been relatively welcome within academic circles, the pathway to incorporating them in practice may follow the delayed and incomplete application of 'Darwinian Medicine' to the practice of medicine (Armbruster, 2008). Indeed, one lesson from Darwinian Medicine is that it may take decades to develop an effective change model that becomes widely adopted. Thus, we should not be too concerned that it may take time to develop a catalog of successful natural security case studies.

Certainly, government and military officials have been briefed by some scientists in these works through individual meetings, staff briefings, seminars at military and homeland security departments and research academies (e.g., Sagarin lectured in evolutionary security at the Naval Postgraduate School) and conferences. There have also been calls for proposals from military organizations specifically looking at biomimetic technologies. How influential these inroads have been on policy remains to be seen and may never be fully quantifiable as policy arises from a complex mix of underlying science and research (which may come from many different fields), ideological struggles, and the particularly political environment surrounding law makers.

Regardless of the pathway to application, there is abundant basic research to be done in this field, which is very much in its infancy. Identifying key security questions that are amenable to an evolutionary approach will be a needed step in properly evaluating the tremendous potential of applying lessons from 3.5 billion years of successful life on Earth to increase the security of its diverse set of global citizens.

See also: Antagonistic Interspecific Coevolution. Intraspecific Coevolutionary Arms Races

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GSRDC Applied Knowledge Services.
www.adaptablesolutions.org
The University of Arizona.

Seedless Land Plants, Evolution and Diversification of

NS Nagalingum, Royal Botanic Gardens & Domain Trust, Sydney, NSW, Australia

Glossary

Allopolyploid A polyploid that formed between individuals of two different species (interspecific).
Arborescent (arborescence) Having a tree-like growth form.
Autopolyploid A polyploid that formed between individuals of the same species (intraspecific).
Bryophytes Lineage including mosses, liverworts, and hornworts. All reproduce via spores, lack vascular tissue, and the gametophyte stage is dominant. Current evidence is unclear whether bryophytes are monophyletic or not.
Cryptospore The first spores found in the fossil record. They lack features indicating they were connected to other spores (compare with trilete spore). They are given the prefix 'crypto' because of the ambiguous configuration with other spores, and the poor knowledge of their parent plants and affinities.
Dichotomous Describes a bifurcation resulting in two equal and symmetric branches.
Epipetric Growing on rock.
Epiphytic Growing on another plant.
Euphyll (also megaphyll) Leaf of a euphyllophyte. The leaves are produced by the apical meristem (a region of active division at the apex of the plant). In the central vascular tissue, in the area where a euphyll arises, there is a distinct region with nonvascular cells (called a leaf gap). Compare with lycophyll.
Euphyllophytes Lineage of plants that bears vascular tissue and leaves that are termed euphylls or megaphylls. It includes all vascular plants except the lycophytes; that is, ferns, horsetails, and seed plants.
Leaf gap A distinct region in the vascular tissue where there are nonvascular cells, and is linked to the formation and position of a megaphyll. Occurs only in euphyllophytes.
Lepidodendrids An extinct lineage of the lycophytes. They formed tall trees with thick trunks, and were particularly common in Carboniferous coal swamps.
Lycophyll (also microphyll) Leaf of a lycophyte. The leaves are produced by an intercalary meristem (a region of active division occurring in the stem, at the base of a leaf). In the central vascular tissue, in the area where a lycophyll arises, the vascular tissues are continuous and uninterrupted, thus lacking a leaf gap. Compare with euphyll.
Lycophytes Lineage including plants in the Lycopodiaceae, Selaginellaceae, and Isoetaceae. The plants reproduce via spores, and leaves are referred to as euphylls or megaphylls.
Megaphyll See euphyll.
Meristem A region of active cell division.
Microphyll See lycophyll.
Monilophytes Lineage including horsetails and ferns. The plants reproduce via spores, and in the central vascular tissues, the first xylem cells (protoxylem) are confined to lobes of the xylem strand.
Tracheophytes Lineage of plants that possesses vascular tissue. It includes lycophytes, ferns, horsetails, and seed plants; that is, lycophytes and euphyllophytes.
Trilete spore Spore that bears a Y-shaped mark. The Y-shape is derived from contact among four spores (tetrad) that were the product of meiosis.
Vascular cambium Actively dividing ring of tissue in the stem, which produces the vascular tissues. A type of meristem.

A large proportion of land plant history has been occupied by spore-bearing plants, that is, plants that reproduce via spores and not seeds. The earliest land plants were spore-bearing plants. These first plants are recovered in the fossil record almost 100 million years before the appearance of seed plants and about 330 million years before the earliest evidence for flowering plants.

This article reviews the evolutionary history of spore-bearing plants (Figure 1) – ferns, lycophytes, mosses, liverworts, and hornworts (the latter three are known as bryophytes). It incorporates knowledge from the fossil record, morphology of living taxa, and analyses of molecular DNA data. This article is not intended to be exhaustive, rather it offers some starting points for further exploration of these fascinating plant groups.

Bryophytes

Bryophytes as the Earliest Land Plants?

The earliest plant life on land reproduced via cryptospores, but there is uncertainty about the taxonomic affinity of these plants. They were thought to be bryophytes (Kenrick *et al.*, 2012; Wellman, 2004), but more recently, they have been called 'cryptophytes' – an extinct assemblage of land plants (a non-monophyletic grade) predating the various modern bryophyte lineages of land plants (Edwards *et al.*, 2014). The affinity or affinities of cryptospore-producing plants and their exact morphology are unclear because the fossils are fragmentary; nonetheless, they were only several millimeters tall with a unique combination of features that are not found in living taxa.

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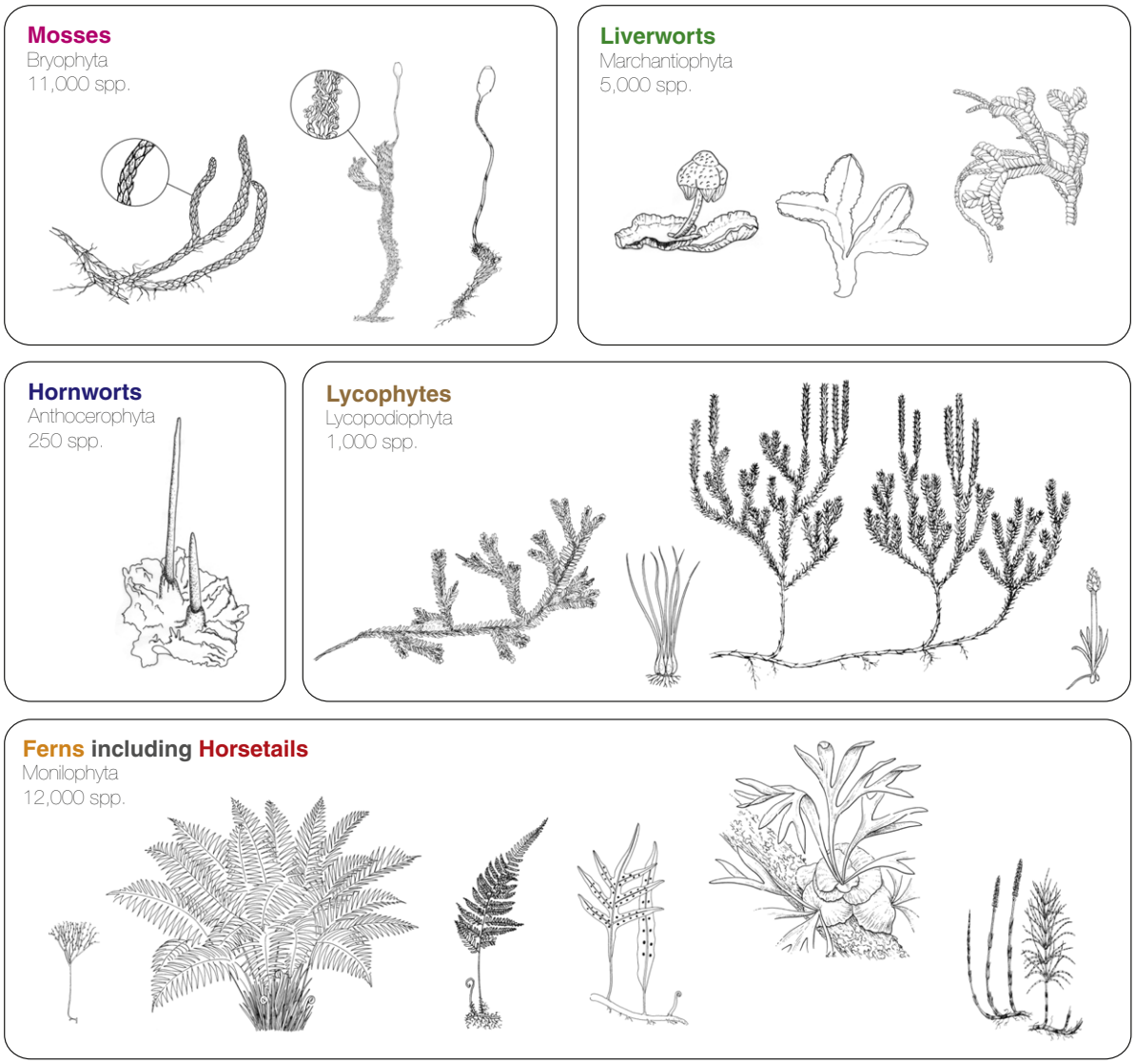

Figure 1 The five living spore-bearing plant lineages. Each box gives the common name, the division, number of species (Chapman, 2009), and representative illustrations of plants in each lineage. Illustrations are not to scale. All images from the Royal Botanic Gardens and Domain Trust archive accessed by Lesley Elkan, except for hornwort and thalloid liverworts images provided by D. Christine Cargill, and *Selaginella* from Stina Weststrand.

The first cryptospores appeared in the Middle Ordovician (roughly 470 mya (million years ago)) and increased in morphological diversity shortly after. For the next 30 million years, from the Middle Ordovician to Early Silurian, the cryptospores were globally distributed, but showed little variation suggesting an evolutionary stasis (Kenrick *et al.*, 2012; Gensel, 2008; Wellman, 2004). A major change in spore floras occured in the Early Silurian when cryptospore diversity decreased and trilete spore diversity increased. By the Devonian, ca. 420 mya, cryptospores virtually disappeared, possibly because trilete spore-producing plants outcompeted the cryptophytes. The plants that produced the early trilete spores were land plants, possibly from bryophyte-like organisms as well as vascular plants.

Alternating Generations and the Haploid Lifestyle

Living bryophytes comprise three groups: mosses, liverworts, and hornworts (**Figure 1**). They are mostly diminutive plants, and share one significant aspect of their life cycle – the haploid stage, in which they have a single copy of each chromosome, is the dominant phase (Shaw *et al.*, 2011; Niklas and Kutschera, 2010). In vascular plants, the diploid stage, in which there are two copies of each chromosome (or more), is dominant. All land plants alternate between these two phases of the life cycle, the haploid and diploid stages (gametophyte and sporophyte, respectively); however, the difference between vascular plants and bryophytes is that the dominant stage differs.

Because the dominant bryophyte plants have only a single copy of each chromosome and each gene, bryophyte evolution is quite different from plants with multiple copies of each chromosome. Every deleterious or beneficial mutation in a haploid plant is immediately expressed. This allows for a rapid spread of beneficial alleles, as well as the rapid elimination of any disadvantageous alleles, ultimately resulting in increased fitness (Otto and Gerstein, 2008).

Ambiguity in Relationships Among Bryophytes

The relationships among the three bryophyte lineages remain controversial. One hypothesis predicts that the three are monophyletic, thus, all sharing a single ancestor, and together are sister to all vascular plants (**Figure 2(a)**). Alternative hypotheses suggest that mosses plus liverworts are sister to vascular plants (**Figure 2(b)**), or that hornworts are sister to all vascular plants, with mosses plus liverworts as the first branching lineage in the land plant phylogeny (**Figure 2(c)**). There is another hypothesis that each group within the bryophytes evolved separately and they do not share a common ancestor, therefore, forming a grade rather than a monophyletic group (**Figure 2(d)**). Furthermore, in some analyses liverworts are the first diverging lineage and are sister to all other land plants (mosses and hornworts can be monophyletic or not) (**Figure 2(d)**). The results depend on the method of phylogenetic analysis used (Cox *et al.*, 2014), and studies using either whole chloroplast genomes or almost a thousand genes have failed to converge on an answer (Wickett *et al.*, 2014; Ruhfel *et al.*, 2014).

Understanding the relationships among bryophytes is critical to interpreting the evolution of land plants and development of morphological innovations (Renzaglia *et al.*, 2007). For example, if mosses are most closely related to vascular plants (Kenrick and Crane, 1997; Kenrick, 2000), the water-conducting cells in the mosses could be precursors to vascular tissue. However, if bryophytes are monophyletic or if hornworts are sister to vascular plants, then these cells in the mosses are an autapomorphy, suggesting vascular tissues arose *de novo* in vascular plants (Kenrick and Crane, 1997). The evolution of vascular tissues was a major evolutionary innovation allowing plants to become larger and explore new habitats.

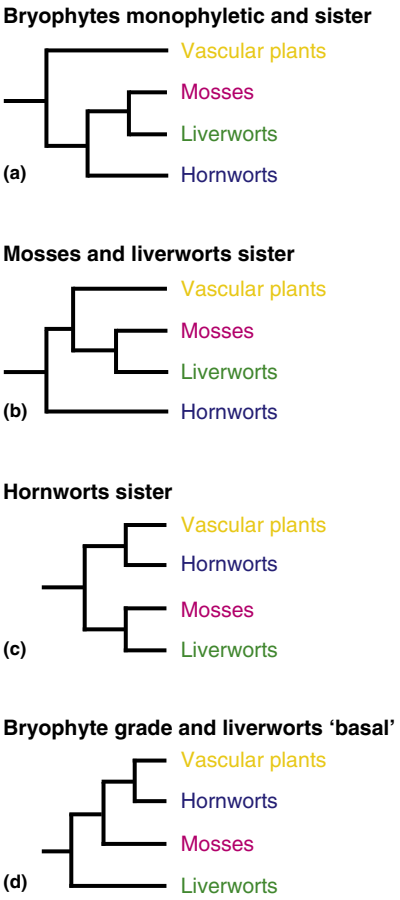

Figure 2 Phylogenetic relationships of bryophytes and vascular plants. (a) Bryophytes are monophyletic and together are sister to vascular plants; (b) hornworts are the first diverging lineage, and mosses plus liverworts are sister to vascular plants; (c) hornworts are sister to vascular plants, and mosses are sister to liverworts; (d) liverworts are the first diverging lineage or 'basal,' and the three bryophyte lineages form a grade with each arising from separate ancestors. The figure depicts the four principal relationships, although alternative relationships have been recovered from various studies. For relationships within vascular plants see **Figure 3**.

Ancient Origins Followed by Later Diversifications

Because of the delicate nature of bryophytes, there are very few macro fossils preserved in the fossil record. This absence of data has hampered our understanding of bryophyte diversification through time. The fossils that are known appear highly similar to extant species and, combined with the persistence of some ancestral morphological features, suggest bryophytes have 'limited evolutionary capacities' (Laenen *et al.*, 2014; Shaw *et al.*, 2011). The available fossils indicate that the modern lineages are ancient in origin, dating from the Paleozoic (although there is no record for hornworts until the Cenozoic) (Heinrichs *et al.*, 2015; Kenrick *et al.*, 2012).

Molecular timetrees are increasingly revealing a more complex history. These timetrees indicate that living diversity is the result of later radiations in the mid- to late Mesozoic and even during Cenozoic (Feldberg *et al.*, 2014; Laenen *et al.*, 2014; Cooper *et al.*, 2012; Fiz-Palacios *et al.*, 2011). For mosses and leafy liverworts, diversification was probably due to the new forest floor and forest canopy habitats created by angiosperms; a similar pattern is also observed for ferns (Schneider *et al.*, 2004; Schuettpelz and Pryer, 2009). Diversification analyses further suggest that mosses and liverworts experienced bursts of diversification from the mid-Mesozoic to the Cenozoic (Laenen *et al.*, 2014), while the hornworts did not (Villarreal *et al.*, 2015). The relatively low extant species richness in the groups appears to be the result of substantial extinction (Laenen *et al.*, 2014).

Lycophytes

Diversity in Morphology and Habitat Preferences

The three lineages, Lycopodiaceae, Selaginellaceae, and Isoetaceae, which collectively form the lycophytes, have divergent

morphologies and habitat preferences (Figure 1). The Lycopodiaceae or lycopods, are the most diverse of these lineages, having species that are epiphytic, terrestrial and creeping with a rhizome, terrestrial and climbing with a rhizome, or terrestrial with a subterranean tuber (Wikström, 2001). Selaginellaceae are prostrate and mat-forming, creeping, or erect, whereas their sister group Isoetaceae are aquatic to semi-aquatic and grow from a corm (Rydin and Wikström, 2002; Korall and Kenrick, 2002; Pigg, 2001).

The First Branch in Vascular Plants

The lycophytes occupy a special position in vascular plant (tracheophyte) phylogeny (Figure 3). They are the first branching lineage of tracheophytes, and consequently, they are sister to all other vascular plants (the euphyllophytes: ferns including horsetails, gymnosperms, and angiosperms) (Pryer *et al.*, 2001; Ruhfel *et al.*, 2014; Wickett *et al.*, 2014; Qiu *et al.*, 2006; Kenrick and Crane, 1997). Using the phylogeny as a framework, the lycophytes are critical to understanding the steps involved in the evolution of plants (Banks *et al.*, 2011; Floyd and Bowman, 2007; Ambrose, 2013). For example, we can compare genomes across the phylogeny to identify genes responsible for evolutionary changes between the major lineages. Comparing a lycophyte genome to genomes of bryophytes and of euphyllophytes reveals that three times as many genes were needed for the lycophyte–euphyllophyte transition as compared to bryophyte–vascular plant transition (516 vs. 1350 genes) (Banks *et al.*, 2011). On a genetic level, this indicates that there is greater complexity involved in becoming a euphyllophyte compared to transitioning to the vascular plant lifestyle.

Converging on the Leaf and Tree Habit

The lycophytes show two remarkable examples of convergence in plants – the evolution of the leaf and of the tree habit. Both have arisen independently in the lycophytes and in their sister group, the euphyllophytes (Figure 3). The earliest evidence of true leaves is in fossil lycophytes, as narrow elongated leaves called microphylls. Microphylls, also termed lycophylls, are quite different from the euphyll, or megaphyll, leaves of the euphyllophytes (Gifford and Foster, 1989; Pryer *et al.*, 2004). Lycophylls arise from an intercalary meristem (a region of active division occurring in the stem at the base of a leaf), whereas euphylls grow from an apical meristem. Also the central vascular tissues in lycophytes are continuous and uninterrupted, whereas those of euphyllophytes have a leaf gap when leaves form (a leaf gap is a distinct region in the vascular tissue where there are nonvascular cells). Such differences in development and morphology indicate that land plants have converged on leaf structures at least twice (Schneider *et al.*, 2002; Donoghue, 2005; Pryer *et al.*, 2004; Ambrose, 2013).

Another example of convergence between lycophytes and euphyllophytes is the ability to grow into large trees, also termed arborescence (Donoghue, 2005; Niklas, 1997). In the lycophyte lineage, the now extinct lepidodendrids were the main components of forests in the Carboniferous, around 320 mya. These plants were up to 35 m tall with trunks 2 m in diameter, dichotomous branches at the crown, and leaves that were 1 m long (Niklas, 1997). They formed enormous stands in swamps, and their decayed, preserved organic matter is the basis of modern coal deposits. One of the many remarkable features of these plants is that they formed tall trees; however, the mechanism by which they attained their height differs from that of most living seed plant trees, indicating separate evolutionary origins. The lepidodendrids and the living seed plants both possess an actively dividing ring of tissue in the stem (a meristem called the vascular cambium) that allows for increased girth and height; however, the divisions of the cells in the ring differ. In the lepidodendrids, the cambium divided in only one direction toward the inside of the stem, while in extant trees, the cambium cells divide in two directions to the inside and outer side of the stem. The tree habit has also evolved within other extinct and living groups, including ferns, horsetails, cycads, and monocots, but in quite different ways (Niklas, 1997).

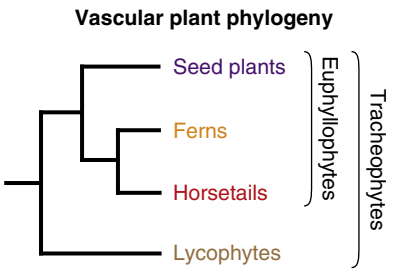

Figure 3 Phylogenetic relationships of the vascular plants. Relationships are based on the consensus from multiple studies (see text).

Ferns

Fern Distributions and Diversifications

In ferns, there are a range of forms and habitat preferences. They can be terrestrial, climbing, aquatic, epipetric (growing on rocks), epiphytic (growing on trees), xerophytic (growing in arid conditions), and also arborescent (Figure 1). Like many other plant groups, ferns are taxonomically diverse in the tropics (Nagalingum *et al.*, 2015; Kessler *et al.*, 2011; Kessler, 2010; Moran, 2008; Kreft *et al.*, 2010). High fern diversity correlates to high rainfall and montane regions (Nagalingum *et al.*, 2015; Kessler, 2010). Ferns share this preference for wet conditions with bryophytes, as shown by the strong correlations between bryophyte and fern diversity (Nagalingum *et al.*, 2014).

The majority of living fern diversity is the result of a relatively recent radiation starting in the mid-Cretaceous about 130 mya, occurring as a response to the diverse and complex forest niches created by the evolution of angiosperms (Schneider *et al.*, 2004; Schuettpelz and Pryer, 2009; Lovis, 1977; Rothwell, 1987; Crane, 1987; Nagalingum *et al.*, 2002). Part of this radiation included diversification of the epiphytic ferns in the Cenozoic (Schuettpelz and Pryer, 2009). Prior to this radiation, there were two earlier periods of fern diversification. The previous radiation was in the late Paleozoic

(approximately 250 mya), resulting in many lineages including some that persist today (such as the Osmundales, Schizaeales, and Gleicheniales) (Tidwell and Ash, 1994; Lovis, 1977; Rothwell, 1987; Pryer *et al.*, 2004). The oldest fern radiation occurred in the early Paleozoic (ca. 360 mya) following the evolution of ferns in the Devonian; however, this radiation produced fern families that are all now extinct (Rothwell, 1987).

Rearranging the Family Tree: Horsetails Among the Ferns

As a result of DNA analyses, fern phylogeny and classification have undergone significant reorganization over the past years, although many groups inferred through morphology have been confirmed (Christenhusz *et al.*, 2011; Smith *et al.*, 2006). There is a general consensus about the major groups in the ferns and their relationships (Rothfels *et al.*, 2012; Schuettpelz and Pryer, 2007; Pryer *et al.*, 2004, 1995; Hasebe *et al.*, 1995; Rothfels *et al.*, 2015; Lehtonen, 2011). However, there have been some outstanding questions, including the relationship of *Equisetum*, the horsetails, within the monilophytes (horsetails and ferns, **Figure 1**; Pryer *et al.*, 2001, 2004; Karol *et al.*, 2010). In some analyses, horsetails are recovered as sister to the Ophioglossales (adder's-tongues, grape ferns, and their allies) and Psilotales (whisk ferns) (Grewe *et al.*, 2013), or as sister to Psilotales alone (Ruhfel *et al.*, 2014). Recent analyses are converging on the position of horsetails as sister to all ferns, making them the first diverging monilophyte lineage (**Figure 3**; Rothfels *et al.*, 2015; Knie *et al.*, 2015; Wickett *et al.*, 2014).

Understanding Why Rates of DNA Evolution Vary

Molecular phylogenies depict the relationships among taxa, and in addition, the length of the branches represents the extent of molecular evolution, usually as substitutions per site. Branch lengths can differ, signifying there have been variable molecular evolutionary rates among the taxa (as well as indicating different ages). Understanding the causes of variation in molecular rates is an intriguing biological question. In animals, generation time is often suggested as a cause of variation, with long-lived species having slower rates simply because there are fewer generations to accumulate mutations. Alternatively, species that have shorter generation times have more generations to acquire mutations. In flowering plants, this phenomenon seems to be echoed by larger plants that take a longer time to reach maturity and so have slower rates, whereas smaller herbaceous plants have shorter generation times and have faster rates (Smith and Donoghue, 2008; Lanfear *et al.*, 2013). But is this true across the plant tree of life, and for plants that have a very different life history, such as ferns that reproduce via spores and undergo a period as a free-living gametophyte?

Ferns have substantial morphological diversity and so it is not surprising that the rates of molecular evolution are not uniform. Phylogenetically, the tree ferns have significantly shorter branches than their nearest relatives (Korall *et al.*, 2010). Analyses clearly indicate that the short branches are due to a slowdown or deceleration in rates in tree ferns. The possible cause is the tree habit, which is associated with a delayed time to maturity as compared to other ferns, mirroring the pattern in animals and in flowering plants (generation time effect).

Polyploidization and the Origin of Species

Polyploidization, the duplication of the whole genome, is prevalent in plants (Otto and Whitton, 2000; Soltis *et al.*, 2003; Soltis and Soltis, 1999; Wood *et al.*, 2009). Polyploid individuals have multiple copies of each set of chromosomes. Humans are diploid with two sets of chromosomes, while taxa with three sets are called triploids, four sets are tetraploids, and so forth. An exceptionally high ploidy level occurs in the adder's tongue fern, *Ophioglossum reticulatum*, which is a 96-ploid with 1440 chromosomes (Khandelwal, 1990).

These polyploids can be categorized to indicate the history of the polyloidy event. Allopolyploids form between two different species (interspecific), whereas autopolyploids occur within the same species (intraspecific). Therefore species are described by the ploidy level as well as the category of polyploidy. For example, *Polypodium hesperium* is tetraploid, and is the result of a cross between the diploids *Polypodium amorphum* and *Polypodium glycyrrhiza*. Thus it is called an allotetraploid (Sigel *et al.*, 2014).

To further complicate matters, allo- and autopolyploids can have repeated origins, where the polyploidization/hybridization event can occur multiple times. Following on from the example above, the chloroplasts of the allotetraploid *P. hesperium* differ across its range (Sigel *et al.*, 2014). This indicates that *P. hesperium* originated from multiple hybridization events between *P. amorphum* and *P. glycyrrhiza*. Based on DNA evidence, it was inferred that *P. amorphum* was the maternal parent (donating its chloroplast) in some cases, and in other cases, *P. glycyrrhiza* was the maternal parent. These different origins occurred in different geographical regions in North America, resulting in populations north and south of 42°N latitude having different plastid genomes. Such recurrent polyploid origins lead to higher genetic diversity, compared to a single origin (Soltis and Soltis, 1999).

Polyploidy allows duplicated, extra gene copies to mutate and explore new functions (Otto and Whitton, 2000; Otto, 2007; Soltis *et al.*, 2003; Soltis and Soltis, 1999, 2000). These new functions potentially enable polyploids to acquire new biochemical properties and expand into ecological niches, resulting in increased adaptability. Given these advantages, it is not surprising there are many polyploid ferns. In the Australian fern and lycophyte flora, 38% of all species are polyploids, with polyploids more common in particular families (Tindale and Roy, 2002). These numbers match estimates for ferns and lycophytes in general, such that 31% of species derive from polyploidy (Wood *et al.*, 2009). Interestingly, some ferns do not appear to have undergone polyploidization, with fossil nuclei showing the same genome size as living plants (Bomfleur *et al.*, 2014). As data from the first sequenced fern genomes are assessed (Sessa *et al.*, 2014), and with further studies investigating differing expression of parental genomes in a polyploid (Sigel *et al.*, 2014), it is likely that we will gain even further insights into the role that polyploidization has played in the evolution of ferns.

See also: Angiosperm Phylogeny and Diversification. Archaeplastida: Diversification of Red Algae and the Green Plant Lineage. Polyploid Speciation. Water Transport, the Role in Plant Diversification of

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Selective Sweeps

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Glossary

Ancestral allele The allele previously fixed in an ancestral population.

Composite likelihood function A simplified version of a likelihood function that treats certain data points as independent even if they are not.

Composite likelihood ratio test A likelihood ratio test (see below) using composite likelihood functions.

Derived allele An allele segregating in a population that differs from the ancestral allele.

Folded site frequency spectrum A site frequency spectrum (see below) based on the minor allele frequency instead of the derived allele frequency. For a sample of n chromosomes, there are n/2 entries if n is even, otherwise there are $(n-1)/2$ entries.

Fst A measure of population differentiation calculated as the amount of genetic variance attributable to population subdivision.

Hard selective sweep The process by which a single adaptive allele arises and spreads through a population to fixation.

Likelihood function A function that gives the probability of the data under a given parametric model.

Likelihood ratio test A statistical test based on the ratio of the likelihood function for an alternative model divided by the likelihood function for a null model, where the null mode is a special case of the alternative model.

Maximum likelihood estimate The parameter values that maximize the likelihood function for a given model and set of observed data.

Minor allele The least frequent allele at a biallelic locus.

Site frequency spectrum (SFS) The random vector $\xi=(\xi_1,\xi_2,\ldots,\xi_{n-1})$, where ξ_i is the number of loci at which i chromosomes carry the derived allele and $n-i$ chromosomes carry the ancestral allele.

Soft selective sweep The process by which multiple adaptive haplotypes rise in frequency, either due to selection acting on a previously segregating allele or selection acting simultaneously on multiple alleles at a locus.

Introduction

A fundamental process in the evolution of species is natural selection. When a novel genetic variant confers a reproductive advantage to an individual in a population, it can undergo positive selection, possibly 'sweeping' through the population by rising quickly in frequency generation over generation. Although the time-course from origin to fixation of an adaptive variant will be faster than a neutral variant, in many cases an adaptive variant will still take hundreds to thousands of generations to achieve a large change in population frequency. It is therefore difficult to directly observe an adaptive variant's frequency trajectory over time.

There are at least two instances where it may be possible resurrect the true dynamics of an adaptive allele. The first is the case of experimental evolution, such as the Lenski Long-Term Evolution Experiment (Lenski, 2011). Here, by following an experimental population subject to a stimulus, it is possible to identify when, where, and how adaptive innovations occur (e.g., Blount *et al.*, 2012). Another case has recently emerged as a result of the development of ancient DNA sequencing (Pääbo *et al.*, 2004), whereby DNA is extracted from the preserved ancestors of a species. Such a technology potentially enables the reconstruction of the natural time-course of adaptive mutations throughout history (Pickrell and Reich, 2014).

However, there are many instances when neither of these cases is possible. In such situations, we may only have genetic data from an extant population. Over the last several decades, population geneticists have devoted much work toward the development of statistics designed to infer where and when positive selection may have occurred from such data collected at a single time point. In this article, we will discuss some basic models of selective sweeps, along with their expected patterns and methods for inferring their historical action from modern sequence data. We will then discuss complications with the selective sweeps model, as well as alternative models of adaptation.

Examples of Selective Sweeps

While selective sweeps are thought to have been rare in human evolutionary history (Hernandez *et al.*, 2011), there are a few strong candidates in the human genome. A now classic example of a selective sweep in humans is the region around the lactase gene (LCT; Bersaglieri *et al.*, 2004; Tishkoff *et al.*, 2006). Ancestrally, adult humans are lactose intolerant – losing their childhood ability to digest lactose – and endure severe intestinal distress should they consume fresh milk. However, certain regulatory variants around *LCT* confer a phenotype that maintains intestinal lactase activity at childhood levels into adulthood, thus allowing adult carriers to digest fresh milk safely (Swallow, 2003). This lactase persistence phenotype may have been advantageous during the development of agricultural pastoralism, allowing adult carriers an additional source of nutrition.

Other species across the great ape phylogeny may have experienced different rates of selective sweeps, both across the autosomes as well as the sex chromosomes. One recent study found a high rate of selective sweeps on the X chromosome across all great apes (Nam *et al.*, 2015). On the autosomes, selective sweeps are thought to be much more common in other organisms such as *Drosophila*, where ~13% of amino acid substitutions are thought to have resulted in selective sweeps (Sattath *et al.*, 2011).

Inference of selective sweeps often informs our understanding of the natural history of species. However, given the putative location of a selective sweep, careful sleuthing is required to understand what the precise adaptive variant may have been (e.g., in the lactase example given above). In domesticated species, we can often use our understanding of the phenotypic diversity across breeds or varieties in conjunction with signatures of selective sweeps to better understand the basic biology governing phenotypic variation. For example, in dogs (the species with the greatest diversity in body size of any terrestrial vertebrate), researchers sought to identify selective sweeps between the smallest and largest breeds (Sutter *et al.*, 2007). They found strong evidence for a selective sweep across a single gene that was nearly exclusive to small breeds compared to large breeds. This gene encodes insulin-like growth factor 1 (*IGF1*), which is thought to influence body size in both mice and humans (Wit and Walenkamp, 2013). Similar study designs have been applied in several different domesticated species such as cattle (Qanbari *et al.*, 2014), rabbits (Carneiro *et al.*, 2014), and rice (Xu *et al.*, 2012) with mixed success.

Basic Population Genetic Models of Natural Selection

The basic model of allele frequency change under selection assumes an autosomal locus in a diploid hermaphrodite species that cycles through a process of random mating and then selection each generation. For two alleles A_1 and A_2 with frequencies p and q, let the absolute fitness of individuals with genotypes A_1A_1, A_1A_2, and A_2A_2 be given by w_{11}, w_{12}, and w_{22}. At the end of random mating, the expected genotype frequencies are given by their Hardy–Weinberg proportions p^2, $2pq$, and q^2. After undergoing selection the expected genotype frequencies are changed to $p^2w_{11}/\overline{w}$, $2pqw_{12}/\overline{w}$, and $q^2w_{22}/\overline{w}$, respectively. The normalizing factor $\overline{w}$ is the mean population fitness and is given by $\overline{w} = p^2w_{11} + 2pqw_{12} + q^2w_{22}$. It is then a simple exercise to derive the expected post-selection allele frequency $p' = (p^2w_{11} + pqw_{12})/\overline{w}$ as well as the change in allele frequency

$$\Delta p = p' - p = \frac{(pq[p(w_{11} - w_{12}) + q(w_{12} - w_{22})])}{\overline{w}} \quad [1]$$

Often, for the sake of simplicity, we consider the relative fitness of genotypes by dividing each individual's fitness by the fitness of one genotype. Thus, under an additive model of selection, if we divide each genotype fitness by the absolute fitness of the A_1A_1 homozygotes, we obtain relative fitnesses of $w_{11}/w_{11} = 1$, $w_{12}/w_{11} = 1 + hs$, and $w_{22}/w_{11} = 1 + s$ for the A_1A_1, A_1A_2, and A_2A_2 genotypes, respectively. Here s is the selection coefficient, and h is the dominance coefficient. Given this reformulation, we can now write eqn [1] as

$$\Delta p = p' - p = \frac{pqs[q(h-1) - hp]}{\overline{w}} \quad [2]$$

with $\overline{w} = 1 + 2pqhs + q^2s$. In the case of $0 \le h \le 1$, we have directional selection, and the most fit genotype will tend toward fixation. For a *de novo* mutation this is referred to as a 'hard selective sweep.'

When $\alpha = 2N_es$ is large (where N_e is the effective population size) the adaptive allele's frequency trajectory over time ($x(t)$) is essentially deterministic (satisfying the differential equation $\frac{dx(t)}{dt} = -\alpha x(t)(1 - x(t))$) when it is not close to 0 or 1 (Kaplan *et al.*, 1989). However, if the adaptive allele is close to 0 or 1 stochastic forces dominate. The frequency at which an adaptive allele transitions from the stochastic phase to the deterministic phase is referred to as the establishment frequency. While the adaptive allele is below the establishment frequency, there is a high likelihood (with probability $1 - 2s$) that the allele will be lost (Ewens, 1979). Kaplan *et al.* (1989) suggest an establishment frequency value of $\varepsilon = \frac{5}{\alpha}$ as a rule of thumb.

Expected Patterns

Under the model of a hard selective sweep, the frequency trajectory of an adaptive *de novo* mutation proceeds in three phases. It arises in a population and undergoes a period of stochastic fluctuations before (possibly) reaching the establishment frequency. The second phase, for large s, is essentially deterministic and the allele quickly rises in frequency. The final phase is a mirror of the first, where the adaptive variant undergoes a period of stochastic fluctuations at high frequency before ultimately reaching fixation. During this process, linked genetic material is also brought to high frequency unless a recombination event intervenes during the sojourn toward fixation. This hitchhiking effect has been the subject of much theoretical analysis (Maynard Smith and Haigh, 1974; Kaplan *et al.*, 1989; Stephan *et al.*, 1992; Barton, 1998), and the local effects on neutral segregating loci are well known, namely

1. a reduction in diversity surrounding the adaptive locus,
2. a skew in the 'site frequency spectrum' (SFS) toward rare variants,
3. a skew in the SFS toward high-frequency derived variants, and
4. long derived haplotypes extending from the adaptive locus.

The length of the physical region affected and the magnitude of the effect are related to the ratio of the selection coefficient to the recombination rate.

This process is illustrated in **Figure 1**. In **Figure 1(a)**, an adaptive mutation (red bar) arises on single haplotype in a population, and in **Figure 1(b)** it begins to rise in frequency. Occasionally a recombination or mutation event will occur during the sweep, but because this process happens on a faster timescale these events are relatively rare. The resulting genetic pattern immediately after fixation (**Figure 1(c)**) is greatly reduced diversity surrounding the adaptive locus. Finally, after the sweep has completed, recombination and mutation events continue to accumulate in the region (**Figure 1(d)**), thus

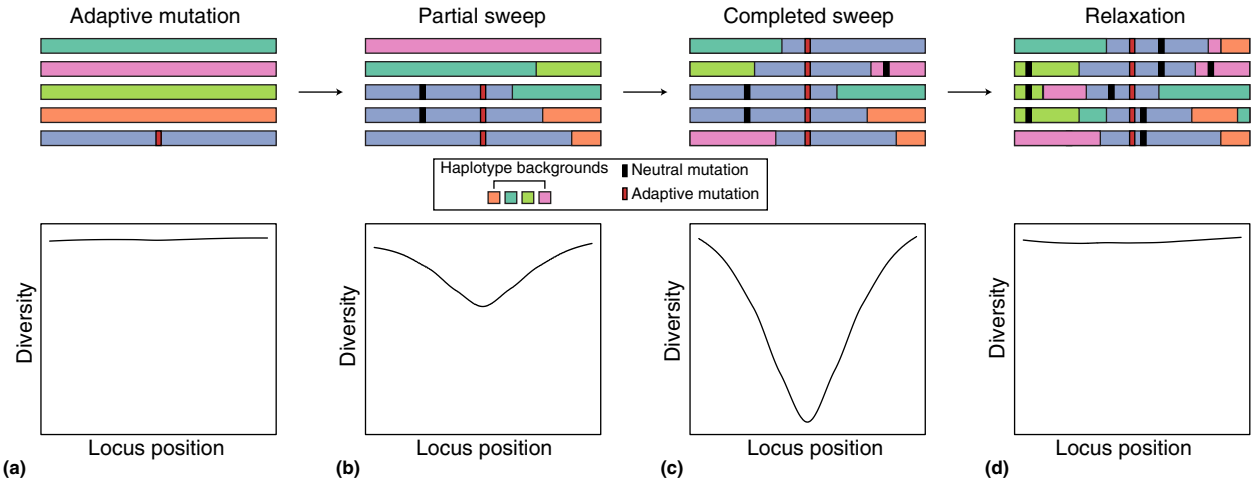

Figure 1 A cartoon illustration of a hard sweep showing haplotype and diversity patterns for (a) the initial adaptive mutation, (b) the hard sweep in progress, (c) the hard sweep at completion, and (d) after relaxation.

increasing diversity among haplotypes and causing a 'relaxation' of the reduced diversity footprint of a selective sweep.

The SFS – the number of observed variants segregating at a given frequency – is also affected by a sweep. As an adaptive allele sweeps toward fixation, linked variants on the adaptive haplotype will rise in frequency, while variants that reside on the nonadaptive haplotype will decrease in frequency. As such, we expect a skew toward rare and high-frequency variants during a sweep and at completion; if no further sweeps occur, the region will eventually relax back to a neutral SFS. Figure 2 demonstrates these patterns in the SFS.

Methods for Inference

Several studies have developed statistics designed to identify putatively selected regions by searching for deviations from neutral expectation of diversity and/or haplotype length (Lewontin and Krakauer, 1973; Tajima, 1989; McDonald and Kreitman, 1991; Fu and Li, 1993; Fu, 1997; Fay and Wu, 2000; Akey *et al.*, 2002; Ramos-Onsins and Rozas, 2002; Sabeti *et al.*, 2002; Kim and Nielsen, 2004; Nielsen *et al.*, 2005; Voight *et al.*, 2006; Jensen *et al.*, 2007; Sabeti *et al.*, 2007; Williamson *et al.*, 2007; Oleksyk *et al.*, 2008; Chen *et al.*, 2010; Pavlidis *et al.*, 2010; Alachiotis *et al.*, 2012; Liu *et al.*, 2013; Pavlidis *et al.*, 2013; Ferrer-Admetlla *et al.*, 2014). Some of these methods have been extended to multiple populations in the search of population-specific selective sweeps. Methods that extend the SFS to multiple populations often consider patterns of F_{st} (e.g., the Population Branch Statistic; Yi *et al.*, 2010), though cross-population extended haplotype homozygosity (*EHH*; see section 'Extended haplotype homozygosity') statistics have also been developed (Sabeti *et al.*, 2007). In this article we focus on two major classes of inference methods for hard sweeps, those based on examining the SFS and those based on examining haplotype patterns.

Site Frequency Spectrum Methods

A number of SFS-based statistics have been developed to test for departures from neutrality, and since the SFS deviates from neutrality during a sweep (Figure 2), these can be used to provide evidence of selection. Formally, we define the SFS of a sample of n chromosomes as ξ_i, the number of loci where the 'derived allele' is observed exactly i times. Similarly we define the 'folded SFS' as $\eta_i=(\xi_i+\xi_{n-i})/(1+\delta_{i,n-i})$ (where $\delta_{i,j}=1$ if $i=j$ and 0 otherwise), the number of loci where the 'minor allele' is observed exactly i times in a sample of size n chromosomes ($i\leq\lfloor n/2\rfloor$). Given the SFS, we can compute a number of useful statistics, including the number of segregating sites $S=\sum_{i=1}^{n-1}\xi_i=\sum_{i=1}^{\lfloor n/2\rfloor}\eta_i$ and the mean pairwise sequence differences $\pi=\binom{n}{2}^{-1}\sum_{i=1}^{n-1}i(n-i)\xi_i=\binom{n}{2}^{-1}\sum_{i=1}^{\lfloor n/2\rfloor}i(n-i)\eta_i$. A detailed treatment of the SFS and its statistical properties are given in Fu (1995).

Tajima's D *and others*

Under the standard neutral model population size is held constant and, in the absence of natural selection, genetic variation is only influenced by mutation and drift. Tajima (1989) devised a test for deviations from the neutral model by comparing the mean pairwise sequence differences (π) and the number of segregating sites (S). Under this model, $E[\pi]=E[S]/a_1=\theta$ (Watterson, 1975; Tajima, 1983), where θ is the population-scaled mutation rate and $a_1=\sum_{i=1}^{n-1}\frac{1}{i}$. Under the standard neutral model, $E[\pi-S/a_1]=0$. Therefore, Tajima (1989) proposed

$$D=\frac{\pi-S/a_1}{\sqrt{Var[\pi-S/a_1]}} \qquad [3]$$

where the denominator is the standard deviation of the difference $\pi-S/a_1$.

Tajima's D is expected to be 0 under the standard neutral model, and departures can shift D both positive and negative. D is roughly beta-distributed (Tajima, 1989), and a rule of thumb for determining significance (at the 5% level) is $|D|>2$, although Simonsen *et al.* (1995) explore this question in more depth. During a sweep, we expect an excess of rare alleles surrounding the adaptive locus sending $D<0$.

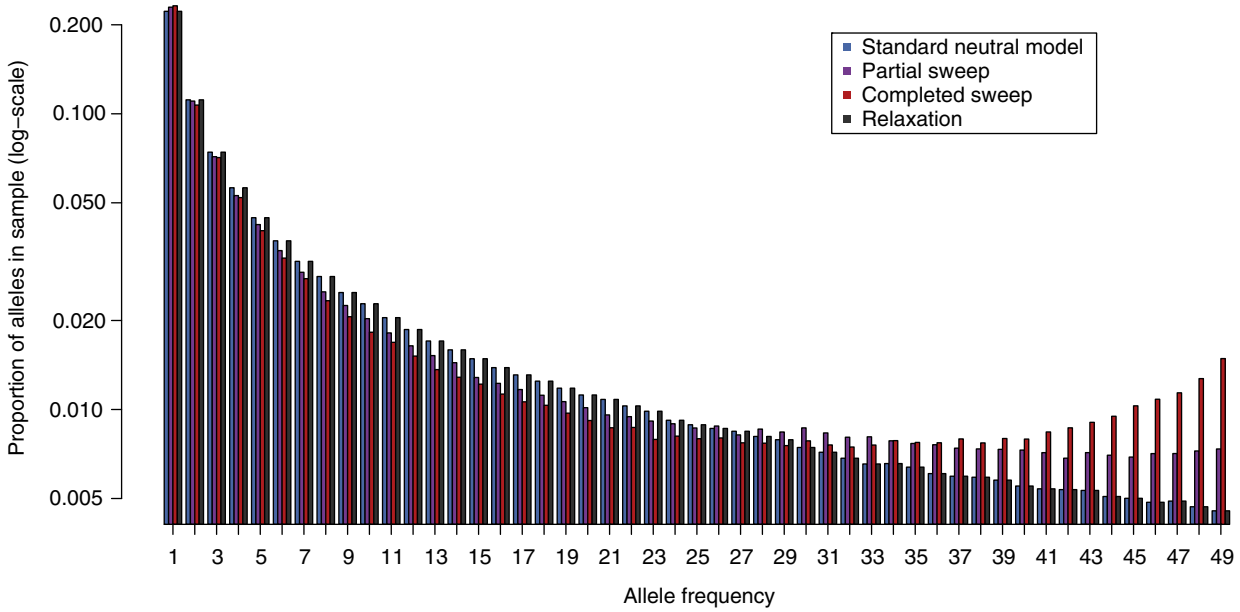

Figure 2 The expected site frequency spectrum for the standard neutral model (blue bars), for a hard sweep in progress (purple bars), for a hard sweep at completion (red bars), and after relaxation (black bars).

We illustrate the effect of a hard sweep on D with simulated genetic data using mssel, a version of the coalescent simulator ms (Hudson, 2002) modified to perform coalescent simulations conditional on an allele frequency trajectory (see Hudson and Kaplan, 1988; Kaplan *et al.*, 1988). We simulate 200 replicates of 50 haploid samples of 3 Mbp in a constant population size (effective population size $N_e = 10\,000$) with a recently fixed positively selected allele placed at the center of the region with a selection coefficient of $s = 0.02$ and a dominance coefficient of $h = 0.5$. We calculate D in a sliding window of 50 kbp with steps of 25 kbp. Figure 3 plots the mean D across replicates in each window along the simulated region and shows a clear dip below $D = -2$ (marked with a red dotted line) in proximity to the selected site indicating a significant departure from neutrality. Importantly, while Tajima's D can be used to detect a recent sweep, it also has power to detect other departures from the standard neutral model, including historical changes in population size that may affect D in ways similar to a hard sweep (Simonsen *et al.*, 1995).

A number of other statistics have been developed along similar lines as Tajima's D, including Fu and Li's D, D^*, and F^* (Fu and Li, 1993), and Fay and Wu's H (Fay and Wu, 2000). Indeed, these are each special cases of two generalized statistics derived by Fu (1997) and based on linear functions of the folded or unfolded SFS.

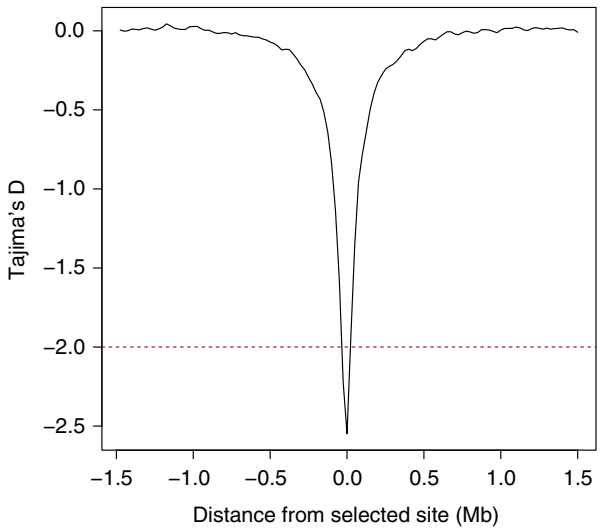

Figure 3 Mean Tajima's D across 200 replicates of 50 haploid samples of 3 Mbp calculated in a sliding window of size 50 kbp with steps of 25 kbps. As the window approaches the location of the simulated sweep, mean Tajima's D drops below 2, suggesting a significant departure from neutrality.

Composite likelihood of site frequency spectrum

A more sophisticated approach to detecting selective sweeps utilizes a 'composite likelihood ratio test' (CLRT) framework to analyze the SFS (Nielsen *et al.*, 2005). For a sample of n chromosomes, let the probability of observing a derived allele j times be p_j for $j \in \{1,2,\ldots,n-1\}$, and let $\mathbf{p} = (p_1, p_2, \ldots, p_{n-1})$. Then the 'composite likelihood function' is given by

$$\mathscr{L}(\mathbf{p}) = \prod_{j=1}^{n-1} p_j^{\xi_j} \qquad [4]$$

for which the 'maximum likelihood estimate' of $\mathbf{p}$ for k single nucleotide polymorphisms (SNPs) is given by $\hat{\mathbf{p}} = (\hat{p}_1, \hat{p}_2, \ldots, \hat{p}_{n-1})$ where $\hat{p}_j = \xi_j / k$ for all $j \in \{1,2,\ldots,n-1\}$. Furthermore, let $\mathbf{p}_R$ represent $\mathbf{p}$ within some genomic region R containing k SNPs, such that $L(\mathbf{p}_R)$ is the composite likelihood for the region R and $\hat{\mathbf{p}}_R$ is the maximum likelihood estimate. A simple CLRT for an aberrant SFS in query region R

is then given by

$$\text{CLRT} = 2[\log\mathscr{L}(\hat{\mathbf{p}}_R) - \log\mathscr{L}(\hat{\mathbf{p}}_0)] \quad [5]$$

where $\hat{\mathbf{p}}_0$ is often computed genome wide. While this test does not specifically test for selection, it will identify regions with a SFS that are significantly different from the genome-wide average, including regions that have undergone a selective sweep.

To directly incorporate selection into this test, Nielsen *et al.* (2005) make two approximations. First, they assume that each ancestral lineage 'escapes' the sweep by recombination off of the sweeping haplotype and that this occurs independently with identical probability. Second, they assume that all recombinations during the sweep occur before any lineages coalesce. Under these assumptions, Nielsen *et al.* (2005) derive the distribution of site frequencies after a sweep and construct another CLRT.

This second test can be used to scan the genome to search for regions that have experienced a sweep and, simultaneously, infer selection strength. In order to determine significance of either of the CLRTs, scores must be compared to neutral simulations under an explicit demographic scenario. Alternatively, the empirical genome-wide distribution of scores can be used.

Using the program SweeD (Pavlidis *et al.*, 2013) to calculate CLR scores from a sample of 102 European human genomes (population code CEU), from The 1000 Genomes Project Consortium (2012), we examine a putatively neutral region of chromosome 2 and the *LCT* locus for evidence of positive selection. In order to determine significance, we use the 95% percentile of the genome-wide distribution of CLR scores.

Figure 4(a) shows CLR scores in a 2 Mbp putatively neutral window on chromosome 2. As expected for a neutral region, the vast majority of CLR scores are below the significance cutoff (CLR = 4.6643 marked with a red dotted line). However, if we examine a region containing the *LCT* locus (**Figure 4(b)**, *LCT* highlighted in blue), we find a substantial enrichment of CLR scores above the critical threshold, indicative of a sweep.

Haplotype Methods

If a hard sweep is in progress or recently completed, we should expect to find a large number of high-frequency variants in the vicinity of the adaptive locus that have hitchhiked to high frequency along with it. In particular, for a small window around the adaptive locus there will be low haplotype diversity as mutation and recombination events are unlikely to have occurred close to the locus during the sweep. However, if we successively widen this window to include more surrounding variants, the likelihood of observing mutation and recombination events increases and we should expect haplotype diversity to increase. Following this idea, a number of statistics have been designed to detect recent or ongoing positive selection by summarizing the decay of haplotype homozygosity away from a given locus. These statistics search for unusually long haplotypes given the putative age of the derived variant (using allele frequency as a proxy). A number of haplotype-based statistics have been implemented by Szpiech and Hernandez (2014) in the program selscan.

Extended haplotype homozygosity

The extended haplotype homozygosity (EHH) statistic was introduced by Sabeti *et al.* (2002) and is defined as the homozygosity of all haplotypes surrounding a core locus. For a sample of n chromosomes, the set C contains all possible distinct haplotypes at a core locus. For simplicity, we assume the core locus and all adjacent markers are biallelic SNPs, thus $C := \{0,1\}$, where 0 represents the 'ancestral allele' and 1 denotes the derived allele. The set $C(x_i)$ contains all possible distinct haplotypes extending from the core locus in one direction (upstream or downstream) to the i^{th} marker. Therefore, $C(x_1) := \{00,01,10,11\}$, $C(x_2) := \{000, 001, 010, 011, 100, 101, 110, 111\}$, etc. The EHH of a sample of haplotypes extending from the core locus to marker x_i is then

$$EHH(x_i) = \sum_{h \in C(x_i)} \frac{\binom{n_h}{2}}{\binom{n}{2}}, \quad [6]$$

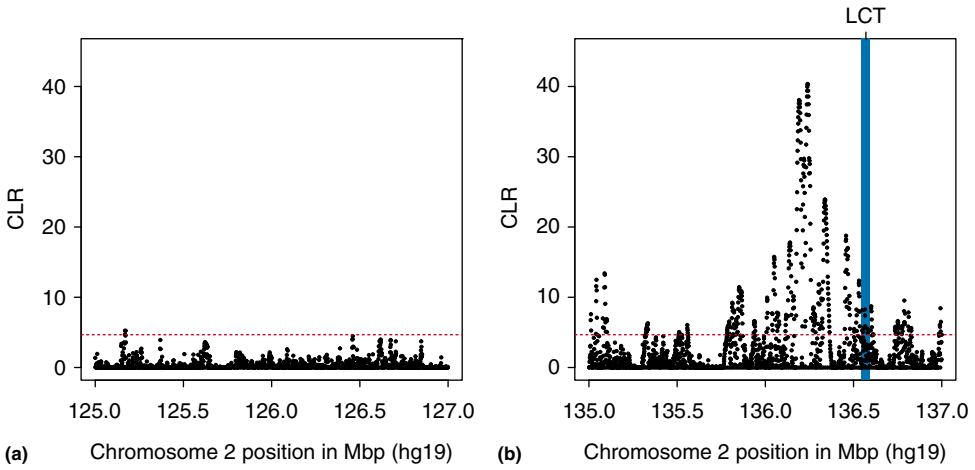

Figure 4 CLR scores calculated across 102 European human genomes for (a) a putatively neutral region of chromosome 2 and (b) a region surrounding the LCT locus (bounded by blue box) on chromosome 2.

where n_h is the number of observed haplotypes of type $h \in C(x_i)$ and by definition $\binom{x}{2} = 0$ for all $x < 2$. EHH(x_i) thus measures haplotype homozygosity within an interval extending from a core locus and is expected to be high for short intervals and monotonically decay as the interval widens since mutation and recombination events break up haplotypes. If the core locus contains an adaptive variant that is sweeping toward or has recently reached fixation, then we expect very long high-frequency haplotypes extending from the core and we should observe EHH(x_i) decay slower than around a neutral locus. In practice, we select a query locus in the genome to test for evidence of a selective sweep and compute EHH at this locus with successively wider genomic intervals. We then compare this to a null distribution of EHH that is computed around a large number of randomly selected, putatively neutral loci, with similar allele frequencies.

We illustrate this using mssel to simulate genetic data and the program selscan (Szpiech and Hernandez, 2014) to compute EHH curves. We simulate 200 replicates of 50 haploid samples of 3 Mbp in a constant population size (effective population size $N_e = 10\,000$) with a recently fixed positively selected allele placed at the center of the region with a selection coefficient of $s = 0.02$ and a dominance coefficient of $h = 0.5$. We also perform 200 replicate neutral simulations of 50 haploid samples in a constant population size ($N_e = 10\,000$). Each EHH calculation was centered on the middle of the simulated region and extended out 100 kbp on either side. **Figure 5(a)** shows the EHH decay curves for both neutral (blue) and non-neutral (red) simulations, demonstrating the slow decay of haplotype homozygosity around a positively selected versus neutral loci.

Integrated haplotype score

If a hard sweep has not reached fixation, the adaptive variant will be at intermediate frequency, and EHH values computed amongst the whole sample will be depressed because of higher overall diversity. In this case we may desire to compute EHH on a subset of haplotypes containing only the derived allele at the core or only the ancestral allele at the core. By separating the EHH computation between the ancestral and derived haplotypes, we can compare the relative size and diversity of ancestral and derived haplotypes at the same locus and search for unusually large differences between the two, thus implying a selective sweep in progress (**Figure 5(b)**). This is the basis for the development of the Integrated Haplotype Score (iHS; Voight *et al.*, 2006).

In order to compare the ancestral and derived EHH curves, Voight *et al.* (2006) proposed to integrate the curves with respect to genetic distance. Unstandardized iHS is then computed as the log-ratio of the ancestral and derived integrated values,

$$\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right) \qquad [7]$$

where iHH_1 is the integrated derived EHH curve and iHH_0 is the integrated ancestral EHH curve. In this formulation, positive values suggest long derived haplotypes relative to ancestral haplotypes and negative values suggest the converse. However, unstandardized iHS is correlated with derived allele frequency (see **Figure 4** in Voight *et al.*, 2006, note that their definition of iHS swaps the position of iHH_1 and iHH_0 in the log-ratio) as low-frequency variants are more likely to be young and therefore reside on longer haplotypes. Thus, unstandardized iHS scores are normalized in frequency bins to give

$$\text{iHS} = \frac{\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right) - E_p\left[\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right)\right]}{\sqrt{Var_p\left[\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right)\right]}} \qquad [8]$$

where $E_p\left[\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right)\right]$ and $\sqrt{Var_p\left[\ln\left(\frac{\text{iHH}_1}{\text{iHH}_0}\right)\right]}$ are the mean and standard deviation in frequency bin p.

Although large positive iHS scores suggest a long derived haplotype, in practice the adaptive variant may not be typed and neighboring ancestral alleles may hitchhike to high

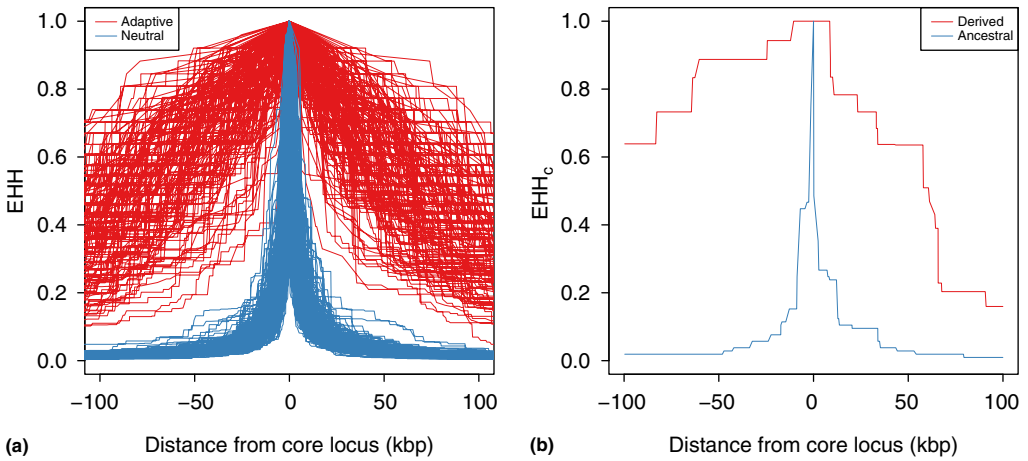

Figure 5 (a) Extended haplotype homozygosity (EHH) calculated from 50 haploid samples for 200 simulated replicates of a hard sweep (red lines) and 200 simulated replicates of neutrally evolving regions (blue lines), illustrating a slower EHH decay away from the adaptive locus as compared to neutral ones. (b) One example of EHH calculated on ancestral (blue line) and derived (red line) haplotypes separately in the vicinity of a hard sweep.

frequency, therefore it is common to search for extreme values of $|iHS|$ instead. Furthermore, Voight *et al.* (2006) note that extreme $|iHS|$ scores can occur under neutrality but tend to be randomly distributed, as opposed to a region undergoing a selective sweep where extreme scores tend to cluster together. With this in mind, Voight *et al.* (2006) devised a procedure to identify putative regions undergoing a sweep.

First, identify all core loci above a critical value, which is typically chosen as $|iHS| > 2$. As the standardized iHS scores are roughly distributed normally with mean = 0 and standard deviation = 1, this approximately represents the most extreme 5% of scores. Then divide the genome up into nonoverlapping windows of 100 kbp, and calculate the fraction of scores within each window that are above the critical value. To account for the variable number of scores within a fixed length window, bin windows based on the number of scores. Finally, examine the distribution of these fractions and select the top 1% (thus bounding the false positive rate to be $\leq 1\%$) from each window bin as putatively under selection. Using the program selscan (Szpiech and Hernandez, 2014) to calculate iHS scores from a sample of 102 European human genomes (population code CEU) from the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2012), we examine a putatively neutral region of chromosome 2 and then the *LCT* locus for evidence of positive selection. **Figure 6(a)** shows $|iHS|$ scores in a 2 Mbp putatively neutral window on chromosome 2. As expected for a neutral region, nearly all of the iHS scores are below the critical threshold ($|iHS| = 2$ marked with a red dotted line). However, if we examine a region containing the *LCT* locus (**Figure 6(b)**, *LCT* highlighted in blue), we find a substantial enrichment of iHS scores above the critical threshold, indicative of a strong sweep in progress.

Cross-population extended haplotype homozygosity

The cross-population EHH (XPEHH) statistic introduced by Sabeti *et al.* (2007) is designed to detect local adaptation within a query population by comparing iHH with a reference population. To do this we use eqn [6], and calculate the integrated haplotype homozygosity of the entire haplotype sample separately for each population. For populations A and B, we have iHH_A and iHH_B, and the unstandardized XPEHH is given by

$$\ln\left(\frac{iHH_A}{iHH_B}\right) \quad [9]$$

which is typically normalized with respect to the genome-wide distribution of *XPEHH* scores. Here, positive scores suggest long haplotypes in population A with respect to population B and a potential sweep in A, whereas negative scores suggest long haplotypes in B with respect to A.

Alternatives to using a genetic map

In many species, recombination events do not occur uniformly across their genome. To accommodate this, the formulations above assume knowledge of a genetic map. When calculating integrated EHH scores, a genetic map is integrated over and acts to weight an extended haplotype based on prior knowledge of recombination rate in that genomic location. For example, if a sweep occurs proximal to a recombination hotspot, then it would be uncommon to observe an extended tract of haplotype homozygosity spanning the region, and thus it is a highly informative event. In genetic distance space, this hotspot will be represented as a long interval, and iHH scores will be upweighted. In contrast, proximal to a recombination coldspot, it would not be uncommon to observe an extended haplotype spanning the region. In genetic distance space this region will appear shrunken, and iHH scores will be downweighted. However, it is not always possible to obtain a genetic map or the map may be of questionable quality. In these cases one could use a physical map as a proxy.

Another alternative would be to use the nSL statistic introduced by Ferrer-Admetlla *et al.* (2014). nSL can be reformulated to conform to the notation given above and is calculated as a log-ratio of the SL statistic calculated for the ancestral and derived haplotype pools. The essential difference from iHS is the removal of genetic map information. Whereas previously we integrate with respect to a genetic map, for the calculation of nSL all gaps between markers are given a weight of 1. After calculating nSL, scores should be normalized in frequency bins as with iHS.

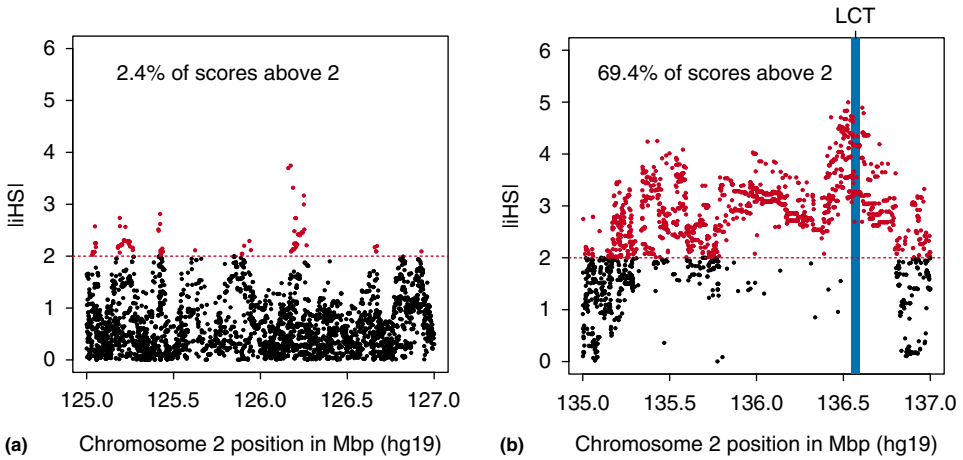

Figure 6 iHS scores calculated across 102 European human genomes for (a) a putatively neutral region of chromosome 2 and (b) a region surrounding the LCT locus (bounded by blue box) on chromosome 2.

Simulation Methods

Structured Coalescent

Kingman's coalescent model for neutral gene evolution (Kingman, 1982) provides a fast and flexible model for simulating neutral genealogies backwards in time and – in concert with a model of mutation, recombination, and migration – allows one to simulate genetic data under complex demographic scenarios. Although in its original formulation, only neutral data can be simulated under this model, theoretical work on the properties of neutral variation linked to a non-neutral variant suggest a way to incorporate selection at a single linked site (Kaplan *et al.*, 1988; Hudson and Kaplan, 1988). By conditioning on the selected site's allele frequency trajectory, $x(t)$, lineages linked to the adaptive derived allele are treated as coming from a population of size $2N_e x(t)$ and lineages linked to the ancestral allele are treated as coming from a population of size $2N_e(1-x(t))$. If a lineage from one background recombines onto the other, this is treated as a migration from one population to the other. A number of software programs exist to simulate this conditional coalescent process including mssel (a modified version of ms, Hudson, 2002), selsim (Spencer and Coop, 2004), mbs (Teshima and Innan, 2009), msms (Ewing and Hermisson, 2010), and cosi2 (Shlyakhter *et al.*, 2014).

Forward Simulations

The structured coalescent for simulating hard sweeps does suffer from some limitations, however. While the coalescent simulations are fast, only a single non-neutral allele can be considered at a time, and a frequency trajectory must also be simulated separately. It is also not possible to simulate the effects of multiple sweeps occurring simultaneously in a population. The primary alternative is a forward simulation, which can be made as flexible as desired (including demography, positive selection on multiple sites, linked negative selection, etc). There are many implementations of forward simulators (Hoban *et al.*, 2012). Some of the simulators that balance flexibility with computational efficiency across a range of parameters SFS_CODE (Hernandez, 2008), SLiM (Messer, 2013), and FWDPP (Thornton, 2014).

Some Caveats

Nonequilibrium Demography

The statistics described above for inferring the genomic location of a hard sweep are quite powerful, as they summarize patterns of polymorphisms that are expected to differ under a hard sweep versus under neutrality. However, the theory supporting these methods assumes a simple constant population size with no subdivision and no migration. Most, if not all, natural populations do not exist with such simple demographic histories and will likely have experienced numerous subdivisions, complex migration patterns, and population size expansions and contractions. These nonequilibrium demographies can create, under neutrality, very similar patterns as a hard sweep in a constant-size population. Indeed, population size contractions have the effect of reducing genetic diversity and generating long haplotypes. Contractions and expansions can create an excess of low- and/or high-frequency variants. Population subdivisions are subject to the Wahlund effect where genetic drift can cause an increase of homozygosity, and migrants between populations can introduce genetic material that has experienced a very different demographic history.

To mitigate these potentially confounding factors, these methods typically do one of two things. The first is to compare observed values to the distribution of values calculated from neutral simulations under an explicit demographic model. As a practical matter though, misspecification of the underlying demographic model could have a profound effect on the distribution of scores and could inflate the false positive rate. Alternatively, under the assumption that recent or ongoing hard sweeps are relatively rare throughout the genome at any given time point, many methods will compare observed scores in a particular region to the genome-wide distribution of scores, highlighting the most deviant ones as putative regions under selection. This, too, suffers from a similar problem of a possibly increased false positive rate. An extreme example would be a genome with absolutely no recent selection, but, through purely neutral deviations, some regions will always fall into the tails of the score distribution, which may then be marked as putative regions under selection.

Background Selection

While our attention has been focused on the action of positive selection, in many circumstances, linked negative selection can result in similar processes (known as background selection). Imagine a locus in the genome subject to frequent deleterious mutations. As natural selection acts to purge these deleterious mutations, linked neutral variation will also be eliminated. This can result in a local reduction in diversity around the deleterious locus, and in some cases result in an excess of both high- and low-frequency derived alleles (Charlesworth *et al.*, 1993). This pattern mimics some signatures of positive selection, and can cause an excess of false-positive inference for statistics that focus on these patterns. However, some of the effects of background selection are easily modeled, and can be incorporated into the statistical procedure for inferring the action of positive selection (Huber *et al.*, 2015).

Alternative Models

Throughout this article we have discussed the properties of hard sweeps and some methods for their detection. However, a selective sweep need not be 'hard.' A mutation that is intially neutral or mildly deleterious may become adaptive after an environmental change or during the colonization of a new niche. Depending on its frequency at the time it became adaptive, it may be segregating on several haplotype backgrounds, which then sweep to high frequency (Hermisson and Pennings, 2005). Similarly, if a locus has a high adaptive mutation rate, multiple adaptive alleles on different haplotype backgrounds could simultaneously sweep to high frequency (Pennings and Hermisson, 2006a). These scenarios result in

'soft selective sweeps,' and may generate different patterns of variation from what would be expected from a hard sweep (Pennings and Hermisson, 2006b; Messer and Petrov, 2013; Wilson *et al.*, 2014). Because of this, many of the statistics developed for detecting hard sweeps are under-powered to detect soft sweeps, although some efforts are being made to detect soft sweeps and distinguish them from hard sweeps (Peter *et al.*, 2012; Ferrer-Admetlla *et al.*, 2014; Garud *et al.*, 2015).

See also: Adaptive Molecular Evolution: Detection Methods. Directional Selection and Adaptation. Natural Selection, Introduction to

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Sensory Systems: Molecular Evolution in Vertebrates

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Introduction

Vertebrates are a strikingly diverse group occupying a wide variety of niches and environments, some astonishingly extreme. Because distinct environmental conditions pose different selective constraints, natural selection is expected to have influenced sensory evolution, shaping the way vertebrates perceive, interact, and respond to their natural surroundings. This has resulted in extraordinary examples of sensory adaptation, such as electroreception in fishes, thermoperception in snakes, and the echolocation abilities of whales and bats. The last few decades have been particularly fruitful in expanding our knowledge of how different sensory systems are structured and how they operate at the level of genes and proteins. Even more remarkable have been the advances in our understanding of the molecular mechanisms underlying sensory receptor physiology as well as the evolutionary processes driving sensory adaptation in vertebrates. This review summarizes our current knowledge of the molecular underpinnings of the diverse array of sensory systems found in vertebrates.

Photoreception

Amidst the diverse array of vertebrate sensory modalities, vision is among the best understood. At the molecular level, all of the components underlying the visual transduction cascade within the photoreceptors of the eye have been identified in model species. Comparative molecular studies have largely centered on visual pigments, light-sensitive receptors found within the highly specialized rod and cone photoreceptor cells of the retina. Visual pigments are G protein-coupled receptors that form the first step in visual transduction, and mediate vision under different light intensities. They are comprised of an opsin protein covalently bound to a vitamin A-derived chromophore in a region known as the binding-pocket. Upon stimulation by light, the chromophore isomerizes, leading to conformational changes in the opsin structure that initiates a biochemical cascade through activation of the G protein transducin.

Five classes of spectrally distinct visual pigments are present in vertebrates, and there is impressive variation in opsin complement across species (**Figure 1**). While rhodopsin (RH1) is expressed in rod cells and mediates vision under dim-light, up to four classes of opsins (RH2, SWS1, SWS2, and LWS) have been identified in cone cells, which are active under bright-light conditions and ultimately mediate color vision. Interactions between the chromophore and amino acids lining the binding-pocket affect the wavelength of maximal absorbance of a visual pigment, which may be shifted to different wavelengths of light as a result of amino acid substitutions in this region. In vertebrates, it is known that the proportion and number of spectral classes of photoreceptors in the eye, and the wavelengths at which they are maximally sensitive (λmax) can vary enormously (**Figure 1**), and are generally thought to represent adaptations to aspects of the light environment such as spectral composition and light intensity (Bowmaker, 2008).

Visual pigment genes evolved through successive duplications from a single ancestral gene. As a result, four classes of visual pigments were thought to be present in the lineage leading to ancestral vertebrates. *RH1* and *RH2* originated later in the evolutionary history of vertebrates emerging from a duplication event that probably occurred before the divergence of agnathans and gnathostomes (Pisani *et al.*, 2006). While several opsin genes were lost in cartilaginous fishes, visual pigments further diversified in bony fishes following a whole genome duplication (WGD) event. This may have spurred the evolution of additional spectrally distinct pigments, allowing bony fishes to explore more varied, spectrally complex environments, and to evolve in concert with sexually dimorphic coloration. For instance, six distinct copies of *LWS* have been found in the adult guppy (Weadick and Chang, 2007), a model system for the study of sexual selection and mate choice. Neotropical cichlids have a reduced number of cone opsin classes (Weadick *et al.*, 2012) relative to their African sister clade famous for their extravagance and diversity in coloration (Seehausen *et al.*, 2008; Miyagi *et al.*, 2012; Weadick *et al.*, 2012). However, ecological factors associated with spectrally distinct fresh water environments may underlie the evolution of *RH1* in both cichlid lineages (Schott *et al.*, 2014). In contrast, deep-sea teleost and cottoid fishes display a reduced visual pigment repertoire and blue-shifted RH1 pigments that match the wavelengths of available light at extreme depths (Hunt *et al.*, 1996; Hunt *et al.*, 2001).

In tetrapods, opsin genes underwent several major evolutionary events that resulted in extremely diverse distributions among lineages. In amphibians, frogs, and salamanders there persists two classes of rod cells, in which *RH1* and *SWS2* are expressed, whereas *LWS*, *SWS1*, and *SWS2* are expressed in cones (Hisatomi *et al.*, 1998; Hisatomi *et al.*, 1999; Ma *et al.*, 2001). Among reptiles, crocodiles and snakes have a reduced cone opsin repertoire while lizards and turtles have retained all five classes of visual genes. In birds, vision is known to have played an important role in the evolution of colored plumage and social communication. Single photoreceptor cells in birds express all five classes of vertebrate opsins, whereas LWS is also expressed in double cones. Interestingly, certain amino acid residues mediating ultraviolet (UVS) to violet sensitivity (VS) in birds may differ from those in other vertebrates. These amino acid substitutions account for large spectral tuning shifts in SWS1, the short wavelength-sensitive visual pigment, in birds, and have been suggested to underlie shifts from a VS ancestor to UVS in a number of bird lineages (Wilkie *et al.*, 2000; Carvalho *et al.*, 2007; Van Hazel *et al.*, 2013). While the influence of particular single amino acid substitutions mediating UVS and VS sensitivity in birds

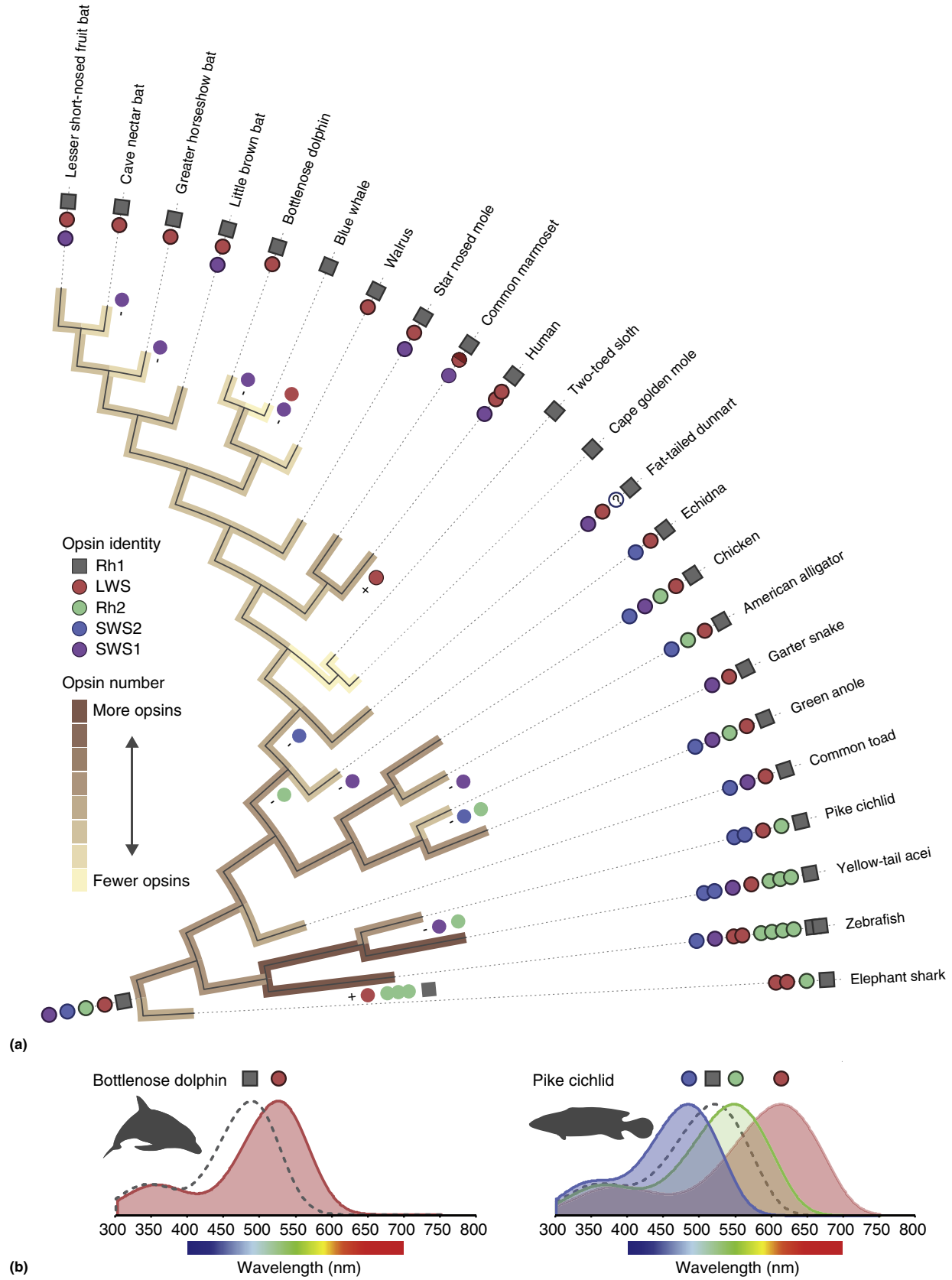

Figure 1 Diversity of vertebrate opsin complements. (a) Phylogeny depicting the gains and losses of specific opsin genes across vertebrate taxa. Branch length and color are indicative of opsin number, and duplications and losses are shown below branches. Cone opsins represented as circles, and rod opsins as squares. Cone opsins with question marks represent the yet-uncharacterized opsin classes, and bicolored cone opsins represent allelic variants providing distinct spectrally sensitive pigments. (b) Spectral sensitivity curves for the bottlenose dolphin (Fasick *et al.*, 1998) and pike cichlid (Weadick *et al.*, 2012).

has been well-characterized, in other vertebrate groups, such as mammals, these substitutions are much more variable and do not predictably shift λmax (Hauser *et al.*, 2014).

Relative to other vertebrates, mammals have a reduced set of opsin genes. While monotremes have functional copies of *LWS* and *SWS2* genes, the majority of marsupials and eutherians have only retained LWS and SWS1 cone pigments (Jacobs, 2009). Intriguingly, a third class of spectrally distinct cones has been identified in some Australian marsupials (Arrese *et al.*, 2002), although the molecular basis of this pigment remains unknown (Cowing *et al.*, 2008).

Within terrestrial eutherian mammals, nocturnality appears to play an important role in the evolution of visual pigments, particularly the *SWS1* gene, which has become pseudogenized in many nocturnal carnivores and nocturnal primates. Curiously, bats, which have long occupied the nocturnal niche, exhibit a complex distribution of the *SWS1* gene; though it is functional in most microbats, *SWS1* became pseudogenized in some megabats and a few related microbat lineages, likely as result of the evolution of refined high-duty cycle (HDC) echolocation as well as cave-roosting behavior (Zhao *et al.*, 2009a). Interestingly, RH1, which mediates dim-light vision, does not appear to have undergone adaptive evolution in bats as a consequence of the photic limited niche, although convergent evolution has occurred between lineages of highly visual bats (Shen *et al.*, 2010; Zhao *et al.*, 2009b).

Remarkably, subterranean eutherian mammals also exhibit variable opsin gene repertoires, ranging from the typical mammalian arrangement in the star-nosed mole to only a functional *RH1* copy in the Cape golden mole (Emerling and Springer, 2014). Nevertheless, a number of visual genes have become pseudogenized in phylogenetically distant lineages, which likely occurred after those lineages invaded subterranean environments (Emerling and Springer, 2014). In contrast, xenarthrans (armadillos, sloths, and anteaters), which diverged very early in the eutherian lineage and occupied a variety of ecological niches, have nonfunctional *SWS1* and *LWS* genes, probably the result of a subterranean lifestyle in the early history of the group (Emerling and Springer, 2015).

Aquatic environments are also thought to have shaped visual pigment evolution in mammals. Pinnipeds and cetaceans have independently lost functional *SWS1* genes, possibly due to relaxation of selective constraints (Levenson *et al.*, 2006). *LWS* is also pseudogenized in most baleen whales and in some deep-diving toothed whale lineages whose representatives inhabit depths in which light is dim and dominated by short-wavelengths (Meredith *et al.*, 2013). Curiously, the RH1 pigment is blue-shifted in most cetacean species (Bischoff *et al.*, 2012), showing functional variation that is likely associated with diving depth.

Unlike the majority of mammals, diurnal primates have regained a middle-wavelength sensitive (MWS) class of cone opsin through evolutionary modification of the *LWS* gene. While this occurred through gene duplication in Old World primates (Nathans *et al.*, 1986), in New World primates it evolved through a polymorphism of the *LWS* gene by allelic variants on the X-chromosome (Williams *et al.*, 1992). Thus, MWS is present in both male and female Old World monkeys, whereas only heterozygous female New World monkeys have a copy of the MWS pigment (Bowmaker, 2008). Opsin polymorphism linked to the X-chromosome has also been identified in diurnal and cathemeral prosimians (Jacobs *et al.*, 2002; Veilleux and Bolnick, 2009). Remarkably, MWS also evolved in Neotropical howler monkeys (Jacobs *et al.*, 1996), though through unequal crossover that allocated two different alleles of the polymorphic New World gene onto a single chromosome (Hunt *et al.*, 1998). Although the adaptive significance of a three-cone opsin system remains unclear, many have argued that it improves the detection and assessment of ripe fruits (Sumner and Mollon, 2000a,b), new leaves (Dominy and Lucas, 2001), and predators (Pessoa *et al.*, 2014) through a better discrimination of red and green signals.

Chemoreception

Odorant Perception

Although not as well studied as vision, the molecular mechanisms of odorant perception are starting to be revealed in detail. Three classes of odorant GPCRs have been identified in vertebrates: olfactory receptors (OR), vomeronasal receptors (V1R and V2R), and the recently discovered trace amine associated receptors (TAAR). Typically, ORs are expressed in the main olfactory epithelium (MOE), and are activated by volatile odorant molecules that enter the olfactory cavity, whereas VNRs are expressed in the vomeronasal organ (VNO) and respond to social odors such as pheromones (Niimura, 2012). The recently identified TAARs are also expressed in the MOE and have the ability to recognize volatile amines present in urine and molecules linked to stress (Liberles and Buck, 2006).

Olfactory receptors

Compared to other sensory receptors, which are tuned to respond to a specific stimulus, OR function in a combinatorial manner: multiple ORs may be sensitive to a single odorant or a single OR may detect multiple odorants. This makes the study of olfactory mechanisms very complex. Two types of ORs have been identified in vertebrates and are predicted to detect odorants with different chemical properties. While ORs that respond to water-soluble molecules are present in fishes and amphibians, ORs sensitive to airborne components have diversified predominantly in tetrapods. Interestingly, amphibians also exhibit a particular group of ORs that likely detect odorants that dissipate both in the air and water, such as alcohols (Niimura, 2012).

In mammals, *OR* genes form a surprisingly high proportion of the genome, but also contain a great deal of pseudogenization. It is broadly accepted that the numerous families of *OR* genes evolved through the process of birth-and-death evolution, in which gene duplication followed by mutation may result in fixation or deletion in the genome, varying the number of functional genes as well as pseudogenes (Nei *et al.*, 2008). Interestingly, expansion and retraction of the mammalian *OR* gene repertoire is thought to be closely associated with ecological factors. For instance, mammals that occupy similar ecological niches but are phylogenetically distant exhibit similar patterns of contraction or expansion of the OR gene families, such as in aquatic, terrestrial and flying groups

(Hayden *et al.*, 2010). Among bats, several *OR* gene families have been linked to frugivory in New World and Old World lineages, indicating another example of niche specialization in the *OR* gene repertoire (Hayden *et al.*, 2014). Primates, on the other hand, have *OR* gene repertoires that are markedly reduced compared to other terrestrial mammals, which has been linked to a trade-off between olfaction and the presence of three classes of cone opsins in some lineages (Gilad *et al.*, 2004; but see Matsui *et al.*, 2010).

Vomeronasal receptors

Unlike ORs, vomeronasal receptors are encoded by only two multigene families, *V1R* and *V2R*, and play an important role in pheromone detection. Upon activation, V1Rs and V2Rs couple with specific G proteins, which leads to the opening of TRPC2 channels, ultimately resulting in signal transduction (Nei *et al.*, 2008). While VNRs are expressed in the vomeronasal system, which is exclusive to tetrapods, genes of the vomeronasal-signaling pathway are expressed in the olfactory epithelium of fishes (Grus and Zhang, 2006).

V1R genes and *Trpc2* genes evolved early in the vertebrate lineage and are less diverse compared to *V2R* genes, which originated after the divergence of agnathans and gnathostomous (Grus and Zhang, 2009). Though *V1R* genes are under strong purifying selection among several fish lineages, sites at putative ligand-binding regions are under positive selection within African cichlids, suggesting an adaptive function of V1Rs for reproductive behavior or social communication (Nikaido *et al.*, 2014). Contrary to *V1R*s, *V2R* genes are much more diverse in teleost fishes and play important roles in feeding behavior and social communication. Although the ligand-binding region of V2Rs exhibits impressive sequence variation within several fish lineages, suggesting adaptation to different ligands, studies have failed to detect signals of positive selection, indicating that sequence variation likely emerges from relaxation of selective constraints or genetic drift (Nikaido *et al.*, 2013).

The transition from aquatic to terrestrial environments in tetrapod evolution was followed by the emergence of the VNO resulting in enormous expansion of VNR genes in some tetrapod lineages as well as numerous pseudogenization events in others. Frogs, which have a rudimentary VNO, display the largest *V2R* gene repertoire among tetrapods and only small number of *V1R* genes. In contrast, no *V1R* genes are observed in birds and the VNO is absent (Grus *et al.*, 2005). Within mammals, there is great variation in VNR gene repertoire. Generally, the *V1R* gene family expanded in lineages that exhibit a morphologically complex VNO, such as in monotremes and rodents where adaptive evolution likely contributed to gene diversification. Nevertheless, no functional V1Rs are observed in several bats, cetaceans, and humans, due to pseudogenization of *Trpc2* (Liman and Innan, 2003; Young *et al.*, 2010; Zhao *et al.*, 2011). Although most *V1R* genes also became pseudogenized in Old World monkeys, several functional copies are observed in New World monkeys and are even more numerous in prosimians. Interestingly, sites at the ligand-binding region of V1Rs in prosimians are under strong positive selection, indicating adaptation of the *V1R* gene repertoire and emphasizing the ecological importance of the VNS in this primate lineage (Hohenbrink *et al.*, 2012; Yoder *et al.*, 2014). Comparatively, *V2R* genes are less diverse and became pseudogenized in several mammalian lineages, likely through relaxation of selective constraints (Shi and Zhang, 2007).

Gustatory Perception

Taste receptors (TRs) are encoded by two multigene families that are in close phylogenetic proximity to *VNR* genes. TRs are expressed in cells located in taste buds on the tongue and palate and respond to organic compounds associated to the perception of sweet, umami (savory), and bitter taste. Similar to other GPCRs, stimulation of TRs mediates the activation of receptor-specific G proteins, leading to a biochemical cascade that ultimately results in the gating of a transduction channel, in this case TRPM5 (Chandrashekar *et al.*, 2006). Conversely, the perception of salty and sour taste is mediated by direct entry of Na^+ and H^+ in taste receptor cells (TRC) through specific ion channels, although molecular mechanisms underlying these gustatory modalities have just begun to be elucidated (Liman *et al.*, 2014). While great variability in TRs is observed among vertebrates, shifts in gene copies numbers, and pseudogenization of *TR* genes generally reflect adaptation to ecological niches and diet, as a way to better assess the quality and nutritional value of food.

T1Rs mediate the perception of umami and sweet taste through different sets of heterometric receptors. Subunits T1R1 and T1R3 form a receptor that detects L-amino acids and nucleotide enhancers (IMP, GMP, AMP), which are perceived as a savory taste known as umami (Nelson *et al.*, 2002), whereas the T1R2 + T1R3 heterodimer responds to simple sugars, artificial sweeteners, and D-amino acids, mediating sweet taste (Nelson *et al.*, 2001). *T1R* genes evolved in the ancestor of cartilaginous and bony fishes, along with the taste-transduction channel gene *Trpm5*. Whereas T1R1 and T1R3 are encoded by single copy genes in bony fishes, T1R2 further diversified through multiple gene duplications (Shi and Zhang, 2006). Interestingly, sites at ligand-binding region of T1R2 are positively selected, indicating that the duplicate genes likely adapted to respond to different tastants (Hashiguchi *et al.*, 2007). Relative to fishes, great divergence is observed in *T1R* genes among tetrapods. Amphibians, for instance, have lost all *T1R* genes and detection of amino acids is mediated by V2Rs, whereas *T1R2* genes were lost in all avian lineages, resulting in inability to perceive sweets (Shi and Zhang, 2006). Intriguingly, this ability was recovered in nectar-eating hummingbirds. In this group, the T1R1 + T1R3 umami receptor has been repurposed by substitutions at the ligand-binding domain of the T1R3 subunit, acquiring a new adaptive function (Baldwin *et al.*, 2014). Similarly, diet specialization and diverse ecological niches act as strong selective pressures on the evolution of T1Rs in mammals. The giant panda is related to carnivorous bears, but lacks the ability to perceive amino acids through umami receptors due to pseudogenization of *T1R1* gene, which occurred concomitantly to a dietary switch to bamboo in the evolution of the species (Zhao *et al.*, 2010b), though other molecular adaptations might also be involved in diet specialization in pandas (Jin *et al.*, 2011). Conversely, *T1R2* became pseudogenized in several carnivores, rendering them unable to detect carbohydrates, which is likely a result of a dietary specialization to obligatory carnivory (Jiang *et al.*,

2012). Curiously, *T1R2* has also undergone pseudogenization in hematophagous bats (Zhao *et al.*, 2010a), and loss of umami taste is widespread among bats, regardless of diet (Zhao *et al.*, 2012). The most striking example of gustatory loss is observed in sea lions and cetaceans, in which feeding behavior and niche specialization contributed to relaxation of selective constraints, resulting in pseudogenization of all *T1R* genes (Jiang *et al.*, 2012; Feng *et al.*, 2014; Zhu *et al.*, 2014).

Unlike other chemosensory receptors, several *T2R* genes are expressed at different levels in a single TCR, rendering taste cells sensitive to a variety of bitter molecules, which maximizes the ability to detect potentially harmful substances, characterized by bitter taste (Yarmolinsky *et al.*, 2009). Compared to T1Rs, T2Rs originated much later in the evolution of vertebrates, just before the divergence of teleosts and tetrapods, in which T2Rs became more diverse through lineage-specific gene duplications (Grus and Zhang, 2009). Amphibians, reptiles, and mammals have large *T2R* gene repertoires, though fewer copies are observed in some avian lineages (Dong *et al.*, 2009). Interestingly, T2Rs in some birds are broadly tuned to a variety of agonists, which compensates for the low diversity of bitter receptors (Behrens *et al.*, 2014). Within mammals, reintrogression into aquatic environments accompanied by a switch in feeding ecology led to pseudogenization of *T2R* genes in sea lions (Jiang *et al.*, 2012) and cetaceans (Jiang *et al.*, 2012; Feng *et al.*, 2014; Wang *et al.*, 2014). In terrestrial mammals, diet plays a major role in the evolution of T2Rs. Herbivorous and omnivorous mammals generally exhibit a larger *T2R* repertoire compared to carnivorous mammals, as a way to detect harmful bitter tasting compounds in plants. However, a large proportion of the *T2R* repertoire of cows and horses became pseudogenized, likely the by-product of extensive artificial selection in these lineages (Dong *et al.*, 2009).

Auditory Perception

Prestin (SLC26A5) is a voltage-sensitive membrane protein expressed in the outer hair cells (OHC) of the auditory system that underlies hearing sensitivity and frequency range through amplification of signal input. This is particularly relevant for bats and whales, in which the ability to acoustically sense the environment, known as echolocation, convergently evolved. The *Prestin* gene has undergone parallel evolution in echolocating bats and continued to undergo adaptive selection in the lineage leading to the more derived HDC echolocating bats (Li *et al.*, 2008). *Prestin* is also thought to have undergone adaptive evolution in toothed whales, suggesting that adaptive changes in *Prestin* are linked to the evolution of echolocation in this group (Liu *et al.*, 2010b). Remarkably, it appears that *Prestin* evolved convergently in HDC bats and echolocating cetaceans, as result of strong selective constraints to detect high-frequency sounds in both lineages (Li *et al.*, 2010; Liu *et al.*, 2010a). More recently, substitutions at two amino acid sites have been identified as critical for functional convergence among high-frequency echolocating mammals (Liu *et al.*, 2014). In addition to *Prestin*, several genes involved in OHC function (*Kcnq4*, *Pjkv*, *Cdh23*, *Pcdh15*), maturation (*Tmc1*) and signal transduction (*Otof*) also experienced adaptive selection and convergent evolution in echolocating bats and dolphins to account for high-frequency hearing (Liu *et al.*, 2011; Davies *et al.*, 2012; Liu *et al.*, 2012; Shen *et al.*, 2012a).

Electroreception and Electrogenesis

The ability to detect electric fields is conferred by electroreceptors, which exhibit a complex pattern of evolution across vertebrate lineages and achieved greater specialization following the evolution of the electrogenic organs in some taxa. Electroreceptors transduce electric signals into action potentials that are processed in the central nervous system, and can convey information of relevance for social communication, navigation, hunting, and defense (Albert and Crampton, 2005). Electroreceptors evolved in the common ancestor of all vertebrates as a submodality of the lateral line system, which also encodes mechanosensory information in fishes and amphibians through specialized neuromast hair cells. In some amphibians, cartilaginous fishes, and non-teleost bony fishes, electroreceptors are organized into ampullary organs that respond to passive low-frequency environmental electric fields (Jørgensen, 2005).

Electroreceptors were lost in amniote and teleost fish ancestors. Air is a poor conductor of electricity, explaining the absence of electroreceptivity in terrestrial taxa. Within amniotes, electroreception was independently regained in aquatic monotremes and river dolphins through neofunctionalization of mechanosensory organs (Pettigrew, 1999; Czech-Damal *et al.*, 2012). Multiple lineages of teleost fishes have independently regained electroreception. In two orders of electrogenic fishes, the South American Gymnotiformes and African Mormyriformes, a sophisticated electrosensory system is mediated by a second class of tuberous electroreceptors. These electroreceptors are sensitive to the higher frequency of self-generated electric fields, enabling fishes to covertly communicate and navigate using electric fields. The waveforms of the electric organ discharges (EODs) vary substantially across gymnotiform and mormyriform species. Avoidance of electroreceptive predators and sexual selection have likely contributed to the evolution of EOD diversity (Stoddard, 1999; Crampton *et al.*, 2013).

The waveform of an EOD is modulated by the rate of depolarization and repolarization of the stacked electrocyte cells forming the electric organ, which is in turn controlled by ion channels lining the anterior and posterior faces of each individual cell. The *scn4aa* and *scn4ab* genes (duplicates of the mammalian *Scn4a* gene), respectively encode the sodium channel proteins Nav1.4a and Nav1.4b, which play a role in determining properties of the EOD (Ferrari *et al.*, 1995). In most teleost fishes, *scn4aa* and *scn4ab* are expressed in muscle; however, in most Gymnotiformes and Mormyriformes, *scn4aa* is exclusively expressed in the electric organ (**Figure 2**; Zakon *et al.*, 2006). This phylogenetic pattern of expression is accompanied by increased rates of molecular evolution and elevated dN/dS values in *snc4aa* from electrogenic fish lineages (**Figure 2**; Arnegard *et al.*, 2010), likely due to natural selection associated with diversification of EODs. Sites found to be positively selected in these lineages likely affect the kinetics of channel activation and inactivation, and

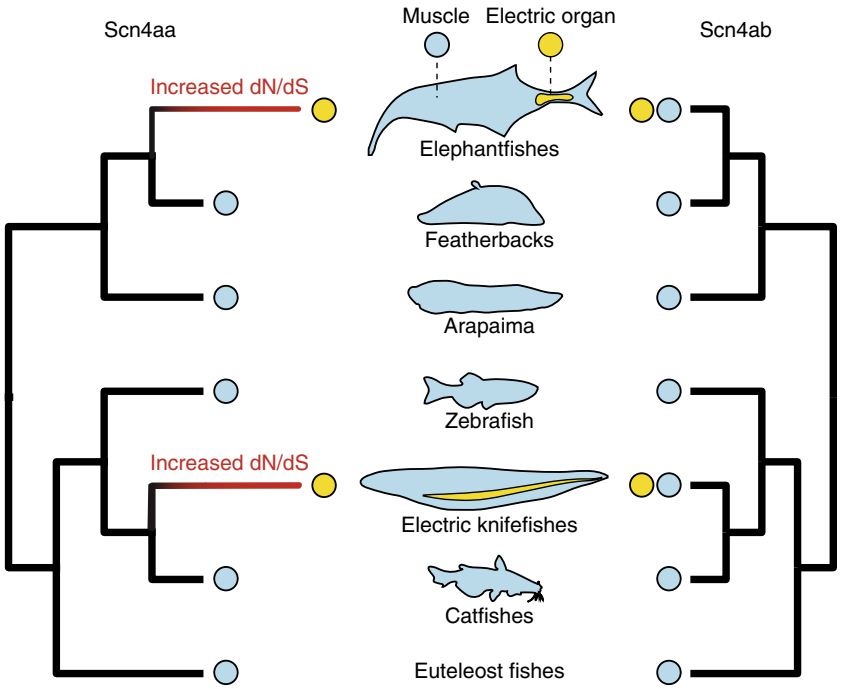

Figure 2 Expression of *scn4aa* and *scn4ab* in teleost fishes. Branch lengths and color are schematic representations of nonsynonymous to synonymous rate variation. Blue and yellow circles identify tissue type where the *scn4a* gene is expressed. Based on data presented in Zakon *et al.* (2006).

thereby EOD waveform parameters. Thus, convergent neofunctionalization of a duplicated sodium channel gene likely contributed to the evolution and diversification of independently derived electrosensory systems.

Thermoreception

The TRP superfamily of ion channels, expressed in neurons of the somatosensory system, responds to temperature changes in peripheral tissues. Remarkably, some *TRP* genes have gained a new function in specialized organs on the faces of snakes and bats, conferring infrared sensing abilities that are crucial for food acquisition. The TRPA1 calcium channel, expressed in the pit organs of snakes, detects infrared radiation, which is transduced into electric signals that are processed in the optic tectum, resulting in a 'thermal vision' (Gracheva *et al.*, 2010). This ability evolved independently in pythons, boas, and venomous snakes through adaptive selection of sites at different domains of the TRPA1 channel, becoming crucial for prey detection and predator avoidance (Geng *et al.*, 2011). Interestingly, infrared perception also evolved in vampire bats, though through a different mechanism. In the leaf pit organs located on the face of vampire bats, a shorter splice variant of the *Trpv1* gene is expressed. TRPV1-S is truncated at the C-terminus end, lowering the threshold to which the leaf pits respond, allowing vampire bats to detect areas where blood flows closer to the skin (Gracheva *et al.*, 2011).

Concluding Remarks and Future Research

Studies throughout the last decade have greatly developed our understanding of the diverse molecular mechanisms underlying the remarkable sensory adaptation observed in vertebrates. This outstanding progress has been particularly fruitful in identifying molecules that act as sensory receptors responsible for transducing a variety of environmental stimuli into neural signals that ultimately result in physiological and behavioral responses. Interestingly, the nature of vertebrate somatosensory mechanoreceptors that mediate our sense of touch remains largely puzzling, although much progress has been made in invertebrates (Schuler *et al.*, 2015). It is also important to note that although vision and particularly visual pigments have been studied in a comparative context (Shen *et al.*, 2012b; Lin *et al.*, 2013; Shen *et al.*, 2013; Emerling and Springer, 2014; Emerling and Springer, 2015), other sensory systems have not received the same degree of attention, and remain largely understudied from a molecular evolutionary perspective. The recent developments in next generation sequencing technologies provide an unprecedented opportunity to not only identify candidate genes that may play a role in sensory transduction pathways (Gracheva *et al.*, 2010, 2011; Gerhold *et al.*, 2013), but also to expand our understanding of the complex mechanisms underlying vertebrate sensory perception. Finally, future research should also be devoted to the intricate interactions among different sensory systems, many of which have likely coevolved to result in the exquisite examples of sensory adaptation observed in nature.

See also: Genome Organization, Evolution of. Systems in Evolutionary Systems Biology

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Sequential Speciation

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Glossary

Cascading or sequential speciation A process by which ecological speciation of one organism creates a new niche for other organisms to potentially adapt to, leading to their speciation.

Co-speciation The simultaneous and interdependent diversification of host and parasite lineages.

Ecological speciation A process by which barriers to gene flow evolve between populations due to ecologically based divergent natural selection between environments or habitats.

Host races Geographically cooccurring populations that are partially reproductively isolated due to divergent ecological selection associated with differential host use.

Inquiline A parasitic relationship between two organisms whereby one species exploits the living space of another.

Niche construction/engineering Modification to the environment by one species in a manner that affects resource availability for other species and that can have lasting effects on the fitness of populations.

Parasitoid A specialized insect that feeds and develops within or on another insect host during the immature stages of its life cycle, typically killing its host in the process.

Reproductive isolation A reduced ability of individuals from different populations to court, mate, or produce viable, fertile offspring.

The Biodiversity Question and Sequential Speciation

'What determines species diversity?' is one of the most important questions in biology (Pennisi, 2005). Two key considerations in this regard involve the ecological adaptation of organisms to different environments and the coevolutionary interplay between organisms themselves. The hypothesis of sequential or cascading speciation attempts to unify these two considerations. Specifically, sequential speciation contends that as organisms diverge they create new niche dimensions for other organisms to exploit, and that if these other organisms experience novel selection pressures, they may speciate in kind. Here, we discuss how the idea of sequential speciation has been generally applied to a number of different questions in biology. We then explain how sequential speciation differs from strict co-speciation and examine the conditions thought to favor sequential speciation. Phytophagous (plant-eating) insects and their natural enemies represent systems where sequential speciation has been most commonly documented. As case studies, we examine evidence from two phytophagous insects–natural enemy systems where sequential speciation has been shown to be occurring. We then discuss several systems where a hypothesis of sequential speciation is not supported.

General Implications of Sequential Speciation

The idea that diversity begets diversity has been applied to several areas in biology. In paleontology, niche construction has been argued to help trigger diversification following mass extinctions, possibly facilitating major diversification events such as the Cambrian explosion (Erwin, 2005, 2008; **Figure 1(a)**). In community ecology, species richness is a strong predictor of diversification rates (Emerson and Kolm, 2005; **Figure 1(b)**). In systematics, sequential speciation may help explain why some clades of plant-feeding insects are more speciose than sister groups of non-plant feeders (Mitter *et al.*, 1988; **Figure 1(c)**). In each of these cases, diversification of organisms into new habitats and life histories may increase the rate of speciation among other species with whom they interact, thereby increasing total species richness in a community.

Distinguishing Sequential Speciation from Strict Co-speciation

Sequential speciation requires that divergent ecological selection pressures cascade across trophic levels to cause linked patterns of diversity. However, sequential speciation is just one process that can increase clade richness across communities. In this regard, it is important to distinguish sequential speciation from strict co-speciation. In co-speciation, concordant divergence across trophic levels is due to (1) host and parasite populations being jointly geographically isolated, resulting in parallel allopatric speciation, and/or (2) parasites being vertically transmitted and lacking a sexually reproductive adult stage independent of the host (de Vienne *et al.*, 2013). Strict co-speciation is therefore not driven by the creation of new biological niches that result in ecological diversification (i.e., by coevolutionary processes), but rather by the concordant geographic and reproductive separation of host and parasite populations (Feder and Forbes, 2010; de Vienne *et al.*, 2013). Distinguishing sequential speciation from strict co-speciation therefore requires study systems having appropriate natural histories (e.g., those possessing free-living reproductive life stages), known biogeographies (to rule out non-ecologically based reproductive isolation as the primary cause for divergence), and well-resolved phylogenies documenting histories of parasite host shifting. An additional consideration for phylogenetic tests of sequential speciation is that it must be shown that a degree of divergent ecological adaptation

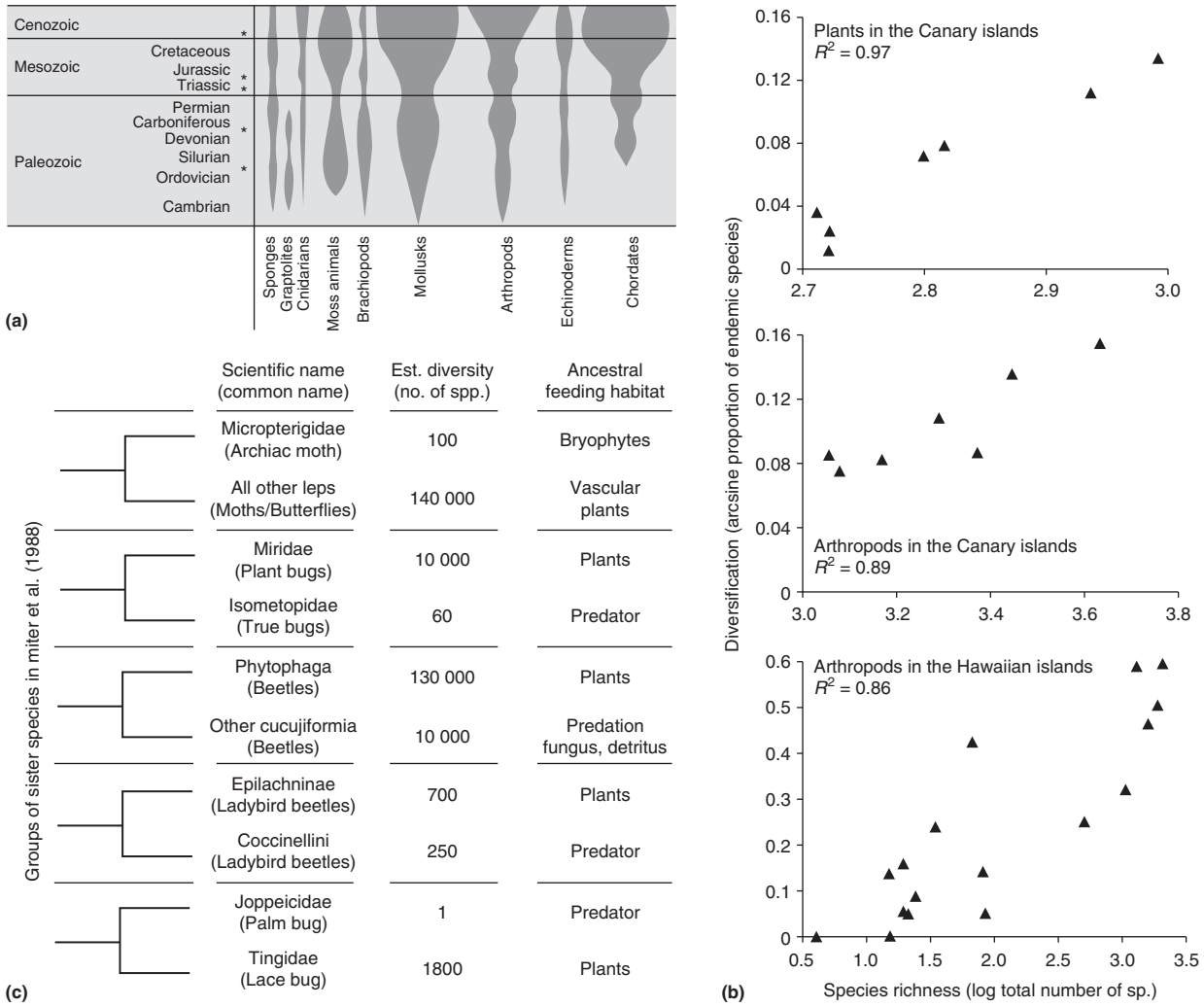

Figure 1 Patterns of biodiversity potentially explained by sequential speciation. (a) Adaptive radiations following the last five major mass extinctions events (indicated by asterisks). Adapted with permission from Purves, W.K., Gordon, H., Raig, H.H.C., 1994. Life: The Science of Biology (fourth ed.). Sunderland, MD: Sinauer. (b) Significant relationship between species richness and diversification rates of taxa in tropical climates. Adapted with permission from Emerson, B.C., Kolm, N., 2005. Species diversity can drive speciation. Nature 434, 1015–1017. (c) Clades of phytophagous insects are more speciose than sister clades of non-plant feeders. Diversity (as indicated by number of species) for five different pairs of sister taxa (scientific and common name given) and their inferred ancestral feeding habitat. Modified from Mitter, C., Farrell, B., Wiegmann, B., 1988. The phylogenetic study of adaptive zones-has phytophagy promoted insect diversification? American Naturalist 132, 107–128.

accompanied (was required for) the host shift, and that parasites are not merely shifting between geographically separated hosts.

Conditions Conducive for Sequential Speciation

For organisms like phytophagous insects and their natural enemies, both of which partition and experience resources on a fine scale, the effects of new niche construction and engineering may be especially prone to cascading through the ecosystem (Bush, 1993). Not all insect systems possess attributes that are conducive to sequential speciation, however. The conditions most favorable for sequential speciation are similar to those important for host race formation via host plant shifting in phytophagous insects (Berlocher and Feder, 2002; Dres and Mallet, 2002 and sources therein). Specific conditions that should promote host race formation and sequential speciation between two or more trophically linked organisms are summarized by Abrahamson and Blair (2008) and Hood *et al.* (2015) and listed (with some modification) below:

1. Recent colonization of a new environment resulting in fitness tradeoffs that reduce gene flow and generate reproductive isolation between populations.
2. Populations at least partially overlap in their geographic ranges such that the opportunity for encountering alternative hosts/habitats exists, i.e., is not totally impeded by physical barriers or distance.
3. Courtship and mating occur on or near the host, linking host choice and mate choice, thereby generating prezygotic

reproductive isolation. A degree of host preference for natal habitats that is at least partly genetically controlled must also exist.

4. Phenology (life history timing) is associated with host habitat such that reproductive overlap between populations is temporally reduced, resulting in prezygotic isolation. This generally requires a univoltine (one generation per year) life cycle with an adult life span that is relatively short compared to the phenological difference between hosts (i.e., individuals cannot span the entire range of seasonal availability of alternate hosts).

Evidence Supporting Sequential Speciation

Empirical evidence for sequential speciation in phytophagous insects and their natural enemies is mixed, with results ranging from apparently negative (Cronin and Abrahamson, 2001; Baer *et al.*, 2004; Althoff, 2008; Lozier *et al.*, 2009; Dickey and Medina, 2012), to uncertain (Henter and Via, 1995; Althoff and Thompson, 2001), to probable (see several sources in Abrahamson and Blair, 2008). Below, we present results for two systems where evidence strongly supports the sequential speciation hypothesis.

Case 1: One Host, One Natural Enemy: The Goldenrod Stem Galler, *Eurosta solidaginis*, and the Tumbling Flower Beetle, *Mordellistena convicta*

Speciation of host

The fly *Eurosta solidaginis* (Diptera: Tephritidae) induces galls on the stems of two species of goldenrods, *Solidago altissima* and *Solidago gigantea*, that geographically overlap in the northern United States (Abrahamson *et al.*, 2003). The shift of *E. solidaginis* from its ancestral host *S. altissima* to *S. gigantea* is a classic example of host race formation via host plant shifting. Several factors contribute to reproductive isolation between host races of *E. solidaginis*. First, *E. solidaginis* emerges from galls induced on *S. gigantea* 10–14 days earlier than galls induced on *S. altissima* (Craig *et al.*, 1993). Second, *E. solidaginis* flies mate on the buds of their respective host plants and exhibit oviposition preferences for stems of their natal host plants. Moreover, if mating and oviposition occur between host races, F1 hybrids and backcrossed individuals suffer a reduction in fitness due to a decreased frequency of successful gall induction on either host plant (Craig *et al.*, 1997). Thus, the link between mate choice, habitat choice, and reduced hybrid fitness ecologically isolate populations of *E. solidaginis* on alternative hosts. Likely a result of these isolating barriers, the two *Eurosta* races exhibit host-associated genetic differentiation at five allozyme loci (Warring *et al.*, 1990) and posses distinct, although not highly diverged, mtDNA haplotypes on *S. altissima* versus *S. gigantea* (Smith *et al.*, 2002).

Sequential speciation of natural enemy

The formation of host races of the tumbling flower beetle, *Mordellistena convicta* (Coleoptera: Mordellidae) represents one of the only examples of sequential speciation that does not involve a host–parasitoid relationship. Beetles feed as inquilines on the gall tissue induced by *E. solidaginis* on goldenrods, resulting in levels of fly mortality >70% (Abrahamson and Blair, 2008). Evidence suggests that host-associated populations of beetles have ecologically and genetically diverged

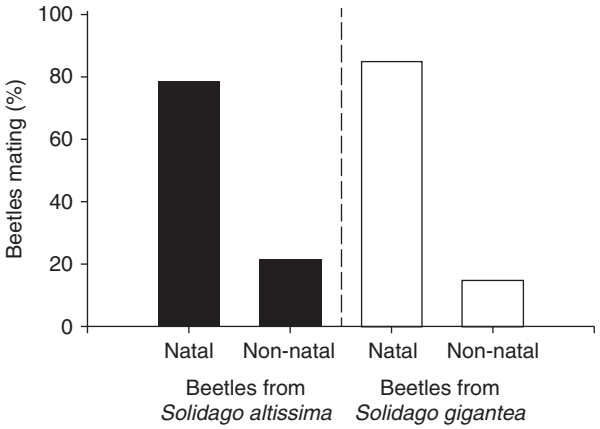

Figure 3 Percent *Mordellistena convicta* originating from *Solidago altissima* (black bars) and *Solidago gigantean* (white bars) goldenrod galls mating with individuals reared from natal and non-natal hosts (Eubanks *et al.*, 2003).

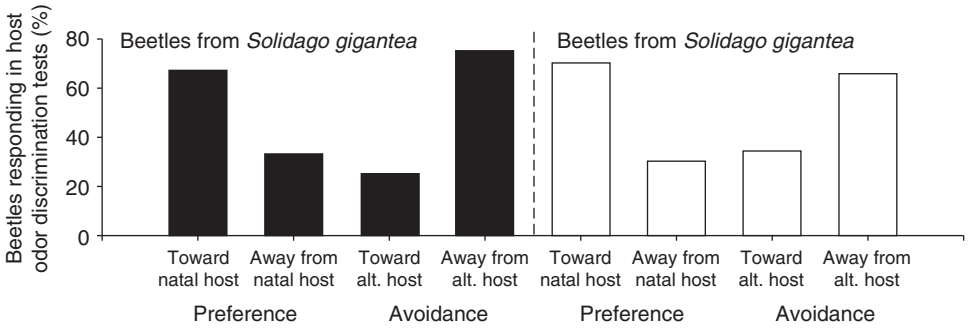

Figure 2 Percent of *Mordellistena convicta* originating from *Solidago altissima* (black bars) and *Solidago gigantea* (white bars) goldenrod galls that prefer and avoid the surface volatiles of galls in host odor discrimination tests. Adapted with permission from Rhodes, B.C., Blair, C.P., Takahashi, M.K., Abrahamson, W.G., 2012. The role of olfactory cues in the sequential radiation of a gall-boring beetle, *Mordellistena convicta*. Ecological Entomology 37, 500–507.

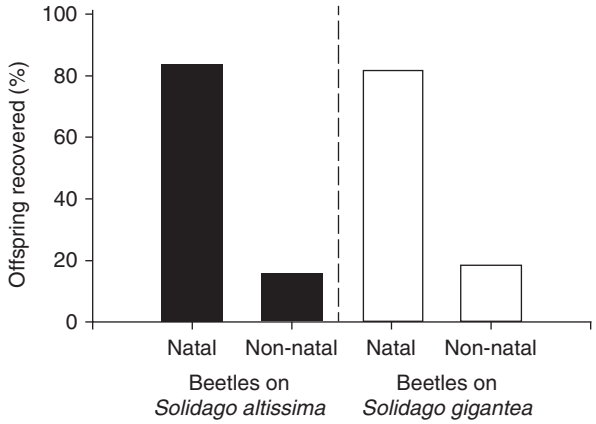

Figure 4 Percent *Mordellistena convicta* offspring originating from *Solidago altissima* (black bars) and *Solidago gigantean* (white bars) recovered the following generation after being reared on natal (control) and non-natal host galls (Eubanks *et al.*, 2003).

due to differential gall formation on alternative host plants. Modest allele frequency differences exist at five allozyme loci between sympatric *M. convicta* inhabiting *S. altissima* and *S. gigantea* galls (Blair *et al.*, 2005). Additionally, a number of host-related ecological adaptations likely contribute to reproductive isolation in *M. convicta*. First, beetles prefer the volatiles emitted from natal galls and avoid non-natal galls in host odor discrimination tests (Rhodes *et al.*, 2012; **Figure 2**). Second, beetles exhibit sexual isolation via mate choice. Adults prefer to mate with individuals from their natal rather than non-natal host plant (Eubanks *et al.*, 2003; **Figure 3**). Third, when host races of *M. convicta* are exposed to the alternative gall habitat and allowed to oviposit, adult emergence the following generation is reduced (Eubanks *et al.*, 2003; **Figure 4**). The reduction in emergence could be due to either reduced survivorship and/or reduced oviposition into non-natal galls; regardless this result implies a degree of host specialization and ecological reproductive isolation. However, the site of mating of *M. convicta* is not known, so it remains to be

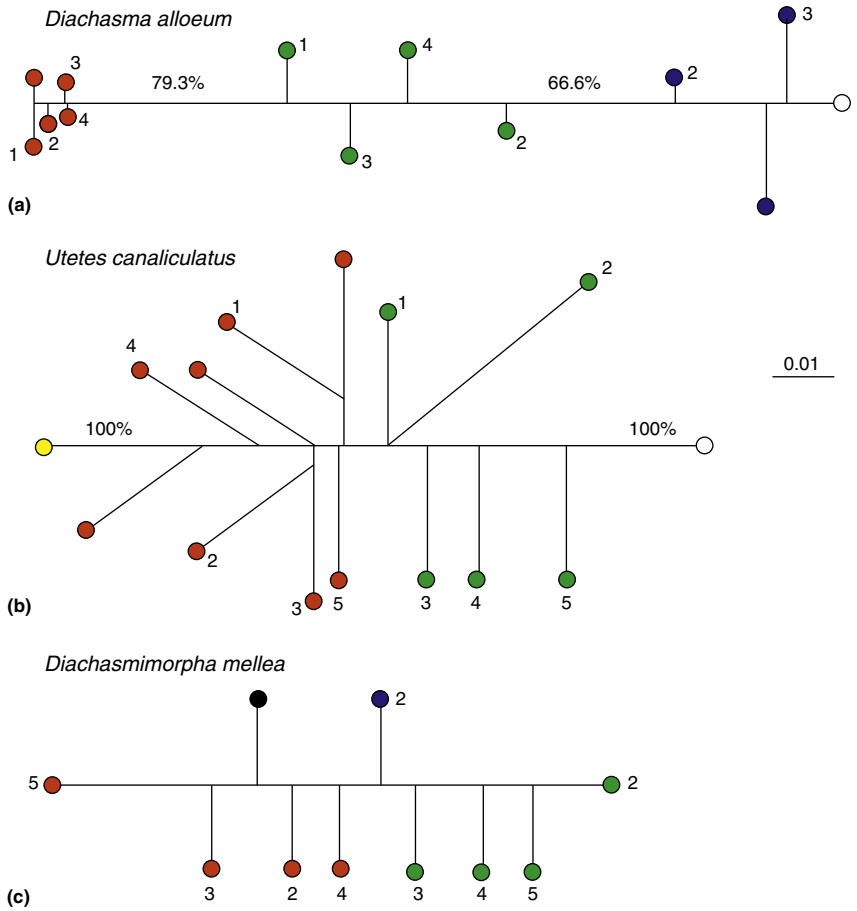

Figure 5 Genetic distance network based on microsatellite loci allele frequency depicting the relationship among (a) *Diachasma alloeum*, (b) *Diachasmimorpha mellea,* and (c) *Utetes canaliculatus* populations attacking apple (green), hawthorn (red), flowering dogwood (yellow) blueberry (blue), snowberry (white), and black cherry (black) flies. The black cherry infesting fly, *Rhagoletis cingulata*, is a more distantly related species to the *Rhagoletis pomonella* group. Populations with the same number represent wasp from the same field site. Adapted with permission from Forbes, A.A., Powell, T.H.Q., Stelinski, L.L., Smith, J.J., Feder, J.L., 2009. Sequential sympatric speciation across trophic levels. Science 323, 776–779 and Hood, G.R., Forbes, A.A., Powell, T.H.Q., *et al.*, 2015. Sequential divergence and the multiplicative origin of community diversity. Proceedings of the National Academy of Sciences of the United States of America. Online early. doi:10.1073/pnas.1424717112.

determined if the beetles experience some degree of prezygotic isolation due to differences in host preference (Abrahamson and Blair, 2008). In addition, *M. convicta* races do not seem to be temporally isolated, as emergence times for adults differ by only 1.1 day (Eubanks *et al.*, 2003; Blair *et al.*, 2005). Consequently, ecological specialization in *M. convicta* appears to be driven by host plant related survivorship and oviposition preferences, with some additional prezygotic isolation due to differential mate choice that is related to host-origin, but independent of host choice.

Case 2: One Host, Many Natural Enemies: The Apple Maggot Fly, *Rhagoletis pomonella*, and Three Parasitoids: *Diachasma alloeum*, *Diachasmimorpha mellea*, and *Utetes canaliculatus*

Speciation of host

The *Rhagoletis pomonella* (Diptera: Tephritidae) sibling species group is a model for ecological speciation-with-gene-flow. The group consists of the hawthorn (*Crataegus* spp.) and apple (*Malus domestica*) infesting host races of *R. pomonella*, as well as several sibling species including the undescribed flowering dogwood fly (host: *Cornus florida*), *Rhagoletis mendax* (host: blueberry; *Vaccinium* spp.) and *Rhagoletis zephyria* (host: snowberry; *Symphoricarpos* spp.). The close morphological similarity, distinct host affiliations, and broadly overlapping geographic ranges of these flies imply that they radiated by shifting and adapting to new host plants in sympatry. In particular, the recent shift (<160 ya) of *R. pomonella* from its native, ancestral host hawthorn to introduced domesticated apple is often cited as an example of incipient ecological speciation in action (Berlocher and Feder, 2002).

Two ecological adaptations are important in generating reproductive isolation among *R. pomonella* taxa. First, each of the flies attack a different, nonoverlapping set of host plants that all fruit at different times of the year (Dambroski and Feder, 2007). *Rhagoletis* uses host fruit as a rendezvous site for mating and oviposition. Eggs hatch and fly larvae feed in host fruit for 2–4 weeks. When fruit abscises, larvae leave fruit, burrow into the ground, and form puparia. Flies overwinter in a facultative diapause, eclosing the following summer as adults to complete their univoltine life cycle. Because adult longevity is short (~28 days), flies must time their diapause phenology to match the fruiting time of their host plant. As different hosts plants fruit at different times, *Rhagoletis* flies eclose at different times of the year, resulting in allochronic premating isolation, as well as a degree of post-zygotic isolation due to migrants and hybrids having diapause phenotypes suboptimal for attacking non-natal hosts (Feder *et al.*, 1994; Berlocher, 2000).

The second factor generating ecologically-based reproductive isolation in *Rhagoletis* is host-specific mating on or near the host fruit, coupled with host fruit discrimination. Differences in host preference lead to assortative mating between fly populations, resulting in prezygotic isolation. The most important long range cue that flies use to find and discriminate among alternative hosts is the volatile compounds emitted from the surface of ripening fruit (Linn *et al.*, 2003). Flies prefer the volatiles of their natal host fruits and avoid non-natal fruit odors (Linn *et al.*, 2003; Forbes *et al.*, 2006). Mark recapture studies have estimated that host fruit choice and habitat specific mating reduce migration 4–6% per generation

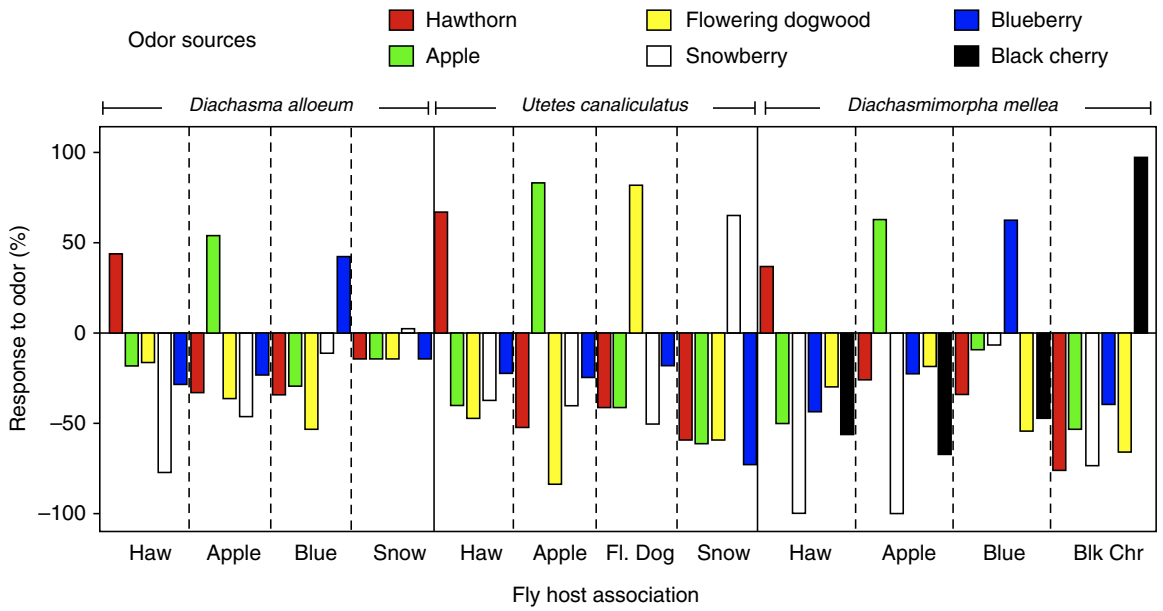

Figure 6 Preference for and avoidance of apple-, hawthorn-, flowering dogwood-, blueberry-, snowberry-, and black cherry-fly origin *Diachasma alloeum, Utetes canaliculatus* and *Diachasmimorpha mellea* to odors of natal and non-natal fruit compared to odorless controls (not shown) in host odor discrimination tests (Forbes *et al.*, 2009). Odor sources: apple (green), hawthorn (red), flowering dogwood (yellow), blueberry (blue), snowberry (white) and black cherry (black). Adapted with permission from Forbes, A.A., Powell, T.H.Q., Stelinski, L.L., Smith, J.J., Feder, J.L., 2009. Sequential sympatric speciation across trophic levels. Science 323, 776–779 and Hood, G.R., Forbes, A.A., Powell, T.H.Q., *et al.*, 2015. Sequential divergence and the multiplicative origin of community diversity. Proceedings of the National Academy of Sciences of the United States of America. Online early. doi:10.1073/pnas.1424717112.

between apple and hawthorn populations at sympatric sites (Feder *et al.*, 1994) and could completely isolate certain sibling species (Feder and Bush, 1989).

Flies in the *R. pomonella* group also display host-related genetic differentiation. Apple and hawthorn flies differ significantly in allele frequencies for 6 allozyme and 26 microsatellite loci, many of which are associated with diapause timing (Michel *et al.*, 2010). The immediate sister taxon to *R. pomonella* that infests flowering dogwood shows an even stronger pattern of allele frequency divergence than the apple and hawthorn hosts races (Powell *et al.*, 2013). Diagnostically fixed genetic differences also do not distinguish between *R. pomonella* and two other siblings attacking blueberry (*R. mendax*) and snowberry (*R. zephyria*) flies. However, these taxa are distinguished by moderate to strong allele frequency differences for a subset of loci (Xie *et al.*, 2008).

Sequential speciation of natural enemies

Rhagoletis pomonella group flies and their associated community of parasitoid wasps (Hymenoptera: Braconidae) represent a case where sequential speciation is multiplicatively amplifying biodiversity across an entire assemblage of species. The wasps, *Diachasma alloeum*, *Diachasmimorpha mellea*, and *Utetes canaliculatus* are each host-specific and attack various immature life stages of *Rhagoletis* developing within host fruit. As with *R. pomonella*, all three species display allele frequency differences for several microsatellite loci among sympatric populations attacking apple, hawthorn, blueberry, snowberry, and flowering dogwood flies, implying that each represents a complex of host races (**Figure 5**; Forbes *et al.*, 2009; Hood *et al.*, 2015). In addition, a lack of fixed differences and shared haplotypes for mtDNA suggests that host-associated wasp populations are of recent origin and/or there is some ongoing gene flow. Moreover, the same ecological adaptations that reproductively isolate *Rhagoletis* flies also isolate members of the parasitoid community. Wasps rendezvous on or near host fruit for mating and display similar host odor preference and avoidance responses to their natal versus alternative non-natal fruit volatiles in behavioral assays (**Figure 6**; Forbes *et al.*, 2009; Hood *et al.*, 2015). In addition, wasps show host-related differences in their mean eclosion times (10–27 days), matching the chronology of when host fruit ripen, and hence when fly larvae are available for oviposition (**Figure 7**; Forbes *et al.*, 2009; Hood *et al.*, 2015). Furthermore, microsatellites that differed significantly among host-associated populations are associated with the timing of adult eclosion in *D. alloeum* and *D. mellea*. Given that adult wasps live only ~2 weeks (half as long as flies), the effects of temporal isolation can be even more pronounced in reducing gene flow (15–100%). The concordant natural history, documentation of recent sympatric host shifting of flies, and evidence for genetic differentiation therefore all support the formation of new wasps host races community-wide and highlight the potential for sequential speciation to multiplicatively amplify diversity.

Systems Lacking Evidence

A number of studies have reported an apparent absence of sequential speciation (Cronin and Abrahamson, 2001; Baer *et al.*, 2004; Althoff, 2008; Lozier *et al.*, 2009; Dickey and Medina, 2012). A common theme among these systems is that the natural enemies in question have multivoltine life histories, a trait which reduces the potential for allochronic isolation (Althoff, 2008; Dickey and Medina, 2012). In addition, four studies investigating sequential speciation (Baer *et al.*, 2004; Althoff, 2008; Lozier *et al.*, 2009; Dickey and Medina, 2012) have attributed negative results to (1) host plant discrimination behavior that is learned, rather than genetically controlled and (2) external feeding of the host on their host plant, elevating the importance of visual cues and decreasing the significance of plant-associated chemical cues for locating and differentiating between hosts. An emphasis

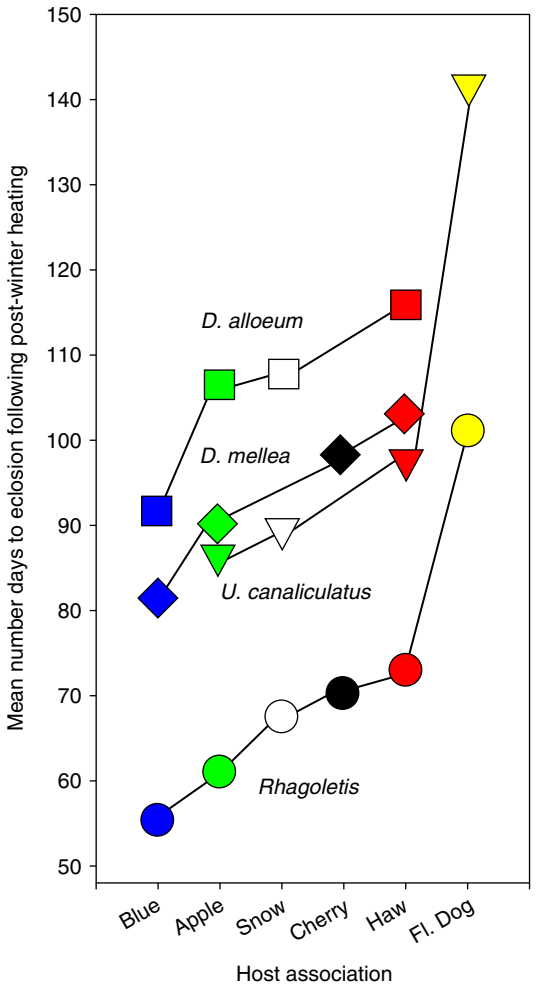

Figure 7 Mean number of days to adult eclosion for *Rhagoletis* (circles) infesting apples (green), hawthorn (red), flowering dogwood (yellow), blueberry (blue) and black cherry (black) flies and *Utetes canaliculatus* (triangles), *Diachasmimorpha mellea* (diamonds), and *Diachasma alloeum* (squares) attacking those fly hosts. Adapted with permission from Forbes, A.A., Powell, T.H.Q., Stelinski, L.L., Smith, J.J., Feder, J.L., 2009. Sequential sympatric speciation across trophic levels. Science 323, 776–779 and Hood, G.R., Forbes, A.A., Powell, T.H.Q., *et al.*, 2015. Sequential divergence and the multiplicative origin of community diversity. Proceedings of the National Academy of Sciences of the United States of America. Online early. doi:10.1073/pnas.1424717112.

on visual, rather than chemical, orientation toward host fly galls may also be a factor in the apparent lack of sequential divergence of the *Eurosta* parasitoid *Eurytoma gigantea* (Abrahamson and Blair, 2008).

Concluding Remarks: Future Directions

Until recently, most evidence for sequential speciation had been inferred from studies of adaptive radiations following mass extinctions, community level studies of species richness, and comparative phylogentic analyses of clade diversity. Here, we have highlighted two test cases from among several empirical studies that have identified candidate systems likely undergoing sequential speciation as well as several instances where no support has been found. To better understand if and how sequential speciation contributes to the genesis of biodiversity, several questions need attention. For example, what is the frequency of sequential speciation, both within and between systems? Is the community-wide amplification of diversity seen in *R. pomonella* more a rule or an exception? Similarly, how far up the trophic web can the effects of divergent ecological selection act to amplify biodiversity? In many systems, hyperparasitoids (parasitoids of parasitoids) are common and may experience the same divergent selection pressures as their lower trophic level hosts. We have focused our discussion on sympatric systems with the potential for high level of gene flow, the most difficult circumstances for divergent selection to drive ecological differentiation. However, sequential speciation may also be a factor in cases of parapatry and allopatry, although in the latter case it is more difficult to disentangle the consequences of divergent selection generated from new biotic opportunities from the effects of geographic isolation and limited or no gene flow. Lastly, while phytophagous insects and their natural enemies have attributes that *a priori* may make them more prone to sequential speciation, a more balanced view of the prevalence of sequential speciation requires study of other types of organismal interactions.

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See also: Cospeciation. Ecological Speciation and Its Consequences. Predation and Parasitism

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Sex and Recombination in Snails

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Glossary

Apomixis Uniparental, asexual reproduction that occurs without meiosis; all offspring produced are genetically identical to each other and the parent (*cf.* automictic asexual reproduction which involved meiosis, hence the opportunity for recombination, but no fertilization).

Asexual reproduction Uniparental reproduction without fertilization; meiosis may occur (automictic) or may not occur (apomictic).

Cladistic A taxonomic perspective that groups organisms by shared evolutionary history; clades are groups of organisms that include the common ancestor and all of the descendants from that common ancestor.

Copulation A physical connection required for transfer of male gametes to the female.

Gonochore/gonochorism (i.e., dioecy/diecious) A sexual system in which males and females are separated into different organisms (i.e., different bodies); *cf.* hermaphroditism. The term 'gonochorism' is typically used by zoologists, while the synonymous term 'dioecy/diecious' is typically used by botanists.

Hermaphrodite/hermaphroditism A sexual system in which males and females are combined into a single organism (i.e., the same body); *cf.* gonochorism. Hermaphroditism can be simultaneous or sequential; simultaneous hermaphrodites express both sexes at the same time (they are simultaneously male and female), sequential hermaphrodites change from one sex to the other during their lifespan (see protandry/protogyny).

Imposex A disorder in some organisms where female individuals develop male sexual structures.

Inbreeding Mating with an individual that is genetically related; the extreme is selfing.

Inbreeding depression The decrease in fitness suffered by inbred offspring relative to outbred offspring.

Lecithotrophic A form of larval dispersal where the larvae are produced with a yolk sac or some other means of being nourished while they disperse. Lecithotrophic larvae may disperse further than direct developing larvae but not as far as planktotrophic larvae.

Life history The relative timing of key aspects of an organism's life, especially with regard to birth/survival/death, growth, sex and reproduction, and the quantitative investment in these aspects. This includes, for example, whether reproduction is attempted only once or in multiple rounds, and how long the reproductive period (and life itself) lasts.

Mating system The pattern of mating among individuals in a population; the mating system can be thought of in terms of the relative number of males and females mating with each other (e.g., monogamy, polygamy, polyandry, polygyny) or in terms of the level of inbreeding/outbreeding in a population.

Metapopulation A set of populations that are linked by migration/gene flow and experience extinction and colonization.

Outbreeding/outcrossing/cross-fertilization Mating with an individual that is not a genetic relative; can be used less strictly in comparison to self-fertilization in simultaneously hermaphroditic species.

Parthenogenesis Literally means 'virgin birth' (i.e., birth from a single female parent with no male involved (no fertilization)); asexual reproduction. Note that some fish species actually require copulation, but reproduce asexually (the sperm do not fertilize the eggs).

Phenotypic plasticity The phenotypic variation expressed by a given genotype (set of genes) across different environmental conditions.

Planktotrophic A form of larval dispersal where the larvae are capable of feeding while they disperse. Planktotrophic larvae may disperse further than both lecithotrophic larvae and direct developing larvae.

Ploidy The number of copies of homologous chromosomes (hence of alleles per gene) that are contained in the nucleus.

Polyphyletic A taxonomic grouping that does not reflect shared evolutionary history; a polyphyletic group may not include the common ancestor of a group, may contain organisms with a variety of different common ancestors, or may exclude some of the descendants from the most recent common ancestor.

Polyploidy Having >2 (e.g., 4, 8) copies of each chromosome. Uneven numbers of copies occur in animals.

Protandry A form of sequential hermaphroditism where an individual starts sexual maturity as a male and switches to female. Some organisms can start as male-only and convert to simultaneous hermaphroditism.

Protogyny A form of sequential hermaphroditism where an individual starts sexual maturity as a female and switches to male. Theoretically, some organisms could start as female-only and convert to simultaneous hermaphroditism, but this is probably rare.

Recombination The production of a new combination of genes in the offspring – different from either parent; primarily occurs through two processes during meiosis: crossing over between homologous chromosomes and independent assortment between nonhomologous chromosomes.

Selfing/self-fertilization Sexual reproduction wherein an organism independently produces male and female gametes (via meiosis) then facilitates their fusion (fertilization) to produce offspring; the most extreme form of inbreeding.

Sex Can refer to the type of gamete an individual produces or to a mechanism of reproduction that involves alternating rounds of meiosis and fertilization. Sexual reproduction may or may not require copulation.
Sex determination A mechanism whereby the sex (male or female) of an individual is determined; can be genetically based (e.g., via a sex chromosome system), environmentally based, or some combination of the two.
Sex ratio The relative number of breeding males to breeding females in a population.
Sexual conflict Any of numerous situations where males and females (or male and female functions in hermaphrodites) maximize their reproductive output by different means; can lead to antagonistic interactions between the sexes where what is good for one sex may actually be detrimental to the other sex.
Sexual dimorphism A consistent difference in phenotype between the sexes (in gonochoric organisms); may involve a size difference or any other ornament/armament/display trait.
Sexual reproduction Reproduction that involves alternating rounds of meiosis and fertilization.
Sexual system The arrangement of male and female gonads into individuals (e.g., gonochorism, hermaphroditism).
Uniparental reproduction Reproduction that only involves a single parent; can be asexual or sexual (self-fertilization).

Introduction

Snails and slugs (Mollusca: Gastropoda) are a very diverse class of single-shelled (or shell-less) mollusks occupying terrestrial, freshwater, brackish, and marine environments. Gastropods are a very old group, with fossils dating to 500 million years ago (Lindberg *et al.*, 2004; Lecointre and Le Guyader, 2006). Estimates for the number of extant snail species range from >100 000 (Lecointre and Le Guyader, 2006) to almost 150 000 (Lindberg *et al.*, 2004) making them second only to insects in terms of the number of animal species in a biological class. New species are very commonly discovered and described, especially in tropical forests and in the sea. Snails, like flowering plants, have long attracted the interest of evolutionary biologists, especially those considering/studying the evolution of reproductive systems, because of their amazing diversity in terms of sexual system, morphology, and behavior. Gastropods also serve as important model species in molecular biology and neurobiology, and they function as intermediate hosts for a number of human diseases (e.g., schistosomiasis). Gastropod reproductive systems have been reviewed in detail in Giese and Pearse (1977) and Wilbur *et al.* (1984); more recent reviews in Leonard and Cordoba-Aguilar (2010) have focused on the reproductive morphology of several major gastropod groups. Ponder and Lindberg (2008) provide an overview of Molluscan evolution including several chapters on gastropods.

All individual snails possess a single gonad (Aktipis *et al.*, 2008), but there is widespread variation in whether this gonad is used to produce one type of gamete or both. Most snails (>75%) are gonochoric (i.e., diecious), meaning that males and females exist as separate individuals. The separation of the sexes, and the mechanism by which this is achieved (e.g., sex chromosomes), brings up immediate questions regarding sex ratios, sexual selection, and sexual conflict – cases where male and female reproductive fitness are optimized in different ways. Some species have evolved radical sexual dimorphism where the males are extremely reduced in size and live within or attached to the females (i.e., dwarf males). Within gonochoric species, some snails utilize a very different form of reproduction – asexual reproduction, where no fertilization is involved (and hence males are not needed). Asexuality in snails is generally associated with an absence of genetic recombination (apomixis) and variation in ploidy level. In some species, asexual females coexist with sexual females and males, meaning that recombination is in competition with asexual reproduction. Some snails, especially the terrestrial and some freshwater ones, are simultaneous hermaphrodites, possessing both fully functional male and female sexual systems. These snails may have the capacity to self-fertilize or outcross (i.e., cross-fertilize) with partners. There are examples of species that prefer one strategy over the other, and there are some that seem to routinely do both (Jarne and Auld, 2006; Escobar *et al.*, 2011). When snails cross-fertilize, copulation is often (but not always) required, and this copulation might be bilateral, where both individuals play male and female at once, or unilateral, where one individual plays the male role and the other plays the female role. Some snails are sequential hermaphrodites, starting their reproductive life as one sex (e.g., male) and switching at some point to be the other (e.g., a fully functional female with no further male gametes produced); protandry seems to be much more common than protogyny (i.e., individuals beginning their life as female before switching to male). Therefore, snails vary from sexual to asexual and span the gamut of sexual reproduction, from self-fertilization to panmictic outcrossing. With all of this reproductive diversity, they serve as an excellent model system to study the evolution of reproductive systems, including sex itself. In this article, we review the diversity of sex and recombination in snails from a taxonomic (cladistic) perspective, highlighting particular examples of unique evolutionary outcomes (e.g., aphally, love darts, parasitic castration, and apophallation).

Gastropod Reproductive Systems

The vast majority of gastropods have separate sexes, which is the case in animals in general (Bell, 1982; Jarne and Auld, 2006). This means that issues bearing on the evolution of sexual systems are to a large part the same as in insects or mammals. For example, sex might be determined through sex chromosomes. However, most gonochoric gastropods are marine species, and our knowledge of these species lags behind, as is often the case when comparing marine and continental biology. No marine species has really been established

as a model system that can easily be manipulated in the laboratory. Much is therefore to be gained by further studies of sexual systems and sexual selection. It is also not clear whether the common ancestor of all gastropods was gonochoric or hermaphroditic. It has been argued, based on out-group comparisons, that the ancestral character state is gonochoric (Ponder and Lindberg, 1997); but until our understanding of the gastropod (and mollusc) phylogeny is settled, a conclusion seems premature. One thing does appear to be clear regardless of what the ancestral character state was: There have been numerous transitions in sexual systems within this group (i.e., several tens of transitions between hermaphroditism and gonochorism; Heller, 1993). Indeed the lability of sexual system is impressive, but so is the fact that several large groups show stasis in terms of sexual systems (e.g., the Hygrophila is almost completely hermaphroditic, see below).

Some gastropods also practice asexual reproduction, and a brief, but important clarification is pertinent. Sexual reproduction requires alternating rounds of meiosis and fertilization (Maynard Smith, 1978; Bell, 1982). Gametes are produced through the process of meiosis, which allows for genetic recombination and reduces ploidy; the normal ploidy level is then restored during fertilization when two gametes fuse to make a zygote. This process may be accomplished by a single individual (if that individual can produce both sperm and eggs) or two individuals (a male and a female in separate-sex species, or two cross-fertilizing hermaphrodites). While an individual may receive sperm from multiple sperm donors, only one spermatozoon is required to fertilize an egg. Asexual reproduction is a uniparental form of reproduction without fertilization. While some forms of asexual reproduction produce offspring that are exact replicas of the parent (i.e., cloning), some forms of asexual reproduction allow for meiosis (and therefore genetic recombination) to occur. Asexual reproduction is not extremely common in gastropods, occurring in a few tens of species, but offers fascinating models. Apomixis (i.e., clonal, where no recombination takes place) seems the norm, but detailed cytological/genetic studies are needed to distinguish automictic parthenogenesis from alternative forms of reproduction. These studies are not always available, and herein we will use 'asexual reproduction' and 'apomictic parthenogenesis' as synonymous.

Aside from variation in sexual systems and mating systems, gastropods also exhibit a great deal of variation in basic life-history traits that influence the evolution of reproductive traits (e.g., spawning, dispersal). While in many species copulation is involved and eggs are deposited either in gelatinous capsules/masses attached to a substrate or laid individually, there are examples of groups that have live birth, brooding, dispersing larvae (planktotrophic and lecithotrophic), and external fertilization (Wilbur *et al.*, 1984; Aktipis *et al.*, 2008). The extent to which the evolution of such life-history strategies is related to the evolution of the reproductive system is a topic ripe for future research. Eppley and Jesson (2008) explored the issue at the scale of all animal groups, and this could fruitfully be downscaled within groups exhibiting wide variety in sexual systems such as the gastropods.

We opt below for a presentation based on the taxonomic organization of gastropods in order to highlight the variety of issues across taxonomic groups and the fact that different questions are addressed in different groups. We used the purely cladistic system of classifying gastropods, recognizing six major clades, of Bouchet and Rocroi (2005). These clades are the Patellogastropoda, Vetigastropoda, Cocculiniformia, Neritimorpha, Caenogastropoda, and Heterobranchia; three of these clades (Vetigastropoda, Caenogastropoda, and Heterobranchia) are considerably larger than the other three, and the vast majority of studies have been on the latter two. The precise phylogenetic relationship among these clades is not clear, which complicates reconstruction of the ancestral sexual-system phenotype. Nonetheless, these clades can be thought of as six semi-independent groups within which the evolution of reproductive systems and strategies can be explored. **Table 1** provides an overview of the presence of hermaphroditism and sexual/asexual reproduction in these groups.

Patellogastropoda

The Patellogastropoda (Nakano and Ozawa 2007; Lindberg, 2008) are the true limpets, a marine group that is thought to be mostly gonochoric with sex ratios approximately 1:1 (e.g., Henninger and Hodgson, 2001), although some examples of protandrous (Lindberg and Wright, 1985; Gray and Hodgson, 2003; Collin, 2013) and even simultaneous (Cunha *et al.*, 2007) hermaphroditism exist. It is not clear whether this latter case is an anomaly, and even less whether self-fertilization occurs, as this species in particular seems to be normally gonochoric (Curdia *et al.*, 2005).

Vetigastropoda

The Vetigastropoda are another marine group of approximately 3700 species (Geiger *et al.*, 2008); they appear to be

Table 1 The prevalence of sexual systems (gonochorism vs. hermaphroditism), sexual reproduction (by outcrossing and selfing), and asexual reproduction among the six major gastropod clades.

Clade	*Sexual system*		*Sexual reproduction*		*Asexual reproduction*
	Gonochorism	*Hermaphroditism (type)*	*Outcrossing*	*Selfing*	
Patellogastropoda	Majority	Minority (sequential; some simultaneous)	Present	Not reported	Not reported
Vetigastropoda	Majority	Minority	Present	Not reported	Not reported
Cocculiniformia	Absent?	Minority (simultaneous)	Present	Not reported	Not reported
Neritimorpha	Majority	Absent?	Present	Not reported	Not reported
Caenogastropoda	Majority	Minority (sequential; some simultaneous)	Present	Not reported	Present
Heterobranchia	Minority	Majority (simultaneous; some sequential)	Present	Present	One species

predominantly gonochoric (e.g., Najmudeen, 2007) although hermaphrodites have been observed (Judge and Haszprunar, 2014). Thiriot-Quiévreux (2003) highlights that one species of *Monodonta* has evolved an X0–XX mechanism of sex determination.

Cocculiniformia

The Cocculiniformia is a group of approximately 130 species of deepwater limpets exhibiting simultaneous hermaphroditism where eggs and sperm are made in a single gonad (Strong and Harasewych, 1999; Lindberg, 2008; Hartmann *et al.*, 2011). Heller (1993) reported that 94% of the genera in this group are hermaphroditic, but noted that it is difficult to distinguish simultaneous and sequential hermaphrodites.

Neritimorpha

The Neritimorpha is primarily a marine group of deepwater limpets (Lindberg, 2008). It also contains some terrestrial and freshwater snails. The group appears to be gonochoric on the whole, but very little else seems to be known about its sexual and mating systems. Sex chromosomes (X0–XX) have evolved in the Neritidae (Thiriot-Quiévreux, 2003). Some species, for example, in the genus *Neritina*, are diecious and have marine larval forms, but freshwater adult forms.

Caenogastropoda

The Caenogastropoda is a very large and diverse group (~ 60% of the Gastropoda) containing marine, terrestrial, and freshwater species. Many snails formerly called Prosobranchs belong to this group; note that Prosobranchia is now considered to be a polyphyletic grouping.

Caenogastropods are almost entirely gonochoric. Studies on Caenogastropods (e.g., *Pomacea canaliculata*; Yusa, 2004a,b, 2006, 2007a) have explored the genetics of sex determination and the evolution of sex ratios. Yusa (2007b) reviewed this work, highlighting that while population sex ratios are often 1:1 the sex ratio within broods is often highly variable. This work, coupled with the discovery that environmental pollutants can cause female snails to develop male reproductive anatomy (i.e., imposex; e.g., Martin, 2002; reviewed in Sternberg *et al.*, 2010) has made Caenogastropod snails an important model for understanding the evolution of sex determination. More work on the ecological and evolutionary implication of imposex is needed. True sex chromosomes (i.e., stabilized gonochorism) have evolved in some species (e.g., Taguchi *et al.*, 2000; reviewed by Thiriot-Quiévreux, 2003). Interestingly, both ZW–ZZ and XX–XY mechanisms of sex determination have evolved in the genus *Viviparus*, while an X0–XX mechanism has been observed in other genera (Dillon, 2000; Thiriot-Quiévreux, 2003). In other groups, sex determination appears to be oligogenic (Yusa, 2007b). This variation suggests that future work on the evolution of Caenogastropod sex-determination mechanisms and transitions between states is likely to be very illustrative.

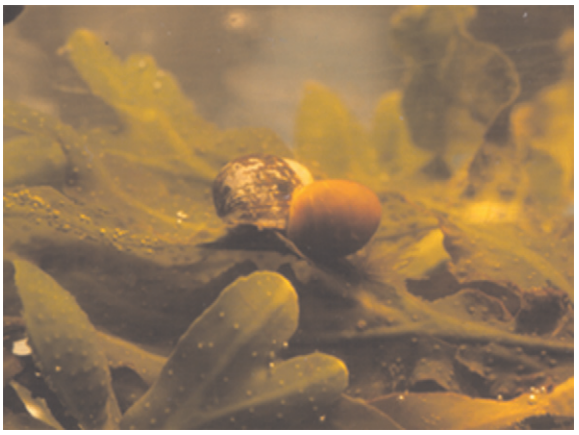

Figure 1 Copulation in *Littorina obtusata*. The diverse, marine genus *Littorina* has been widely studied in evolutionary ecology (Rolán-Alvarez *et al.*, 2015). Photo courtesy of E. Rolán-Alvarez.

Other Caenogastropods (e.g., *Littorina*; **Figure 1**) have served as models for studying the evolution of mate choice (Zahradnik *et al.*, 2008; Saltin *et al.*, 2013) and the process of sexual selection (Erlandsson and Johannesson, 1994; Rolán-Alvarez *et al.*, 2012). Many of these studies have focused on an intertidal hydrid zone in *Littorina saxatilis*, and illustrate the role of mate choice in the speciation process (Johannesson *et al.*, 1993; Pickles and Grahame, 1999).

While the Caenogastropoda is almost exclusively gonochoric, a few hermaphroditic species have been reported (Webber, 1977; Fretter, 1984; Heller, 1993; Ponder *et al.*, 2008; Hodgson, 2010). The eulimids, a group of echinoderm parasites, exhibit both hermaphroditism and gonochorism with dwarf males (Elder, 1979; Heller, 1993; Takano and Kano, 2014). Here, males are much smaller (e.g., one-tenth the size or smaller) than females and live attached (externally or internally) to the female. Heller (1993) also brings up the possibility of environmental sex determination in this group, where a female may recruit a larval individual and induce masculinity. This sort of environmentally induced sex determination is known to exist in the sequentially hermaphroditic genus *Crepidula*, the slipper limpets (Yonge and Thompson, 1976; Heller, 1993; Collin, 1995; Mérot and Collin, 2012). These organisms form stacks on top of one another and change sex so that the individuals on the bottom function as females but newly arriving individuals (on top) function as males. This illustrates an interesting overlap between sequential hermaphroditism, environmental sex determination, and phenotypic plasticity. Often, it is not clear what the exact stimulus is that induces a change in sex, but the availability (and position) of potential mates is certainly important. In her review, Collin (2013) highlighted that protandry has evolved in a few different Caenogastropod groups (Calyptraeids and Coralliophilids; see also Calvo and Templado, 2005); she proposes that aspects of protandry itself (growth repression of males by females) may facilitate the evolution of dwarf males in these groups.

Caenogastropods have also attracted some attention because of the occurrence of asexual reproduction in at least three families (i.e., Hydrobiidae, Viviparidae, and Thiaridae;

Heller, 1993). All of these asexual species dwell in freshwater. This suggests an association between freshwater and asexuality since the vast majority of Caenogastropods live in marine environments (Johnson *et al.*, 1995). One particularly well-studied species with regard to the evolution of sex, for example, its stability and loss, is the New Zealand mud snail (*Potamopyrgus antipodarum*). In this species, diploid, sexual males and females coexist with triploid, apomictic, asexual females, sometimes in the same populations, especially in the native area (Lively, 1987). All else being equal, asexual individuals enjoy the benefit of not needing to produce sons: all offspring are daughters, which doubles the reproductive rate (see, e.g., Maynard Smith, 1978). Nonetheless, sexual individuals are maintained in the population. Research has demonstrated that sexual reproduction is more common when the prevalence of a digenetic trematode is high (Lively, 1987, 1992; Jokela and Lively, 1995a,b; King *et al.*, 2009). When infected by this trematode, the snails are sterilized (castrated). Importantly, long-term studies have revealed oscillations in snail genotype frequencies over time (Dybdahl and Lively, 1998); these results have been viewed as supporting the Red Queen hypothesis for the evolution of sex (Bell, 1982; King *et al.*, 2009; Brockhurst *et al.*, 2014).

Two other Caenogastropod genera that have been studied are *Campeloma* and *Melanoides*. Both of these snails, like *Potamopyrgus*, have sexual and asexual morphs, often coexisting within the same 'population.' That is, they exist as three different reproductive 'types' (asexual females, sexual females, and sexual males), where asexual clones arise from sexual individuals. *Campeloma* is an ovoviviparous snail that is a widespread inhabitant of the southeastern USA. The asexual morphs appear to have arisen by hybridization (Johnson and Leefe, 1999). Some research has shown that asexual morphs of this snail occupy a narrower array of habitats than the sexual morphs (Crummett *et al.*, 2013). Studies of *Melanoides tuberculata* (**Figure 2**), which is a widespread invasive species throughout the tropics, have explored the distribution of asexual reproduction in particular environmental conditions (Ben-Ami and Heller, 2007). Interestingly, we do not know how asexual reproduction arises in *Melanoides*, but we do know that it is associated with an increase in ploidy level and that clones include a variable fraction of males and can hybridize to generate new clones (Samadi *et al.*, 1999). In this case, hybrid clones inherited the full maternal chromosome set (hexaploid) and half that of their father, such that hybrids were nonaploid. As such events have been observed at least twice at a small geographic scale (Martinique island, Lesser Antilles), it can be suggested that clones recurrently exchange genetic material leading to an increase in genetic variance and therefore maintaining some evolutionary potential (Facon *et al.*, 2008). The same long-term studies have shown that clones vary in their competitive ability, which might explain their succession in invasive series in the Lesser Antilles (Facon *et al.*, 2008).

Figure 2 *Melanoides tuberculata*, an invasive Caenogastropod species throughout the tropics. This species is capable of reproducing by parthenogenesis. Photo by Jean-Pierre Pointier.

Heterobranchia

The Heterobranchia is a diverse group, also containing marine, terrestrial, and freshwater species. There are three 'informal' groups within the Heterobranchia: the lower Heterobranchia, the Opisthobranchs, and the Pulmonata, the latter group has been studied to a much greater extent than the others (e.g., Dayrat *et al.*, 2011). The lower Heterobranchia have not received a great deal of attention.

Opisthobranchia

The Opisthobranchs are a marine group of ~6000 species that are now recognized to be paraphyletic. They are essentially all simultaneous hermaphrodites (Beeman, 1977; Hadfield and Switzer-Dunlap, 1984; Heller, 1993; Wägele *et al.*, 2008; Valdes *et al.*, 2010), where cross-fertilization with elaborate courtship behaviors appears to be the predominant mode of reproduction (e.g., Anthes and Michiels, 2005; Hamel and Mercier, 2006). Opisthobranchs can have very specific courtship behaviors that coordinate reciprocal sperm transfer (i.e., sperm trading; Anthes *et al.*, 2006; Sprenger *et al.*, 2008, 2009). In some species sperm are injected hypodermically (Anthes and Michiels, 2007; Schmitt *et al.*, 2007), an observation that has prompted research into the role of sexual conflict in the correlated evolution of male and female reproductive morphologies and behavior (Anthes *et al.*, 2008). Work on the sea hare *Aplysia californica* (a popular model system in neurobiology), and other species in this genus, has contributed to our understanding of gender-role preferences and size-assortative mating (Pennings, 1991; Yusa, 1996, 2008; Angeloni and Bradbury, 1999; Angeloni *et al.*, 2003; Ludwig and Walsh, 2008). The prospect of future research of the neurobiology of copulatory interactions in this group seems particularly exciting.

Despite the fact that Opisthobranchs are simultaneously hermaphroditic, self-fertilization is rare. Only a few examples are known, for example, in *Alderia willowi* individuals are capable of selfing following hypodermic insemination (Smolensky *et al.*, 2009). Valdes *et al.* (2010) recently reviewed the reproductive anatomy of this group; they have separate reproductive tracts for incoming and outgoing sperm, which is perhaps a mechanism to ensure outcrossing. They also display elaborate courtships and complex reproductive anatomy, which have been understudied.

Pulmonata

The Pulmonates are largely a group of hermaphroditic terrestrial or freshwater snails; the vast majority are simultaneously hermaphroditic (Tompa, 1984; Geraerts and Joosse, 1984; Heller, 1993; Jarne and Stadler, 1995; Mordan and Wade, 2008). There are about 25 000–30 000 species of Pulmonates (Mordan and Wade, 2008), most belonging to the Stylommatophorans (i.e., land snails). Pulmonates in particular have been extensively studied in terms of their mating system (Berry, 1977; Jarne *et al.*, 1993; Jarne and Auld, 2006; Baur, 2007, 2010; Jordaens *et al.*, 2007; Jarne *et al.*, 2010; Escobar *et al.*, 2011) with some species reproducing primarily by self-fertilization and others primarily by outcrossing. Apparently, asexual reproduction has evolved in at least one Pulmonate, the slug *Deroceras laeve* (Nicklas and Hoffmann, 1981; Hoffmann, 1983; Reise, 2007; but see Lebovitz, 1998). Escobar *et al.* (2011) reviewed the available data on inbreeding depression and the rate of self-fertilization in animals; the majority of these data come from Pulmonates (and specifically, the Hygrophila (freshwater snails – formerly the Basommatophorans)). Interestingly, there was approximately as much variation in the selfing rate within Hygrophila as among hermaphroditic animals in general. In general, inbreeding depression is thought to be a major factor operating on the evolution of the selfing rate (Charlesworth and Charlesworth, 1987; Ronfort, 1999; Husband *et al.*, 2008; Charlesworth and Willis, 2009). Numerous studies on Pulmonates have increased our understanding of mating system evolution and how it is affected by inbreeding depression (e.g., Wethington and Dillon, 1997; Jarne *et al.*, 1991, 2000). Polyploidy, which is generally rare in animals compared to plants, has evolved in a few Pulmonate genera (e.g., *Bulinus*, *Succinea*, *Gyraulus*; Wu, 1972; Burch and Jung, 1993; Anisimov *et al.*, 1995; Jarne and Stadler, 1995; Doums *et al.*, 1998b; Jorgensen *et al.*, 2011). The relationship between polyploidy and inbreeding depression is a complicated topic that requires further study.

Pulmonates have a single gonad (the ovotestis) that produces both eggs and sperm. These gametes are produced in different 'islets' (acini) throughout the organ, but stored in distinct places (Jarne *et al.*, 1993, 2010; Baur, 2010). In the Hygrophila, there are separate openings for the male and female reproductive systems, while in the Stylommatophora the male and female reproductive systems share a common opening. Copulation in the Hygrophila is usually unilateral meaning that when individuals copulate, one individual plays the male role (i.e., sperm donor), while the other plays the female role (i.e., sperm recipient; **Figures 3** and **4**). Sex role alternation (i.e., reciprocal gender-role switching) may follow in this situation. Some species are capable of bilateral copulation (i.e., mating partners play both roles at once; Soldatenko and Petrov, 2012; see also Lamy *et al.*, 2012). In some circumstances, female-role *Lymnaea* and *Physa* have been observed to attach themselves to their partner's shell before copulation has finished – thereby they ensure an opportunity to assume the male role and donate sperm once copulation as a female has ended. In the Stylommatophora, for example, in the garden snail *Cornu aspersum* (*Helix*; Adamo and Chase, 1988), bilateral copulation is more common than in the Hygrophila, but unilateral copulation also occurs, depending in part on shell shape (Davison *et al.*, 2005). Sperm are deposited into the reproductive tract of the recipient via a phallus, and may be used to fertilize eggs, stored for later fertilization (Wethington and Dillon, 1991; Beese *et al.*, 2009; Nakadera *et al.*, 2014a), or diverted to the bursa copulatrix for digestion. Sperm received from a partner (i.e., allosperm) are stored in a different location than sperm made by the focal

Figure 3 Copulation in *Physa acuta* (Heterobranchia: Hygrophila). There are four individuals in this picture; the individual on the left is copulating as a female with the individual that is mounted on its shell (the phallus is visible). Individuals can only copulate in a single gender-role with a given partner at the same time, but a single individual can simultaneously be involved in two separate copulations (i.e., with two separate partners) where it can act as both sperm donor and sperm recipient. Photo by Josh Auld.

Figure 4 Copulation in *Lymnaea stagnalis* (Heterobranchia: Hygrophila). The individual on the left is playing the male role (with phallus clearly visible), the individual on the right is playing the female role. Picture provided by Joris M. Koene based on Koene *et al.* (2010).

individual (i.e., autosperm). This provides the opportunity to preferentially use allosperm for cross-fertilization, and still transfer autosperm during copulation. The location of self-fertilization may also differ between the Hygrophila and Stylommatophora: Selfing appears to occur in the ovotestis in Hygrophila, but in a pouch down in the seminal vesicles in Stylommatophorans; more work on this topic is needed. In some Pulmonates, a polymorphism has been observed where some individuals completely lack a phallus (i.e., aphally, **Figure 5**; Jarne *et al.*, 1992; Schrag *et al.*, 1994; Jordaens *et al.*, 2006a). Aphally has evolved once in Hygrophila, and at least 12 times in Stylommatophorans (Schrag and Read, 1996). Aphallic individuals are capable of reproducing through selfing and outcrossing via the female role, but they cannot function as outcrossing males because they cannot transfer sperm to a partner during copulation (Doums *et al.*, 1996a, 1998a, b). Interestingly, the production of a phallus can be affected by environmental stimuli including temperature, linking this phenomenon with environmental sex determination (Schrag and Read, 1992, 1994; Doums *et al.*, 1996b; Ostrowski *et al.*, 2000, 2003). Some Stylommatophorans (e.g., *Cornu aspersum*) possess a calcareous 'love dart' (**Figure 6**) that is used to stab the partner during courtship and copulation (Davison *et al.*, 2005; Baur, 2010), sometimes thousands of times during a single copulation bout (Koene and Chiba, 2006). While it was initially thought that this dart (which is dislodged in some species, but retained in others) was a form of nuptial gift, recent research has suggested that it may be a tool for partner manipulation stemming from sexual conflict (Koene and Schulenburg, 2005). Here it appears that the dart may introduce accessory gland proteins into the sperm recipient that increase paternity of the sperm donor (i.e., the stabbing snail). This apparently has led to counter-adaptations on the female side of the hermaphrodite (e.g., elaborate reproductive tracts, sperm digestion), an example of antagonistic coevolution and sexual conflict (Beese *et al.*, 2009; Nakadera and Koene, 2013). Such dynamics are not limited to Stylommatophorans; in the freshwater snail *Lymnaea stagnalis* accessory gland proteins that manipulate the sperm recipient are transferred in the ejaculate along with the sperm (Nakadera *et al.*, 2014b; van Iersel *et al.*, 2014). Another oddity in Stylommatophorans is the occurrence of giant phallus in some slug species from the *Limax* genus (**Figure 7**). Why an almost one-meter phallus and external copulation based on twisted (double helix) phallus has evolved in species no longer than 15 cm remains mysterious, but certainly has to do with sexual selection.

Because of their elaborate copulatory behaviors, Pulmonates have served as models for studying sex allocation and sexual selection (Schärer, 2009; Anthes *et al.*, 2010). This includes analyses of gender-role preferences, and, for example, how size, age, and previous mating history affect the social interactions leading to copulation (DeWitt, 1996; Wethington and Dillon, 1996; Koene, 2006; Baur, 2007; Minoretti *et al.*, 2011; Nakadera *et al.*, 2015). In some species, the phallus can become lodged within the sperm recipient and it may be

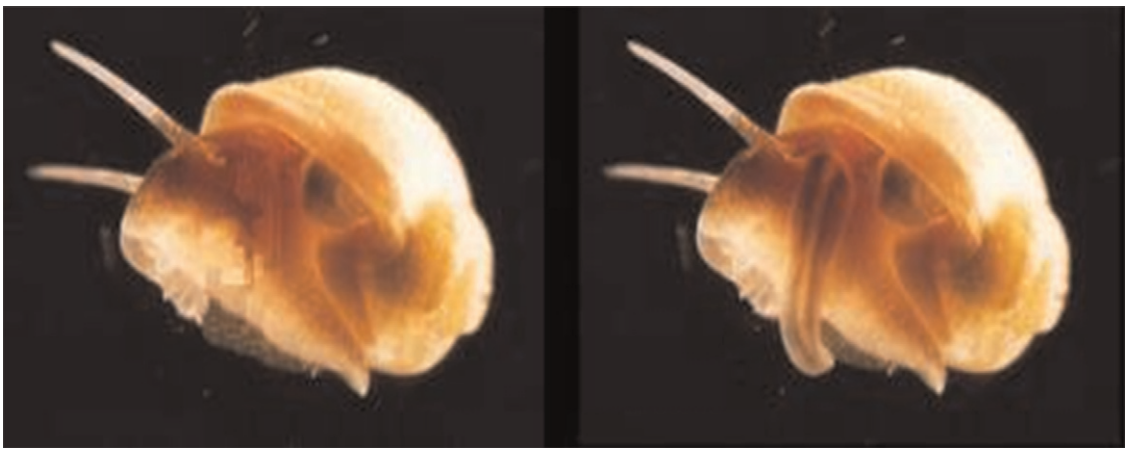

Figure 5 Phally polymorphism in *Bulinus truncatus* (Heterobranchia: Hygrophila). Some individuals have the same reproductive tracts as regular hermaphrodites in Hygrophila (e.g., the individual on the right with an everted phallus). Others are deprived of the male tracts, including the phallus (e.g., the individual on the left). Photo by Philippe Jarne.

Figure 6 Love darts of land snails (Heterobranchia: Stylommatophora); from left to right: *Xerarionta kelletti*, *Leptaxis erubescens*, *Cepaea hortensis*, *Monachoides vicinus*, and *Everettia corregata*. Pictures provided by Joris M. Koene based on Koene and Schulenburg (2005) and Koene *et al.* (2013).

Figure 7 Giant phallus in *Limax* slugs. The phallus of the two individuals are intertwined. Sperm is exchanged at the tip of the phallus, and crawled up the partners' phallus to reach its 'female' tracts. Individuals are about 10 cm in length. Photo by Gerhard Falkner (http://wirbellose.at/limax-bivonae).

Figure 8 Banana slugs (*Ariolimax buttoni*) engaged in reciprocal copulation. Both phalluses are visible. Photo courtesy of Janet Leonard.

chewed off (i.e., apophallation; Leonard *et al.*, 2002; **Figure 8**). Pulmonates have also been recently used as models for understanding sex-specific inbreeding depression and reproductive senescence (Janicke *et al.*, 2013; Auld *et al.*, 2014). Further, despite the fact that the sexes are united in the same body, sexual selection has been shown to operate differently on male and female functions (Charnov, 1979; Pélissié *et al.*, 2012). Evidence that resources may be differentially allocated to one sex function over the other (e.g., De Visser *et al.*, 1994; Koene *et al.*, 2006; Jordaens *et al.*, 2006a,b; Minoretti *et al.*, 2011) has revealed the potential for adaptive plasticity in sex allocation (Charnov, 1996; Schärer, 2009). Collectively this work has shown that while these snails are simultaneously male and female, and the gametes are made within the same organ, expression, and evolution of the two sex functions can occur semiautonomously (Charnov, 1982). This work has significantly advanced research on sexual selection and sexual conflict by clearly illustrating that these phenomena are not restricted to diecious species; male and female reproductive systems can evolve semi-independently within the same body and potentially have reciprocal effects on each other (Anthes *et al.*, 2010; Pélissié *et al.*, 2012).

Conclusions

Due to their plethora of reproductive strategies, snails provide a microcosm within which we can observe a great variety of reproductive strategies at play. They have been and will continue to be an excellent model system to study the evolution of reproductive systems and strategies, including topics such as the evolution of sex-determination mechanisms (Thiriot-Quiévreux, 2003), the evolution of hermaphroditism (Charnov, 1982; Avise, 2011), the evolution of the mating system (Jarne and Charlesworth, 1993; Jarne and Auld, 2006; Jordaens *et al.*, 2007), and the interaction between these factors and other aspects of the life history (Cheptou, 2012; Auld and Rubio de Casas, 2013; McClain *et al.*, 2014). However, we still only know a fraction of what we would like to know. One serious limitation to knowing more about gastropod reproductive systems is that a large fraction of the species, especially marine ones, are very difficult to maintain under laboratory conditions. However, gastropods offer some relevant models, especially among freshwater hermaphrodites, such as *Physa acuta* or *Biomphalaria glabrata*, easy to breed, with decent generation times (6–8 weeks) and improving genomic resources. Note though that, in many cases, a more detailed investigation of the situation in the field is also relevant, especially for model species that are mainly studied in the laboratory.

One very interesting observation to emerge from this review is the apparently independent, repeated evolution of uniparental reproduction in freshwater systems. We have highlighted a variety of instances where asexual parthenogenesis or self-fertilization have evolved in these systems; comparably these mechanisms of uniparental reproduction are not known to occur in the sea. Selfing also occurs widely in terrestrial gastropods. Currently, we do not know the extent to which uniparental reproduction facilitated an invasion into freshwater systems or evolved *de novo* after colonization. If uniparental reproduction in any way enabled the colonization of new habitats (e.g., freshwater systems), it would provide an interesting comparison/example of Baker's law (Pannell *et al.*, 2015). Likewise, the evolutionary persistence of both mechanisms of uniparental reproduction varies within snails. In some species of *Lymnaea* (*Galba*; Correa *et al.*, 2010) selfing has persisted for a very long time (~20 million years) while in others (e.g., *Biomphalaria pfeifferi*), the transition to selfing appears to be rather recent (DeJong *et al.*, 2003). The persistence of selfing in snails appears to be much longer than has

been observed in plants. Similarly, the persistence of parthenogenesis in Thiarids and *Potamopyrgus* appears to vary substantially. Future work that explores the origin and maintenance of these different mechanisms of uniparental reproduction will be very important.

With the advent of better molecular and genomic tools, further analysis of loci affecting sex determination and expression will be extremely useful. Future work that clarifies the phylogenetic relationships among the major gastropod clades will facilitate our understanding of where (and how often) shifts in sexual systems, sex-determination mechanisms (including sex chromosomes), mating systems (e.g., sex ratios, self-fertilization), and other traits have occurred. While molecular phylogenies are available for particular groups (e.g., Cerithioidea within the Caenogastropoda (Strong *et al.*, 2011) and Pulmonata within the Heterobranchia (Dayrat *et al.*, 2011), they are generally lacking – a situation that seriously hinders our ability to understand transitions in reproductive systems (and other traits). Furthermore, continued population genetic data will reveal information on levels of inbreeding including the potential for biparental inbreeding. Lastly, future work that explores the evolution of sex and recombination within a spatially explicit framework, for example, in metapopulation models (Pannell, 2015), will be particularly useful, especially for understating the dynamics of mating-system and life-history evolution.

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See also: Sex Determination. Sex, Evolution and Maintenance of

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Sex and Selfish Genetic Elements

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Glossary

Androdioecy A situation where individuals are either male or hermaphrodite.
Chromosome A large DNA molecule found within a cell, often carrying large numbers of genes. An organism may contain many different chromosomes.
Dioecy A situation where there are two sexes, and individuals are either male or female.
Diploid When a cell contains two copies of each chromosome. In clonal species, all cells may be diploid, whereas in other organisms there may be a haploid gamete phase, one sex may be haploid, or there may be alternation between haploid and diploid generations.
Endosymbiont An organism that lives within the cells or body of other organisms. These can be obligate endosymbionts, whereby they can only complete their life cycle with the use of their host organism.
Gametes These are reproductive cells which fuse (fertilization) to form a zygote during sexual reproduction. For example, sperm, pollen, and ova are gametes.
Gene A unit of genomic sequence that contains regulatory regions, sequences that are transcribed, and other functional regions.
Gynodioecy A situation where individuals are either female or hermaphrodite.
Haplodiploidy A genetic system whereby diploid individuals develop as females, whereas haploid individuals develop as males.
Haploid When a cell contains only one copy of each chromosome. This can apply to gametes of a diploid species, to a haploid sex, or to species where every individual is haploid.
Hermaphrodite An individual capable of producing both male and female gametes. Hermaphrodites are not necessarily able to self-fertilize.
Intragenomic conflict Conflict which arises within the genome of an organism due to different genetic elements having opposing interests, and being selected to increase their own success despite the costs this causes to other genes in the genome.
Parthenogenesis A form of asexual reproduction. Embryos can develop in the absence of sexual fertilization, with offspring only inheriting DNA from their mother.
Spermatogenesis The process by which functional spermatozoa are generated from reproductive germ cells in males.

Introduction

Sexual reproduction is extremely widespread across eukaryotes. This process, by which genetic material is inherited from generation to generation, involves the production of haploid gametes that subsequently fuse into (predominantly) diploid offspring (Figure 1). This fusion of genes from two parents, alongside recombination that allows genetic exchange between chromosomes, provides the offspring with a novel and diverse genetic assemblage. Within this broad definition of sex, a huge degree of complexity and variety exists, with important implications for biology. For example, many organisms are split into male and female sexes that invest in either many low-cost gametes or fewer expensive gametes respectively (anisogamy), as is the case with sperm and eggs in mammals. Mating systems, which describe how individuals interact with one another sexually, also show a remarkable variety in nature (Emlen and Oring, 1977; Goodwillie *et al.*, 2005). For example, two closely related plant species can have completely different mating systems, where one can self-fertilize while the other requires gametes from two different parents. Mating system diversity ranges far beyond this, including individuals that change sex depending on age or the presence of rivals, parthenogenetic species that require sperm from males of other species to reproduce, and unusual genetic systems such as haplodiploidy (Figure 2). How this diversity has come about, and the impacts it has, is a major focus of biological research. Increasingly, the causes and consequences of mating systems are being found to be intimately related to the existence and proliferation of selfish genetic elements (SGEs) – rogue genes that disobey Mendel's laws of inheritance.

From the process of sex, Mendel established our early understanding of genetic inheritance. He suggested that gametes are generated and recombined in a random manner, resulting in an equal chance for any part of the parental genome to reach the next generation. This 'fair' Mendelian ratio of segregation during the production of gametes ensures that the interests of genes are aligned and all will benefit when the collective (diploid organism) is more successful. However, there has been a growing realization that this cooperative view of the genome is not the whole story. Increasing numbers of cases have been found where genes act selfishly within the genome and it is now recognized that the living world contains a wide range of SGEs, ubiquitous across the tree of life (Burt and Trivers, 2006). The character that unifies these diverse elements is that they all increase their own frequency in subsequent generations to a degree greater than that expected by Mendelian inheritance, without increasing the fitness of the organism that carries them. The methods for achieving this, however, vary widely between different SGEs: from transposable elements (TEs) that can replicate and proliferate within the genome of a cell to killer chromosomes that sabotage the production of gametes that do not carry a copy of themselves

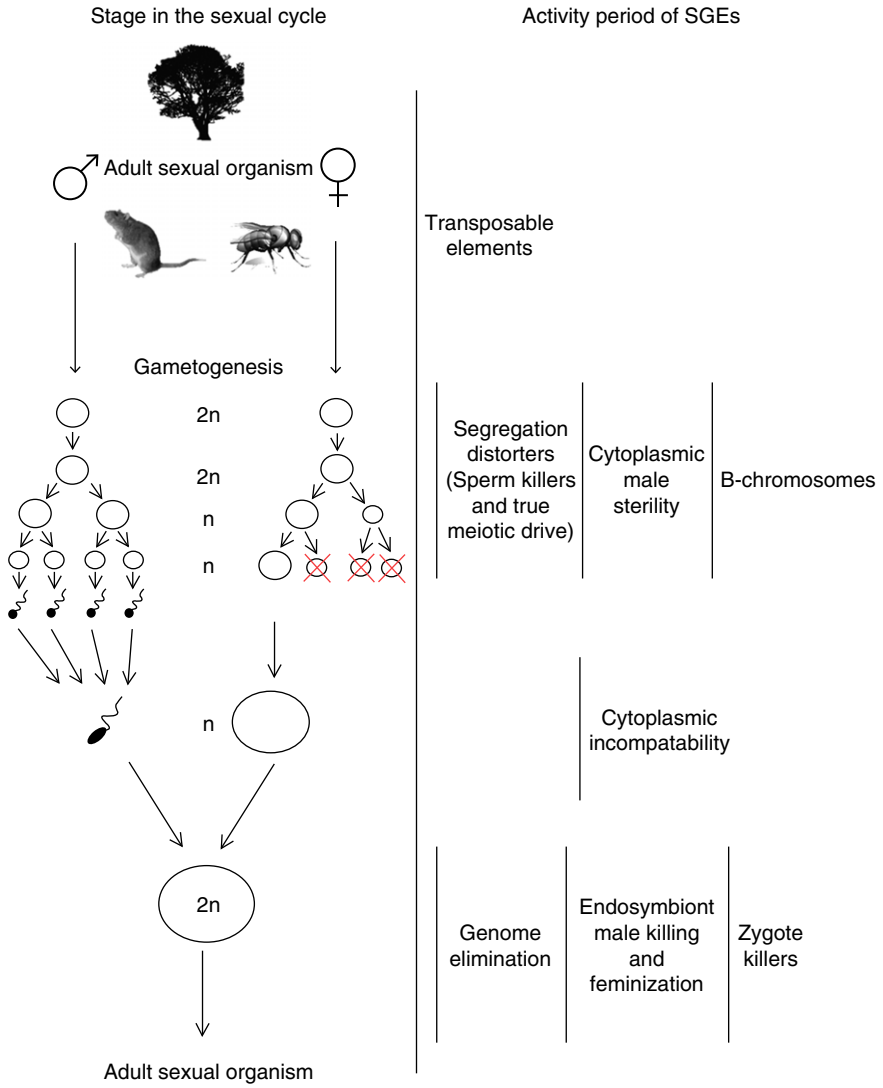

Figure 1 A schematic of the stages of sexual reproduction and the different points at which selfish genetics elements can be active.

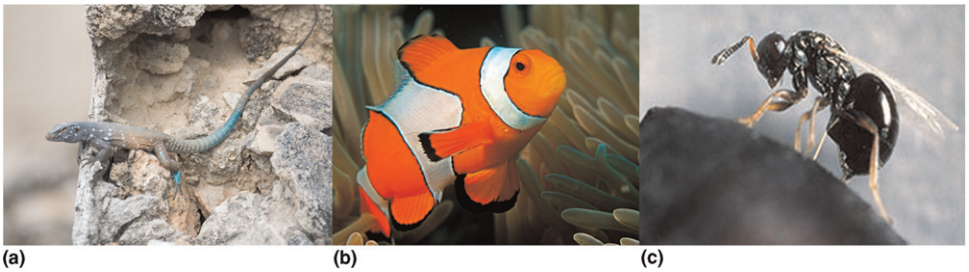

Figure 2 Example of different mechanisms of having sex. (a) Whiptail lizards can have species with two sexes that reproduce sexually, or have a single sex and reproduce parthenogenetically. (b) Clown fish have males that can turn female when the dominant female dies. (c) *Nasonia,* an example of a wasp species that has haplodiploid sex determination. It is this wasp genus that harbors the paternal sex ratio (PSR) system where the paternal genome is eliminated.

(Burt and Trivers, 2006; **Table 1**). In their most extreme form SGEs can result in 50% of the genome being lost. One of the best examples of this is the paternal sex ratio (PSR) system in the wasp genus *Nasonia*. PSR is an extremely small extra chromosome that is not essential for the survival of males and does not appear to increase the fitness of males in any way. Such supernumerary chromosomes are widespread in nature, and referred to as 'B-chromosomes.' However, what is (so far)

Table 1 Some classes of selfish genetic elements and some of their potential interactions with sex. Note that selfish genetic elements (SGEs) are highly variable, and cannot easily be separated into distinct classes. Transposable elements, B-chromosomes, and segregation distorters are all defined here by their genetic basis. In contrast, zygote killers, paternal genome elimination, cytoplasmic incompatibility, and cytoplasmic male sterility are all defined by the impacts they have on their host. This list of SGEs is not exhaustive, and we expect many new kinds of SGE to be discovered in the future

Type	*Description*	*Interaction with sex*
Transposable elements	DNA sequences that are capable of moving their location within an organism's genome. This frequently results in an increase in the copy number within the genome	• Whether an organism is sexual or asexual is expected to alter the evolutionary pressures on TEs • The degree to which organisms self-fertilize is also expected to impact the frequency of transposable elements
Segregation distorters	Elements that are active in increasing their frequency during gametogenesis. These elements distort inheritance at the level of the chromosome. These elements can be active in males or in females, either by killing non-self sperm or biasing the likelihood of their own type being present in mature female gametes	• Sperm-killing segregation distorters could be negatively affected by polyandry and sperm competition. This could select for increased polyandry to avoid the costs of mating to meiotic drive males • The spread of sex-linked drivers can rapidly alter the sex ratio of populations, thereby altering the behavior of each sex • Mating systems that allow segregation distorters to spread may be eliminated by sex chromosome drivers
Cytoplasmic male sterility (CMS)	When male sterility is driven by non-nuclear genes, for example, those contained within mitochondria. These genes are inherited solely through the maternal lineage. These genes gain a transmission advantage despite causing male sterility	• CMS results in shifts between hermaphrodite populations and gynodioecious ones. This may be a stepping stone toward evolving dioecy • Cyclical evolution between organelle CMS genes and nuclear suppressor genes can generate incompatibilities between populations
B-chromosomes	These are extra units of DNA within a cell that are not necessary to survival and replication. These supernumerary chromosomes can build up in cells during meiosis, increasing their own frequency in that lineage, despite often being deleterious to fitness	• High numbers of B-chromosomes can reduce male fertility, potentially creating sperm or pollen limitation in a population. This might also drive the evolution of increased investment in female gametes in hermaphrodites
Paternal genome elimination	This occurs when genetic material inherited from the father is either inactivated or lost after the formation of the zygote. This can result in the males being effectively haploid (parahaplodiploidy) and occurs in species of mites, mealybugs, and beetles. Maternal genome elimination is also possible	• High costs to the eliminated genome are expected to drive the rapid evolution of resistance. This coevolution may alter the genetic sex determination systems of species
Zygote killers	These systems result in a failure of the zygote to develop after fertilization. This is thought to be caused by a toxin–antidote system. The best known example of this is the medea system in flour beetles	• In a brood where many offspring die due to zygote killer incompatibility, the surviving offspring carrying the zygote killer may gain a slight advantage, due to decreased competition with their siblings. This might drive the evolution of increased polyandry
Cytoplasmic incompatibility	These incompatibilities occur when gametes are unable to form a zygote and is driven by intracellular endosymbionts. Sperm of carrier males are only compatible with eggs of infected females, meaning the infection has a transmission advantage	• Some evidence that endosymbiont carrying males have poor sperm competitive ability. Hence females might evolve to remate more often, thereby reducing their exposure to cytoplasmic incompatibility

unique about PSR is that it is transmitted only through males, making daughters a dead end for PSR. It has therefore evolved an extraordinarily damaging method of ensuring it is passed on only to sons. In *Nasonia*, males are haploid, carrying a single copy of each chromosome, while females are diploid, inheriting one copy of each chromosome from each parent. PSR eliminates all paternally derived chromosomes following the fusion of gametes, which means that individuals carrying the B-chromosome always develop into males. This ensures the continued transmission of the PSR B-chromosome, but reduces the fitness of all the other genes carried by the male to zero.

Across these diverse examples, it is the transmission advantage gained to the next generation that is central in defining SGEs. Sex, and all the diversity and variation associated with it, is responsible for how genetic material reaches the next generation. Therefore it follows that SGEs and sex are inextricably linked. This article explores four case studies of how sex and SGEs interact. Using these, we hope to highlight the diversity of interactions that occur across a range of organisms. The time frame in which these processes occur can be extremely wide and the interactions between SGEs and sex can be bidirectional (Figure 3). These emerging fields of research offer many unresolved questions and there remains exciting scope for future discoveries. More broadly, the interactions of SGEs and sex have the potential to inform a wide range of subject areas in evolution and ecology: from behavior and population ecology to genome architecture, speciation, and extinction.

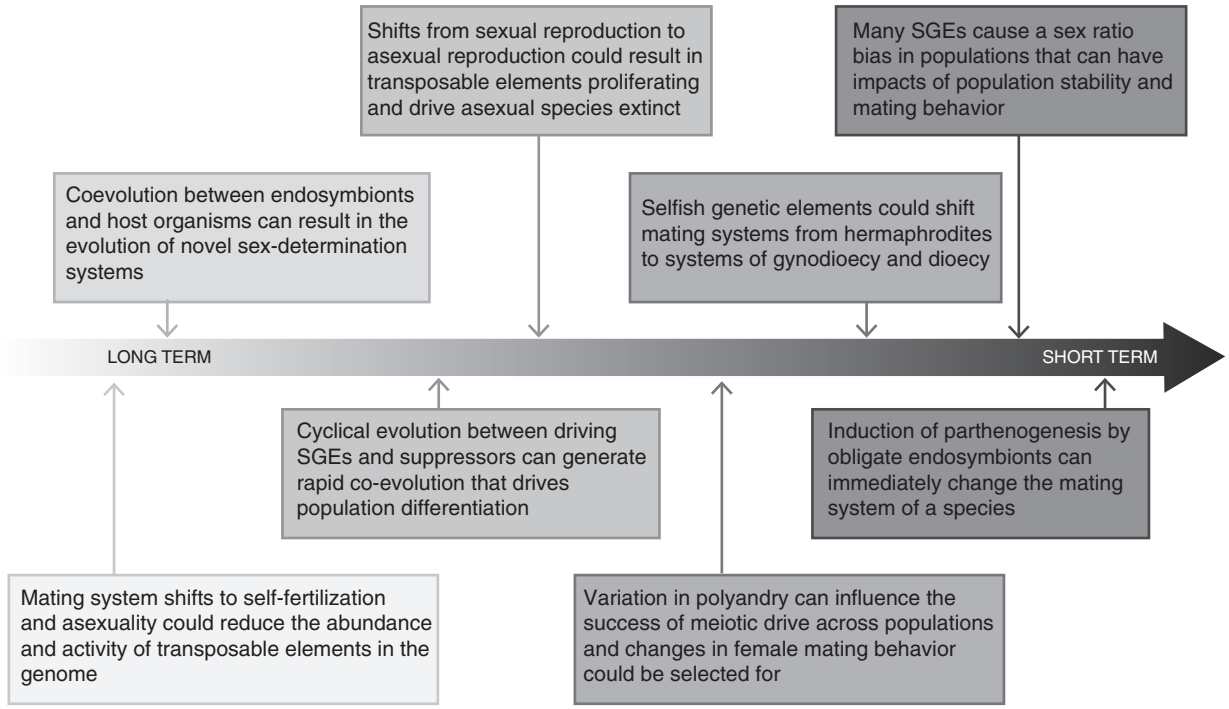

Figure 3 A timeline showing examples of the different interactions and impacts that selfish genetic elements and aspects of sex can have on each other.

Short-Term Impacts of Mating Behavior on SGEs: Sperm-Killing Meiotic Drive and Polyandry

The interaction between female remating behavior (known as 'polyandry') and sperm-killing meiotic drive provides a compelling example of how mating system can impact on an SGE. Sperm-killing meiotic drive occurs when one chromosome selfishly increases its own transmission by eliminating sperm that carry the rival chromosome during spermatogenesis. These drivers can be located on sex chromosomes, which has the added consequence of producing sex-ratio biases in broods (Jaenike, 2001). These systems have evolved repeatedly in a broad range of organisms, with classic examples in mice, mosquitos, *Drosophila*, and stalk-eyed flies (Burt and Trivers, 2006; Jaenike, 2001), with pollen drive being a parallel system in plants (Taylor and Ingvarsson, 2003). Sperm-killing meiotic drive typically results in the carrier producing only half as many sperm as a standard male. Males, however, typically produce vastly more sperm than females produce eggs. As males generally produce such huge numbers of sperm, losing half to the action of a selfish meiotic-drive element may not result in a significant fertility cost when females mate once. This is because a male will typically still be able to transfer sufficient sperm to fertilize all the females' eggs. However, in an estimated 90% of internally fertilizing animal species, females mate with more than one male, allowing sperm from multiple males to compete within females to fertilize her ova (Taylor *et al.*, 2014). In general, males that transfer more sperm to females are more successful in this competition between sperm, and father more offspring (Simmons, 2001). Therefore, if a male loses half of his sperm due to carrying a meiotic drive element, his fitness could be severely impaired if females remate frequently (Haig and Bergstrom, 1995).

Wu (1983a,b) was the first to examine sperm competition in the 'sex-ratio' ('SR') drive system in the fruit fly *Drosophila pseudoobscura*. It was shown that males carrying SR transfer half the number of sperm to female than standard males (Price *et al.*, 2014), and as a result father far fewer offspring than standard males when females mate with multiple males (Wu, 1983a,b). Subsequently, this pattern of meiotic drive males being poor sperm competitors has been found in other *Drosophila* (Angelard *et al.*, 2008; Unckless and Clark, 2014), other fly genera (Wilkinson and Fry, 2001), and mice (Sutter and Lindholm, 2015). Experimental work has also shown that when female remating is artificially suppressed then meiotic drive elements can spread rapidly through laboratory populations (Price *et al.*, 2010). A meiotic drive element at high frequency can also result in biased population sex ratios in nature (Bryant *et al.*, 1982). These changes in population sex ratio could alter per capita birth rate, affecting a population's ability to compete with neighboring populations or other species within the community (Unckless and Clark, 2014). Alternately, extreme sex-ratio biases could destabilize populations, potentially causing population extinctions (Price *et al.*, 2010).

Polyandry is extremely variable in nature, with differences in the level of polyandry between species (Taylor *et al.*, 2014), populations (Pinzone and Dyer, 2013; Price *et al.*, 2014), seasons (Torres-Vila *et al.*, 2005), and even individual females within a population (Price *et al.*, 2011). Recently, studies in two distantly related species of *Drosophila* have shown that patterns of polyandry and the frequency of meiotic drive are

Figure 4 The distribution of X-chromosome meiotic drive (outer circles) and polyandry (inner circles) in populations of two species of fruit fly, *Drosophila neotestacea* (gray circles) and *Drosophila pseudoobscura* (black circles), across North America. In both species, the frequency of meiotic drive decreases to the north, and the frequency of polyandry increases in parallel, suggesting that higher polyandry may reduce the success of the driving X (Pinzone and Dyer, 2013; Price *et al.*, 2014).

linked in populations across North America. Populations of *D. pseudoobscura* and *Drosophila neotestacea* across North America carry sperm-killing X-chromosome drivers, but the driver in each species has evolved independently. Recent work has shown that in both species the frequency of meiotic drive in a population could be predicted by the prevalence of polyandry in that population (Pinzone and Dyer, 2013; Price *et al.*, 2014; **Figure 4**). This strongly suggests that high levels of polyandry results in sperm competition that eliminates the meiotic drive from natural populations. A study of a semi-natural population of house mice in a barn in Switzerland also suggested that polyandry could explain the observed population extinction of the t-haplotype meiotic drive element (Manser *et al.*, 2011). Overall, there is compelling evidence that sperm competition plays a major role in determining the frequency of meiotic drive in males in wild populations.

The influence of mating systems on meiotic drive, however, does not necessarily have to be in a single direction. Currently, the reasons why polyandry varies within and between species are poorly understood (Taylor *et al.*, 2014). Are drivers playing a role in the evolution of mating behavior? A number of SGEs which are costly to females (including meiotic drive), also reduce the sperm competitive ability of males (Price and Wedell, 2008). As a result, increased polyandry may allow females to reduce the costs of mating with SGE-carrying males. In populations of *D. pseudoobscura* kept in the laboratory, females rapidly evolved increased polyandry in populations where they were at risk of mating with meiotic drive-bearing males (Price *et al.*, 2008). The question remains, however: can polyandry evolve as a response to the presence of an SGE in nature?

More broadly, the interaction between polyandry and meiotic drive could play a role in population stability and extinction. In the laboratory, sex-linked meiotic drive is observed to rapidly drive populations extinct through the extreme sex-ratio bias it creates (Price *et al.*, 2010), if females are forced to mate only once. The likelihood of observing localized population extinction events caused by sex-linked meiotic drive in the wild is probably low. Despite this, there is some evidence of a sex-linked meiotic driver causing a population collapse in *D. neotestacea*, in a population with little polyandry (Pinzone and Dyer, 2013). If novel sex chromosome meiotic drivers regularly evolve, then there is a risk that these could spread to a high enough frequency to drive the species extinct (Carvahlo and Vas, 1999). Recently, an X-chromosome drive system in *Drosophila simulans* originating in Southeast Africa has spread across the continent and into Europe and Asia, rapidly creating female-biased population sex ratios (Bastide *et al.*, 2011). In this case, within a few years a genetic resistance allele that prevents the killing of Y-bearing sperm also spread from the same origin, returning sex ratios to approximately equal numbers of males and females throughout Africa (Bastide *et al.*, 2013). In polyandrous species, pre-existing genetic variation in predisposition to polyandry (Price *et al.*, 2011) might allow species to rapidly evolve higher levels of polyandry in the presence of costly sex ratio distorting drive. However, in species where females remate extremely rarely or not at all (monandry), there may be little or no ability to evolve increased polyandry, potentially increasing their vulnerability to extinction by sex-ratio drive. Hence the prevalence of polyandry as a mating system might not simply be due to increased fitness of polyandrous females, but might also be influenced by selection at the population or species level (Price *et al.*, 2010). Are monandrous species more likely to go extinct than polyandrous ones, if novel sex chromosome drivers regularly evolve and spread through the species unchecked by polyandry?

Summary

Males carrying sperm-killing meiotic drivers typically have low success when females remate, because they produce fewer sperm than standard males. As a result, in populations where

Figure 5 (a) A bdelloid rotifer feeding through algae. These anciently asexual organisms harbor extremely low numbers of retrotransposons in their genome. (b) The maize genome is composed of 85% transposable element (TE)-derived genetic material. Maize is also the organism where TEs were first characterized by Prof. Barbara McClintock in the 1950s.

females mate with many males, meiotic drive is rare or absent, showing that mating system can determine the prevalence of an SGE. Monandrous species might be particularly vulnerable to extinction caused by the spread of a sex chromosome meiotic driver, potentially creating species-level selection for polyandry. However, the SGE can also impact on the evolution of mating systems – meiotic drive can cause the evolution of increased polyandry, because polyandrous females avoid many of the costs of mating with drive-bearing males.

Long-Term Impacts of Mating Systems on SGEs: Transposable Elements, Sex, and Mating Systems

TEs have been described as the most abundant genes in nature (Aziz *et al.*, 2010). They are self-replicating units that can copy themselves into other locations in the genome. As a result, they can proliferate within the genome of an individual, with TE-derived material comprising 10–90% of the genome in various species. The maize genome, for example, contains approximately 85% TE-derived material (Schnable *et al.*, 2009; **Figure 5**), while our own genome is around 50% TEs (Human Genome Consortium 2001). Meanwhile, in bdelloid rotifers many retrotransposon TEs appear to be completely absent from their genomes (Arkhipova and Meselson, 2000; **Figure 5**). Despite this abundance, harboring TEs is usually harmful (Pasyukova *et al.*, 2004), and they are implicated in human diseases, including cancer and hemophilia (Burt and Trivers, 2006; Callinan and Batzer, 2006; Hancks and Kazazian, 2012). Nevertheless, variation generated by TEs can sometimes be adaptive (Kidwell and Lisch, 1997). For example, some TEs have been implicated in telomere repair in *Drosophila* (Biessmann *et al.*, 1992). Given the huge range of TEs that exist, and the importance of their activity, understanding the general forces that influence their abundance and distributions remains a central goal in evolutionary genetics. One factor that can have a large impact on the TE dynamics in a population is the mating system of an organism (Arkhipova and Meselson, 2005; Charlesworth and Wright, 2001; Wright and Finnegan, 2001).

Both the presence of sexual versus asexual reproduction, as well as the mating systems of sexual organisms, vary enormously across eukaryotes. A species may be an obligate sexual (e.g., Humans and *Drosophila*), cyclically sexual and asexual (e.g., yeast and daphnia), or purely asexual (e.g., bdelloid rotifers). These differences in the presence and frequency of sex will impact on TE dynamics (Arkhipova and Meselson, 2005; Dolgin and Charlesworth, 2006; Wright and Finnegan, 2001). In one respect, sex allows the movement of TEs between genetic lineages. In disease, increased transmission rates tend to select for higher virulence, whereas diseases that tend to persist long term in a single host are selected for lower harm and lower virulence. Similarly, higher transmission between lineages via sex is likely to select for higher rates of transposition by TEs (Charlesworth and Langley, 1986). This suggests that asexual lineages might harbor lower numbers of TEs with lower transposition rates (Charlesworth and Langley, 1986). However, sexual lineages are also predicted to have a greater capacity for removing harmful TEs from the population via purifying selection, while the absence of sex in obligate asexual species could allow the rapid proliferation of TEs (Arkhipova and Meselson, 2005; Dolgin and Charlesworth, 2006). This potential proliferation of harmful TEs in asexual lineages, and inability to remove them via recombination, could lead to such high costs that it results in population extinction (Arkhipova and Meselson, 2005; Dolgin and Charlesworth, 2006).

In sexual species, how sex occurs is also likely to impact on the success of TEs in the genome. Sexual mating systems vary in how gametes are mixed, with some species requiring gametes of two different parents (obligate outcrossing), while others can combine gametes from the same parent (self-fertilization) (Charlesworth, 2006). Shifts between these systems have evolved repeatedly, and are expected to have important effects on the dynamics of TEs within a species (Charlesworth and Wright, 2001). For example, recessive costs of TE insertions might be more frequently exposed in self-fertilizing species due to increased homozygosity. Hence, these TEs may be purged by selection more effectively from a self-fertilizing population (Byers and Waller, 1999). The spread of TEs may also be inhibited by a lack of outcrossing, and may rapidly be lost in species with high levels of self-fertilization (Morgan, 2001; Le Rouzic and Capy, 2005; Boutin *et al.*, 2012). On the other hand, increased self-fertilization will have the effect of reducing the effective population size of a group, thereby increasing the effect of genetic drift (the stochastic fluctuation in allelic frequency due to random sampling across generations) (Charlesworth, 2006; Wright *et al.*, 2008). This reduced effective population size of self-fertilizing populations could also result in selection being less effective at removing TEs (Charlesworth and Wright, 2001; Tenaillon *et al.*, 2010). Following this argument, TE numbers might be more stochastic

immediately following the evolution of self-fertilization, while over longer time periods outbreeding populations will harbor lower numbers of TEs. Therefore, although differences between sexual and asexual species and variation in mating systems within sexual species will influence TE dynamics, there are competing theories about the direction of these effects.

The effects of asexuality on the dynamics of transposable elements have been examined in a number of model organisms. A study that introduced a TE into sexual and asexual lineages of yeast found the spread was faster and more consistent in sexual lineages, supporting the theory that sex facilitates the spread of TEs (Zeyl *et al.*, 1996). Asexual strains of the water flea, *Daphnia pulex* also carried lower numbers of TEs than cyclically sexual types (Schaack *et al.*, 2010). In contrast, an exciting study in the wasp *Leptopilina clavipes*, where infection by an endosymbiont *Wolbachia* induces parthenogenesis, found evidence of TE proliferation in the asexual types consistent with the initial spread of TEs following a shift to asexuality (Kraaijeveld *et al.*, 2012). Interestingly, this proliferation was specific to certain families of TEs. Meanwhile, a recent comparative study of the evening primrose *Oenothera* failed to find evidence for a reduction in TE abundance linked to functional asexuality (Agren *et al.*, 2014a,b). Studies focussing on how differences in outbreeding or self-fertilizing affect TE dynamics have also shown mixed results. While some studies show increased TE copy number in outbreeding species (Hu *et al.*, 2011; Morgan, 2001), others have shown either little effect or the opposite results (Dolgin *et al.*, 2008; Tam *et al.*, 2007). Across three genomes in the genus *Capsella*, the older self-fertilizing species *Capsella orientalis* (self-compatible) did show lower numbers of TEs, while the two more recent sister species *Capsella rubella* (self-compatible) and *Capsella grandiflora* (outcrossing) showed little difference in TE abundance, possibly indicating that the age of mating systems shifts is important (Agren *et al.*, 2014a,b). Overall, the relative importance of outcrossing facilitating the spread of TEs and reduced efficacy of selection allowing them to accumulate in self-fertilizing lineages remains unclear.

Sex and mating systems clearly play a role in facilitating the spread of TEs in a number of instances, but there remain exceptions. Some of these differences may be explained by the fact that these studies observe only a snapshot of the genome in time, which may be at different stages following a mating system shift. Short-term dynamics of TEs may be more stochastic, and the forces governing TE dynamics could change over time (Dolgin and Charlesworth, 2006; Boutin *et al.*, 2012; Agren *et al.*, 2014a,b). Refining the phylogenies, and better determining the evolutionary timings of mating system shifts, as well as increasing the number of study systems, will be vital to gain a broader understanding. Population parameters are also likely to be important in influencing the level of variation and the importance of genetic drift (Dolgin and Charlesworth, 2006; Tenaillon *et al.*, 2010). A number of studies reported differences between classes of transposable elements, making it likely the genomic ecology and behavior of different TEs a crucial factor. Nonetheless, how TE dynamics interact with mating systems is important to consider when approaching broader questions. What role does TE proliferation and the distribution of mating systems across species play in explaining variation in genome size (Whitney *et al.*, 2010; Agren *et al.*, 2014,a,b)? How do TE dynamics and mating system shifts contribute to differences in gene expression between related species (Hollister *et al.*, 2011)? If mating system shifts consistently change the short- or long-term genomic burden of TEs in a species, how could this impact on speciation and extinction rates (Ågren, 2013; Arkhipova and Meselson, 2005; Oliver and Greene, 2009)?

Summary

Despite their deleterious fitness effects, TEs constitute a huge proportion of the genome for many species and their numbers are highly variable between species. Models suggest that mating systems shifts have major impacts on TE dynamics, with a number of models supporting the loss of TEs in asexual or highly selfing species. A number of empirical studies have found that self-fertilizing or asexual species harbor lower number of TEs. This interaction between mating system and TE dynamics may have an impact on genome size, gene expression, mutation rate, and speciation.

Long-Term Impacts of SGEs on Mating Systems: The Case of Cytoplasmic Male Sterility

Conflict within the genome fundamentally arises when a genetic element can increase its own transmission without benefitting the rest of the genome. When genes carried by an individual have different patterns of inheritance, this can create conflicts of interest, potentially resulting in selfish behavior. A classic example of this is uniparental inheritance of organelles. Eukaryote genomes include nuclear genes, arranged on one or more chromosomes contained within the nucleus, and genes contained within other organelles (additional membrane bound cell structures other than the nucleus). Although not all organelles contain DNA, some vitally important ones such as mitochondria and plastids do. These DNA carrying organelles are typically inherited through the maternal line, and almost never from the father, which means the DNA in these organelles are not passed on through male gametes such as sperm or pollen. These organelles therefore gain no fitness by being carried by a male. Instead, these organelles increase their representation in the next generation by maximizing the number of daughters they produce. As nuclear genes are passed on through both male and female gametes, nuclear genes have a clear evolutionary interest in producing sons. This imbalance, with sons having high value for nuclear genes, but no value for organelle genes, creates a perfect scenario for intragenomic conflict and the evolution of selfish genes.

Mitochondria are found in all eukaryotes, and are essential for a range of key metabolic processes. In particular, the synthesis of adenosine triphosphate (ATP), the main molecule responsible for intracellular energy transport, is dependent on genes found only in the mitochondria. By interfering with these vital pathways, mitochondrial genes might be able to increase the proportion of offspring produced that are female, or increase the success of daughters, at a cost to the individual's ability to produce sons. In cytoplasmic male sterility

(CMS), this is exactly what happens. CMS occurs when a normally hermaphroditic plant has its ability to produce pollen drastically curtailed by the selfish action of the mitochondria it carries. In many cases, CMS eliminates all pollen production in individuals carrying CMS causing mitochondria. In these species, populations consist of hermaphrodites with non-selfish mitochondria, and females carrying CMS mitochondria, fundamentally altering the mating system in that population.

CMS is found in a very wide variety of plant species. However, the molecular mechanisms by which CMS occurs is poorly understood for most species, with only a few model systems having been well characterized (McCauley and Olson, 2008). The elimination of pollen production occurs in very distinct ways in different species, including CMS strains that convert pollen producing stamens directly into seed producing carpels, thereby clearly increasing seed production and increasing the transmission of the CMS mitochondria (Chase, 2007). However, in some CMS strains stamens are converted to petals, or pollen is produced but degrades as it matures. In these cases it is not really clear how this damage to pollen actually increases seed production or benefits the CMS mitochondria.

The costs of CMS to nuclear genes often drives the evolution of suppressors of CMS in the nuclear genome. As a result, CMS is often cryptic, and only revealed when distantly related individuals crossbreed (Budar *et al.*, 2003). This coevolution of CMS and suppressors can occur rapidly and independently, even in nearby subpopulations. Hence, CMS and suppressors can create enormous variation in mating system in different populations and over time (Bailey and Delph, 2007). There is substantial evidence that this conflict may also help produce reproductive incompatibilities between populations, and hence drive speciation (Ågren, 2013). Moreover, as suppressors seem to be effective only against one CMS type and populations can harbor several different strains of CMS, there is the potential for cycles of different CMS strains to become locally abundant, and then be suppressed by the increase in frequency of a specific nuclear suppression allele (Taylor *et al.*, 2001). Hence, it is possible that mating systems in many species may be determined by stochastic factors involving which CMS strains and suppressors happen to have been present in the founders of the population (Nilsson and Ågren, 2006).

However, CMS is also implicated in longer term changes in mating system. Gynodioecy, a mating system in which females and hermaphrodites coexist, is found in 5–10% of angiosperm plants (Renner and Ricklefs, 1995). In some cases this is likely to be driven by a balance between CMS and suppressors. However, in other cases, gynodioecy appears to be controlled by nuclear genes, with no mitochondrial involvement (Dufay and Billard, 2012). It is likely that CMS drives the initial evolution of gynodioecy in most cases, but then details of the ecology of the population can either select strongly for suppressors and a return to pure hermaphroditism, or can stabilize gynodioecy. The benefits of gynodioecy, accrued by nuclear genes as well as mitochondria, are likely to involve removing the risk of self-fertilization. CMS has not only driven a change in mating system in many species from all hermaphroditic to gynodioecy, it is also thought to be a major step in the evolutionary transition from hermaphroditic to dioecious (Touzet, 2012). However, it is still not clear how a gynodioecious species would then transition to full dioecy (Spigler and Ashman, 2012). Nevertheless, transitions from hermaphroditism to gynodioecy, and then to dioecy, are tentatively supported by the available phylogenetic data (Spigler and Ashman, 2012).

An enduring mystery of CMS is why mitochondrial elimination of male gametes in hermaphrodites seems so rare in animals. A reduction in sperm should benefit mitochondria in hermaphroditic animals if this results in a reallocation of resources and the production of increased numbers of eggs. Around 5% of animal species are hermaphroditic, so there is ample opportunity for CMS to have evolved in animals (Jarne and Auld, 2006). In contrast to this expectation, hermaphroditic animals are actually far more likely to evolve androdioecy, a mating system in which individuals are either hermaphrodite or male. The transition from hermaphroditism to androdioecy is ten times as common as the transition to gynodioecy (Weeks, 2012), with only nine gynodioecious animal species recorded, including corals, sponges, two worms, and a hagfish. The reasons why animal mitochondria seem to be unable to create gynodioecy are unclear. Most reviews suggest that the genomes of animal mitochondria are too small to evolve CMS. However, although plant mitochondrial genomes can be several megabases, compared to 16 kilobases in most animals (Touzet and Delph, 2009), and have more complex genomes with potential for recombination (McCauley and Olson, 2008), the number of proteins, rRNAs, and RNAs produced by the mitochondria are similar between plants and animals (Chase, 2007). Moreover, transmission manipulation by selfish mitochondria has been found to

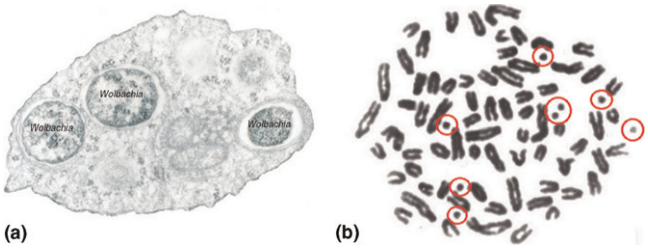

Figure 6 (a) An example of a moth cell that contains a number of bacterial cells within it from the obligate endosymbiont *Wolbachia*. (b) An image showing the karyotype (chromosome compliment) of a Siberian roe deer. Multiple supernumerary B-chromosomes carried by this individual are circled in red.

be widespread in natural populations of a roundworm (Clark *et al.*, 2012). The reasons why gynodioecy is so uncommon in animals therefore remain mysterious.

Summary

The transmission of organelle DNA only through females creates a conflict of interest between organelle and nuclear DNA, resulting in mitochondria that eliminate male gametes. These are very common in plants, and can create a mating system where individuals are either female or hermaphroditic. Rapid evolution, chance, and local ecology can cause this conflict to create differences in mating systems between subpopulations, and changes in the mating system of a single population over time. In the long term, this conflict can play a major role in shifting a species mating system from one where all individuals are hermaphrodites, to one where there are males and females.

Short-Term Impacts of SGEs on Mating Systems: Endosymbiont Manipulation

Many organisms carry intracellular endosymbionts, such as *Wolbachia*, *Cardinium*, *Rickettsia*, and *Spiroplasma* (**Figure 6**). These are microorganisms that infest cells of the host organism, and are extremely widespread and in some clades can be extremely common. They are typically inherited in the same manner as mitochondria, and have the same conflict of interest with nuclear genes, benefiting from transmission through female gametes, but gaining nothing from sons. As a result, they have evolved a wide variety of mechanisms for increasing their transmission through female gametes (Werren *et al.*, 2008). However, endosymbionts can also be beneficial to the host, with some endosymbionts providing protection for their hosts from attack by parasitoids (Jaenike *et al.*, 2010) or viruses (Hedges *et al.*, 2008). Despite the benefits of endosymbiont infection in some cases, in many or most species endosymbionts reduce the fitness of the rest of the genome. In many cases, this occurs because the endosymbiont manipulates the host to ensure it is transmitted through more female gametes. The form of this reproductive manipulation is heavily dependent on the details of the mating system of the species. However, endosymbiont infection can also transform the mating system of the host.

In many organisms, infection by endosymbionts can feminize the host individual, converting genetic males into functional females. As a result, the endosymbiont is passed on through female gametes in an individual that would usually produce endosymbiont-free sperm. However, similar to the X-chromosome meiotic drive case, feminization is likely to cause a heavily female-biased population sex ratio, and nuclear genes will lose fitness by not being expressed in males. Endosymbionts may also kill males in which they occur, if this results in the concentration of resources on their sisters and improves their fitness. In the butterfly *Hypolimnas bolina* in Polynesia, male killing endosymbionts created populations where females outnumbered males (100:1) for decades. Recently a nuclear suppressor of the male killing mechanism spread through Polynesia, rapidly returning sex ratios to a 1:1 ratio (Charlat *et al.*, 2007a; Hornett *et al.*, 2014). Although in continental Asia, males of this species compete with one another for access to females, and females mate with only their preferred males, the male killer completely altered the mating system in Polynesia, with males unable to mate with all the females they encountered and evolving to be extremely choosy, while females evolved to compete with one another for access to males (Charlat *et al.*, 2007b). Hence endosymbionts can rapidly distort sex ratios and modify mating systems, but the rapid evolution of suppression or removal of the endosymbionts may make this transitory, at least in some species.

Summary

Endosymbionts, parasitic bacteria living in the cells of their hosts, are typically passed on only through female gametes, creating a potential conflict of interest with the nuclear genome. As a result, endosymbionts have evolved a wide range of mechanisms for manipulating reproduction that reduce investment in sons and male gametes, and increase it in daughters and female gametes, an evidence that this can rapidly drive changes in sex ratio and mating system.

Final Thoughts

These four case studies demonstrate that the mating system of the host organism plays a key role in determining the arena in which SGEs operate, and so greatly influences their evolution. Moreover, SGEs can themselves alter the mating system of their host, through mechanisms such as changing sex ratios and sex determinations systems, altering the costs and benefits of multiple mating, and eliminating populations, species, or lineages that display mating systems that make them vulnerable to the spread of SGEs.

Beyond those already mentioned in this article, there exists an enormous range of known SGEs that manipulate reproduction (Burt and Trivers, 2006). SGEs have the potential to evolve whenever there is a conflict of interest over transmission of the DNA carried by an organism, making it likely that SGEs occur in all sexual organisms, and potentially all life. The vast majority of organisms have never been examined for the presence of SGEs, and many types of SGE are hard to detect, requiring multigenerational examinations of transmission and fitness. Hence it is likely that an enormous array of novel SGEs await discovery. Recent discoveries of selfish mitochondria (Clark *et al.*, 2012), sperm-based zygote killers (Seidel *et al.*, 2008) in model nematodes, and a novel form of meiotic drive in human oogenesis (Ottolini *et al.*, 2015) also support the idea that many novel types of SGE have not even been thought of, let alone searched for. As a result, it is likely that the interrelationship of mating system and SGE evolution, action, and prevalence, may be even more closely linked than currently known.

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See also: Sex, Evolution and Maintenance of. Transposable Elements, Population Genetics of

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Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth

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Glossary

Androdioecy Reproductive system in which individuals are either male or hermaphroditic.
Dioecy Reproductive system in which individuals are either male or female.
Gynodioecy Reproductive system in which individuals are either female or hermaphroditic.
Heterogamety Production of gametes with different sex chromosomes (e.g., X- and Y-carrying sperm in humans).
Homogamety Production of gametes with similar sex chromosomes (e.g., X-carrying egg cells in humans).
Recombination Exchange of genetic content between chromosomes during mitosis or meiosis.
Sex chromosomes The chromosome pair on which the master sex determination gene is located, leading to these chromosomes being transmitted differently through both sexes.
Sex determination Process directing development into a sexually functional individual.
Sexual conflict Differences between sexes in optimal fitness strategies.
Sexually antagonistic genes Genes that are under different selective forces between males and females.

Introduction

Eukaryotic genomes typically consist of several chromosomes that contain an individual's genetic information. Sex chromosomes carry the master sex-determining genes, causing them to be transmitted differently through the sexes. Consequently, they are subject to different selection forces compared to their autosomal counterparts (see **Table 1**) and follow an evolutionary trajectory that deviates from the rest of the genome (Rice, 1988). It is generally accepted that sex chromosomes originate from autosomes by acquiring a sex determination function.

In diploid heterogametic systems, which are found in most animals and some plants, one sex chromosome, referred to as the major sex chromosome, is transmitted through both sexes. The other sex chromosome, referred to as the minor sex chromosome in the heterogametic sex, is sex-limited in its inheritance. For example, in humans the X chromosome is transmitted through both sexes, whereas the Y chromosome is limited to males. Over time, the minor sex chromosome reverses to a mostly haploid and clonal lifestyle and adapts to its sex-limited role (Rice, 1988; Bachtrog, 2013). However, the lack of recombination leads to the degeneration of the chromosome, as mutations accumulate and genes are lost. In humans, the Y chromosome is much smaller than the X chromosome, and carries only few functional genes. Due to their specific genomic niche and inheritance pattern, sex chromosomes can play important roles in processes such as speciation, sexual selection, and genomic conflict (e.g., Werren and Beukeboom, 1998; Kirkpatrick and Hall, 2004; Presgraves, 2008; Demuth *et al.*, 2014).

Sex chromosomes are found in a wide variety of organisms, ranging from fungi to plants and animals. They are associated with diverse sex determination mechanisms, though several sex determination mechanisms without sex chromosomes also exist (reviewed in Beukeboom and Perrin, 2014; Bachtrog *et al.*, 2014). Systems with sex chromosomes can be classified in three distinct groups. First, in XY systems, which are found

Table 1 Features of sex chromosomes in comparison to autosomes. Sex chromosomes are responsible for sex determination in the haploid (U and V) or diploid (W, X, Y, and Z) phase of the life cycle and are transmitted through one or both the sexes. Recombination between different types of sex chromosomes is suppressed. Sex chromosomes have different effective population sizes compared to autosomes

	Y/W	*Z/X*	*U*	*V*	*Autosome*
Phase in life cycle in which sex is determined	diploid	diploid	haploid	haploid	not applicable
Sex-specific inheritance	yes, Y through males, W through females	no, but X twice as often through females, Z twice as often through males	yes, through female gametophyte	yes, through male gametophyte	No
Recombination	suppressed	unsuppressed in the homogametic sex, suppressed in the heterogametic sex	suppressed	suppressed	yes
Effective population size	1/4	3/4	1/2	1/2	1

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in most mammals and insects, sex is determined in the diploid phase of the life cycle. In these systems, males are the heterogametic sex. Second, ZW systems are similar to XY systems, but instead the female is the heterogametic sex. Organismal groups in which ZW systems are found include birds, butterflies, and moths. Third, in UV systems sex is determined in the haploid phase of the life cycle, with the U chromosome in females and the V chromosome in males. UV systems are found in, for example, fungi, algae, and mosses. Some organismal groups, such as fish and amphibians, are rather diverse when it comes to sex chromosome systems. In such groups, some species have XY systems, whereas others have a ZW system. In some cases, the Y or W chromosome is absent. Such systems are referred to as XO and ZO, meaning that the heterogametic sex produces one type of gamete with and one type of gamete without the major sex chromosome. XO systems are frequently found in insects such as beetles and grasshoppers; ZO systems are less common. Finally, systems with multiple sex chromosomes are also known, but these are rare.

The division in homo- and heterogametic sexes stems from the fact that one sex carries two identical (homomorphic) sex chromosomes that are inherited equally to sons and daughters, whereas the other sex carries one sex chromosome that inherits to sons and one that inherits to daughters only. In male heterogametic systems, the male has one X and one Y chromosome, and the female two X chromosomes; the Y chromosome is male-determining. In female heterogametic systems, the female carries one Z and one W chromosome, and the male two Z chromosomes; the W chromosome is female-determining. As the sex-limited chromosomes are often different in size and genetic composition (heteromorphic), this is called the heterogametic sex.

Acquisition of a Sex-Determining Function

Several phases can be distinguished in the evolution of sex chromosomes. Sex chromosomes are formed when an autosome gains a sex-determining function (Charlesworth *et al.*, 2005; Pease and Hahn, 2012, but see Carvalho *et al.*, 2009), typically by acquiring genes causing carriers to develop into a specific sex. This can happen through translocation of existing sex determination (SD) genes from other chromosomes, fusion of an autosome with an existing sex chromosome, or through de novo origin of an SD gene, such as the mammalian *SRY* (*sex-determining region Y*) gene which came into being by mutation of the *Sox3* gene in the ancestor of therians some 150 million years ago. Chromosomes that have recently gained a sex-determining function are referred to as proto-sex chromosomes or neo-sex chromosomes.

Hermaphrodites may give rise to separate sexes, thereby producing proto-sex chromosomes (Charlesworth and Charlesworth, 1978; Charlesworth, 2013). In this scenario, a loss-of-function or sterility mutation may cause hermaphroditic individuals to develop into one sex; a subsequent mutation in the remaining hermaphrodites may then cause offspring to develop into the other sex. Such mutation may first lead to development of females (gynodioecy) or males (androdioecy) depending on which sexual traits are affected by these loss-of-function mutations, before complete dioecy is established (see Charlesworth and Charlesworth, 1978; Charlesworth, 2013 for further details).

Sex chromosomes may support different forms of sex determination. One possibility is that they carry a dominant gene for maleness (Y) or femaleness (W), such as the mammalian *SRY* (Berta *et al.*, 1990), *DMY* in fish (Matsuda *et al.*, 2002), or M-factors in houseflies (reviewed in Dübendorfer *et al.*, 2002). Another possibility is that sex chromosomes are being counted, one sex having two copies and the other only one (X or Z). This is, for example, the case in *Drosophila* (reviewed in Cline, 1993; see also Ericksson and Quintero, 2007), and may be rather common given the frequency of XO and ZO systems in nature.

Though the associated genetic mechanisms of sex determination and the selective forces driving their evolution may differ substantially between species, clear similarities can be distinguished in the evolution of proto-sex chromosomes (reviewed in Bachtrog *et al.*, 2014). After they have been formed, proto-sex chromosomes may undergo rapid evolution as a result of their different transmission through the sexes (Rice and Holland, 1997; Charlesworth, 2013), with the minor sex chromosome becoming haploid and restricted to one sex.

Sexual Conflict and Sex Chromosomes

Males and females often experience different selective pressures. Because sex chromosomes are transmitted differently through the sexes, they are a potential hotspot for genes that are under different selective forces in males and females (Rice, 1984). A locus that is selected differently depending on its genetic background experiences genetic conflict (Rice, 1987a; Werren and Beukeboom, 1998; Stewart *et al.*, 2010). Selection on autosomes will be toward optima intermediate to those experienced by both sexes, whereas for sex-linked genes selection will lead to the optimum experienced by the sex to which these genes are linked. Rice (1984, 1994, 1998) showed that exclusive transmission of a chromosome through one sex can lead to accumulation of alleles on that chromosome that are beneficial for that sex but detrimental for the other sex. Such genes that experience differences in selection sign or strength between sexes are referred to as sexually antagonistic (SA) genes. When linked to an SD gene, SA allele frequencies may shift between the sexes, allowing for the resolution of genetic conflict (Rice, 1987a). However, resolving genetic conflict between the sexes may not necessarily require translocation to the sex chromosomes. Gene expression may already be dependent on sex (Stewart *et al.*, 2010), allowing for divergence of gene expression and conflict resolution. The association between SD and SA genes may be explained by an SD gene evolving near SA genes, which may be favorable to the spread of the new proto-sex chromosome (Van Doorn and Kirkpatrick, 2010). However, enrichment in SA genes on sex chromosomes relative to autosomes suggests that these genes were translocated from other genomic regions to the sex chromosome (Mank, 2012).

Though genetic conflict theory predicts that the X and Y chromosomes become enriched for female- and male-beneficial genes respectively, this need not necessarily be true. A male-beneficial gene may spread when X-linked provided that it is at least partially recessive (Rice, 1988; Graves, 2006). In males, such a gene may easily have its beneficial effect as it is

hemizygous, whereas in females the deleterious effects may be sheltered by dominant alleles. Similarly, the X chromosome may be enriched for female-beneficial genes, though these are only expected to spread easily when sufficiently dominant (reviewed in Ellegren and Parsch, 2007).

Suppression of Recombination

Why Does Suppression of Recombination Occur?

The suppression of recombination along the minor sex chromosome is a key step in sex chromosome evolution. It prevents gene flow between the sex chromosomes and leads to the non-recombining region becoming sex-limited and thus effectively haploid (Charlesworth, 1978). This causes progressive divergence between the sex chromosomes and leads to the degeneration of the minor sex chromosome (see also below).

Late-stage minor sex chromosomes typically no longer recombine along the majority of its length, except for in a small terminal region known as the pseudoautosomal region (PAR, reviewed in Otto *et al.*, 2011). The PAR may be involved in obligate recombination during meiosis; however, its absence in some species shows this is not a universal requirement. The discovery of several evolutionary strata on therian X chromosomes (Lahn and Page, 1999; Nicolas *et al.*, 2005; Roesti *et al.*, 2013), as well as differing degrees of divergence between X- and Y-linked genes (Chibalina and Filatov, 2011; Zhou and Bachtrog, 2012b; Natri *et al.*, 2013) suggest that recombination does not halt abruptly, but rather spreads along the chromosome in a stepwise or gradual fashion. Suppression of recombination will start at the sex-determining region (SDR) for two possible reasons. First, crossovers in the SDR may be selected against because it results in improper sex determination. Second, breaking up the association between SD and SA genes (Rice, 1987a) leads to SA genes being passed on to offspring that will develop into the sex in which they have a deleterious effect, which will be selected against. Tight linkage between SD and SA genes allows for stronger sex-specific adaptation and thus suppressed recombination may be favorable (Muller, 1964; Nei, 1969). As more and more SA genes accumulate on the sex chromosomes, this suppression of recombination is believed to spread outward, until the full chromosome experiences suppressed recombination. It should be noted that this theoretical model of SD and SA gene interaction is broadly accepted but that the experimental support is still weak.

How Is Recombination Suppressed?

The proximate causes of recombination suppression are not well understood, though several mechanisms have been proposed that may explain how it evolves. First, recombination rates often differ between sexes (Burt *et al.*, 1991), which may suggest the presence of recombination-suppressor genes as postulated by Nei (1969). Such a (local) suppressor gene may spread provided that it is linked to a beneficial combination of genes (e.g., SD and SA genes). A sex-linked global recombination suppressor would explain the marked differences in recombination rates between sexes, as this would lead to reduced recombination in one sex.

Second, extensive methylation is known to inhibit recombination (Maloisel and Rossignol, 1998; Melamed-Bessudo and Levy, 2012). Increased methylation in the SDR of the proto-sex chromosome may lead to reduced recombination levels between SD and SA loci (Maloisel and Rossignol, 1998; Gorelick, 2003; Melamed-Bessudo and Levy, 2012). This is in accordance with findings that in some species, for example sticklebacks, recombination between the sex chromosomes stopped in a very gradual fashion (Chibalina and Filatov, 2011; Natri *et al.*, 2013; Roesti *et al.*, 2013).

Third, sufficient levels of sequence divergence between homologues reduce recombination rates. Though sequence divergence appears unlikely to evolve between recombining chromosomes, such divergence can be accelerated by chromosomal inversions (Andolfatto *et al.*, 2001). Inversions have been proposed as an explanation for evolutionary strata on sex chromosomes in various species (e.g., Lahn and Page, 1999; Wright *et al.*, 2014).

Extensive methylation and inversions can cause local suppression of recombination and can be regarded as specialized forms of the suppressor-gene model (Nei, 1969). These mutations may spread provided they are linked to a beneficial combination of genes. Theoretical studies have shown that this is indeed true for inversions (Kirkpatrick, 2010), but similar results are expected in case of extensive methylation.

Sex Chromosome Degeneration

Recombination and Selection

Though suppressed recombination may allow for faithful transmission of co-adapted gene complexes, it also induces the degeneration of the minor sex chromosome. Various models describe how suppressed recombination induces degeneration (**Figure 1**). These can be classified according to two effects of recombination: (1) the creation of novel genotypic combinations and (2) the ability to select on loci separately. Genome-wide effects include Muller's ratchet, a process that leads to the random loss of mutation-free chromosomes, and Hill–Robertson interference, the inability to combine beneficial mutations if these arise on separate chromosomes. Locus-specific processes refer to the interdependence between fitness effects of loci and their genetic background.

Muller's ratchet is considered to be the main cause of mutation accumulation (Muller, 1918; Muller, 1964). It assumes that mutations occur readily, that these mutations are typically near-neutral and thus do not suffer from selection until the mutational load transgresses a certain threshold (see also Rice, 1996). Recombination may lead to the formation of chromosomes with a lower mutational load compared to the ancestral chromosomes. Without recombination, each chromosome will accumulate mutations over time, mutation-free chromosomes will become increasingly rare, and may eventually be lost due to genetic drift. When such mutation-free chromosomes have been lost, the ratchet has turned a single click; compared to the ancestral chromosome, the least-loaded chromosome now carries at least one mutation. Subsequent clicks of the ratchet then lead to loss of all chromosomes carrying one mutation, etc.

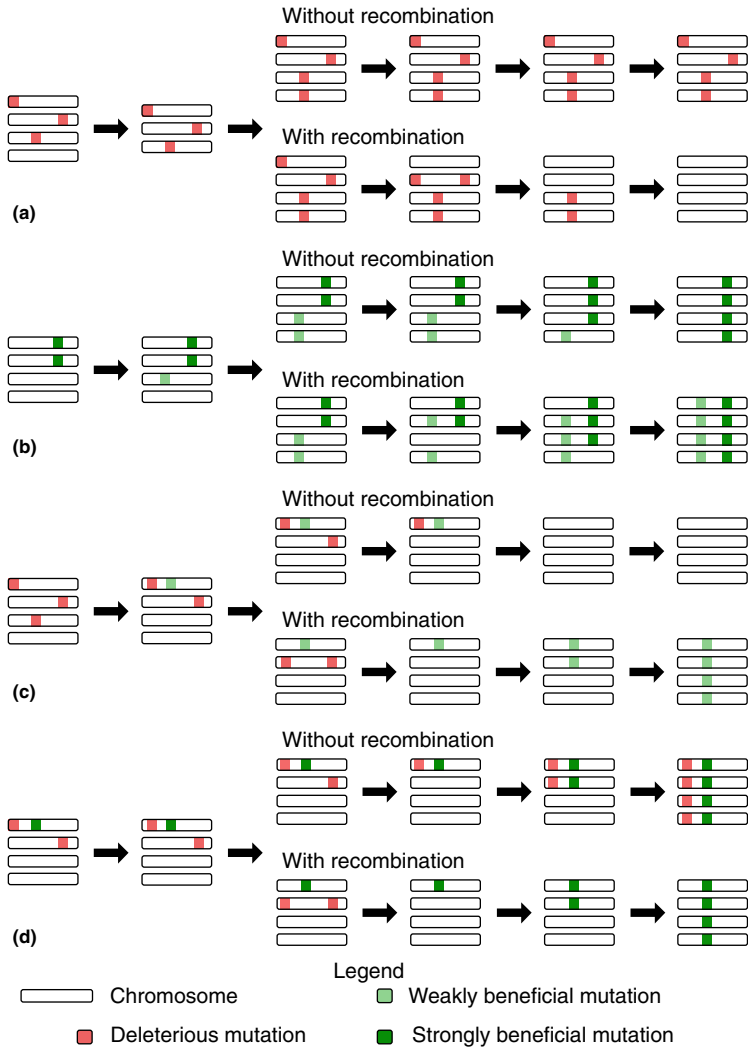

Figure 1 Recombination and chromosomal degeneration. Different models may contribute to degeneration of non-recombining chromosomes. (a) Muller's ratchet. Mutation-free chromosomes cannot be restored when lost stochastically. (b) Hill–Robertson interference. Beneficial mutations cannot be combined if they arose on different chromosomes. (c) Background selection. Beneficial mutations may not spread if they are linked to deleterious mutations. (d) Genetic hitchhiking. Deleterious mutations may spread when linked to beneficial mutations. See text for further details. Adapted from Bachtrog, D., 2013. Y-chromosome evolution: Emerging insights into processes of Y-chromosome degeneration. Nature Reviews Genetics 14, 113–124, with permission from Macmillan Publishers Ltd, © 2013.

Hill–Robertson interference refers to the inability to combine beneficial mutations in the absence of recombination (Hill and Robertson, 1966). In natural populations, beneficial mutations spread as a result of selection. In case such mutations have not yet become fixed, a novel beneficial mutation may occur on a chromosome which does not yet carry a beneficial mutation. Recombination in double heterozygotes may then create a chromosome harboring both beneficial mutations, whereas without recombination, these mutations will compete with one another (Hill and Robertson, 1966; Charlesworth and Charlesworth, 2000). Natural selection will then lead to fixation of the more beneficial mutation, effectively purging the other mutation.

The second effect of recombination, the ability to select on loci separately, is akin to Hill–Robertson interference. Without recombination, ancestral combinations cannot give rise to novel combinations and thus each mutation is restricted to the genetic background it arose in. Consequently, selection can only act on the combined effects of mutations and their respective backgrounds (Rice and Chippindale, 2001); beneficial mutations may be lost due to linkage to deleterious mutations (background selection, Charlesworth *et al.*, 1993), whereas deleterious mutations may spread when linked to strongly beneficial mutations (genetic hitchhiking, Rice, 1987b).

Background selection occurs when a weakly beneficial mutation is lost because it is unable to overcome the negative selection it faces from linked deleterious mutations (Charlesworth *et al.*, 1993; Rice, 1996). An example would be when a weakly beneficial mutation arises on a chromosome already experiencing a relatively high mutational load due to Muller's ratchet. Initially, this may spread, but as time progresses, each copy will only become associated with higher mutational loads as Muller's ratchet turns. As a result, it will eventually transgress the selection threshold and thus, the beneficial

mutation will be lost. With recombination, such a weakly beneficial mutation may end up on a chromosome with a smaller load, where it is able to spread due to natural selection.

Inversely, genetic hitchhiking describes how strongly beneficial mutations may spread and drag along deleterious mutations (Rice, 1987b). Similar to the scenario for background selection, it assumes that several weakly deleterious mutations may already be present on a chromosome due to Muller's ratchet. A strongly beneficial mutation on this chromosome may still become fixed, provided that its net fitness transcends the fitness of chromosomes lacking this mutation, even when having a lower deleterious mutation load. This in turn may help to accelerate Muller's ratchet (Bachtrog, 2008); the mutational load of a chromosome may increase, but a strongly beneficial mutation reduces the selection pressure on this chromosome. As a result, it spreads through the population, eventually outcompeting the ancestral mutation-free chromosome.

The effects of these population genetic processes are amplified by the reduced effective population size of the sex chromosomes (**Table 1**). The ratio between autosomes and X chromosomes is 4 to 3 per mating pair, whereas this is only 4 to 1 for Y chromosomes (and 2 to 1 for both U and V chromosomes; Rice, 1988). This strongly increases the effect of genetic drift and may further accelerate Muller's ratchet.

Males typically experience stronger sexual selection than females. These effects may be further intensified for the male-limited Y and V chromosomes (reviewed in Bachtrog *et al.*, 2011), whereas they may be relatively relaxed on female-limited U and W chromosomes. However, in UV systems and plants, the degeneration of sex chromosomes is often slower compared to diploid animals. This is likely due to purifying selection in the haploid phase (Bergero and Charlesworth, 2011; Chibalina and Filatov, 2011). In plants, gene expression during meiosis of sex-linked genes is much higher than in animals, whereas species with UV systems are haploid during most of their life cycle (e.g., Ahmed *et al.*, 2014). This would prevent sheltering of deleterious mutations as seen in diploids, resulting in higher selection pressure against deleterious mutations, thereby slowing down degeneration (Bergero and Charlesworth, 2011; Chibalina and Filatov, 2011; Charlesworth, 2013).

Intralocus Competition and Selective Gene Silencing

The overall genetic inertness of the Y chromosome (and likely also the W chromosome, though much less data are available) suggests that its degeneration is associated with gene inactivation. Gene silencing may readily occur through transpositional inactivation (Steinemann and Steinemann, 1992, 1998). Though typically deleterious, high mutation rates induced by transposons may allow for rapid inactivation of genes involved in intralocus competition (Rice, 1987b). However, silencing of Y-linked alleles may only be favored if there are no negative dosage effects, or if dosage compensation has readily evolved (Kaiser *et al.*, 2011; see also Mank, 2013). Some genes may be dosage-sensitive and silencing Y-linked alleles without upregulation of X-linked alleles may cause deleterious effects, preventing gene silencing. The link between the evolution of dosage compensation and mutational silencing of Y-linked genes requires further theoretical and empirical study.

Gene Content of Late-Stage Sex Chromosomes

Sex chromosome maturation is associated with major changes in their gene content. Depending on the genetic mechanism of sex determination, variable numbers of sex determination genes may be present on the sex chromosomes. Sex determination genes may be single dominant loci such as *SRY* in mammals (Berta *et al.*, 1990), or they may represent quantitative loci that direct sex determination depending on their dose (e.g., X-linked signal elements as found in *Drosophila melanogaster*, Cline, 1993).

Though predicted to play a major role in sex chromosome evolution, SA genes have proven difficult to be identified. An excessive number of genes involved in male reproductive functioning are sexually antagonistic in *D. melanogaster* (Innocenti and Morrow, 2010) and *Drosophila miranda* (Zhou and Bachtrog, 2012a). Late-stage sex chromosomes are often enriched for genes involved in reproductive processes, such as fertility genes or genes expressed specifically in the gonads (e.g., Skaletsky *et al.*, 2003; Bellott *et al.*, 2010). However, whether such genes are commonly under sexually antagonistic selection is unknown and requires further investigation in other species. In addition, some sex chromosomes show enrichment for genes that are under sexual selection, such as color genes in poeciliids (Kallman, 1970), cichlids (Streelman *et al.*, 2003), and medaka fish (Wada *et al.*, 1998). Possibly, such genes represent additional types of SA genes, as they may be harmful in females for their increasing conspicuousness.

SD genes or genes that confer large fitness benefits are less prone to degeneration. Due to strong selection on these genes, mutations are more likely to have large deleterious effects and thus Muller's ratchet may not operate efficiently, as natural selection will purge such mutations quickly. However, other genes may degenerate, leading to increased sex-specific bias in the remaining gene content on the minor sex chromosome that is typical of late-stage minor sex chromosomes (Y chromosome masculinization or W chromosome feminization).

Though sex chromosomes may acquire genes through translocation, they may also lose genes. Typically, this concerns genes that are located on the major sex chromosome, but are beneficial to or only functional in the heterogametic sex (Wu and Xu, 2003). Genes expressed specifically in the gonads of the heterogametic sex or genes involved in their gamete production were shown to move away from the major sex chromosome in several species (e.g., Emerson *et al.*, 2004; Vibranovski *et al.*, 2009). Coupled with the accumulation of SA genes that confer fitness benefits to the homogametic sex, this leads to feminization, respectively masculinization, of the X and Z chromosomes.

Degeneration-Driven Loss and Turnover of Sex Chromosomes

Does Degeneration Lead to Loss of the Minor Sex Chromosome?

With the plethora of degenerative forces acting upon the minor sex chromosome, it has been predicted that the Y

chromosome will fade away and eventually be lost (Graves, 2006). As a result, males would be lost from the population and extinction would be imminent. These predictions are however based on several incorrect assumptions.

First, degeneration is not constant. It may be rapid on young proto-sex chromosomes, but slows down over time as gene numbers dwindle (Rice, 1988; Bachtrog, 2008; Hughes *et al.*, 2012). Consequently, the probability that a deleterious mutation occurs becomes increasingly small, asymptotically approaching zero. Though degeneration may lead to a highly reduced gene content on the Y chromosome, those genes that remain are usually crucial for male reproduction (e.g., Lahn and Page, 1997). These genes are under strong purifying selection and thus, mutations in such genes may be purged very rapidly, preventing the loss of such genes. Second, gene content of the Y chromosome need not only decrease (see also Graves, 2006); translocation of genes may increase gene content, though the effect on halting degeneration may be limited. The degeneration cycle may be reset by formation of neo-sex chromosomes by fusion between a sex chromosome and an autosome. This increases gene content and allows for partial rejuvenation of the minor sex chromosome (Kitano and Peichel, 2012). Alternatively, sex chromosome turnover leads to creation of novel sex chromosomes, effectively resetting the cycle (see also below). Finally, degeneration may not be as inevitable or irreversible as implied. Novel insights reveal that in many species, sex chromosomes remain homomorphic for extended periods of time, suggesting the existence of mechanisms which counteract degeneration.

Barring all else, loss of the Y chromosome need not initiate population extinction. The widespread occurrence of X0 systems in nature suggests that not all Y chromosomes are essential for male development. Such Y chromosomes may be readily lost, provided that any essential genes originally contained on the Y chromosome have translocated.

Escape from Degeneration and Neo-Sex Chromosomes

Several mechanism may allow for sex chromosomes to escape the degenerative forces acting upon them. Recombination rates often depend on phenotypic rather than genotypic sex, and in some organismal groups, such as amphibians and fish, sex reversal may occur occasionally (reviewed in Mank, 2012). In sex-reversed individuals, recombination may occur between the sex chromosomes provided that divergence between the sex chromosomes is still relatively minor. Occasional rounds of recombination may effectively reverse Muller's ratchet and stop other degenerative processes. Sex reversal may thus function as 'foundation of youth' (Perrin, 2009), effectively allowing the sex chromosomes to circumvent degeneration and decrease their mutational load.

Intra-chromosomal recombination is another mechanism that could prevent degeneration of vital genes. The human Y chromosome contains eight palindromic regions with duplicated genes (Skaletsky *et al.*, 2003). Gene conversion by non-homologous crossover between these duplicated copies allows for removal of mutations (Rozen *et al.*, 2003), conserving duplicated Y-linked genes and reducing degeneration.

Sex Chromosome Turnover and SD System Transitions

As degeneration continues, the mutational load of the Y chromosome increases. At some point, sex chromosome turnover may become selectively favorable compared to retention of ancestral sex chromosomes. Such turnovers can come about in a variety of ways, such as translocation of the SD gene to an autosome resulting in a novel proto-sex chromosome or fusion of a sex chromosome with an autosome resulting in neo-sex chromosomes. High rates of sex chromosome turnover have been described in fish, reptiles, and amphibians (reviewed in Kikuchi and Hamaguchi, 2013) and translocation of the SD gene occurs spontaneously in some species (e.g., Traut, 2010).

Turnovers in sex chromosomes are hindered by the fact that the ancestral Y chromosome often contains many male-essential genes (see also above). Turnover may therefore only occur if the novel Y chromosome confers an especially large fitness benefit, for example when the SD gene maintains an association with one or more SA genes (van Doorn and Kirkpatrick, 2007). As such, it is still unknown whether the spread of novel proto-sex chromosomes occurs when a sex determination gene lands next to an SA gene or whether SA genes land next to SD genes.

Additionally, sex chromosome turnover may be caused by changes in sex determination mechanisms induced by meiotic drive (Kozielska *et al.*, 2010). If sex chromosomes exhibit meiotic drive, sex ratios will become distorted. Balancing sex ratio selection will then favor drive-suppressing mutations or mutations which cause individuals to develop into the rarer sex. In the latter case, this may lead to transitions in sex determination systems and sex chromosome turnover if a different chromosome acquires the master sex-determining gene.

Conclusions and Open Questions

Sex chromosomes have their own genomic niche and evolve in peculiar ways (**Figure 2**). Starting as autosomes, they become proto-sex chromosomes when they acquire a sex-determining function. They mature as sex chromosomes through a complex interplay between SD and SA genes and recombination suppression, ultimately resulting in their degeneration. Much of what we know about sex chromosome evolution is inferred from theory. Experimental investigation of sex chromosome evolution has been hampered by lack of tractable systems and variation within or between closely related species. However, some promising systems that have recently yielded important new insights are several fish species, such as medaka (Wada *et al.*, 1998) and sticklebacks (Natri *et al.*, 2013) as well as insect species such as houseflies (Hamm *et al.*, 2009; Feldmeyer *et al.*, 2008) and *Drosophila* species (Zhou and Bachtrog, 2012a, 2012b).

The role of SA genes in sex chromosome evolution has been firmly established by theoretical models, but the empirical evidence is still meager. It is still disputable how widespread SA genes are, as they have to date been identified in only a limited number of species, despite the fact that many genes are under different selection pressures between the sexes (reviewed in Cox and Calsbeek, 2009).

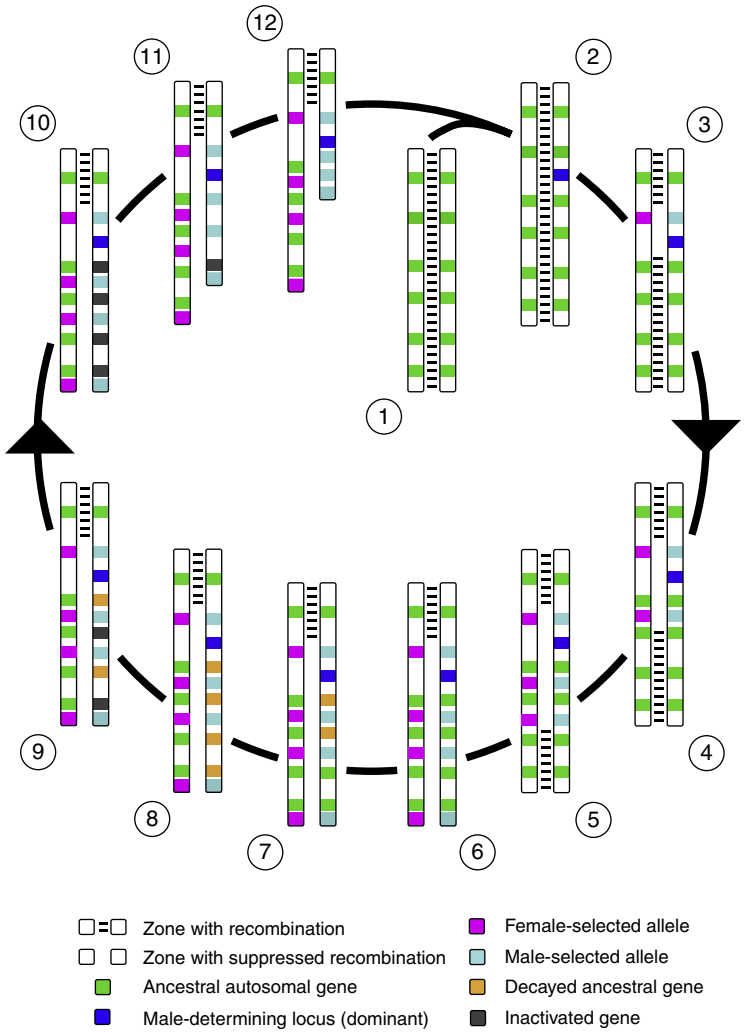

Figure 2 Steps in sex chromosome evolution. Sex chromosomes are derived from autosomes (1), and become proto-sex chromosomes when they acquire a sex-determining function (2). They mature as sex chromosomes as SA genes accumulate and recombination becomes suppressed (3–6). Non-essential genes may decay as they accumulate mutations, leading to reduced functionality (7–8). Decayed genes will be selectively silenced (9–10) and large deletions may lead to chromosome shrinkage (11–12). A novel sex chromosome may be formed when another autosome becomes a proto-sex chromosome, thereby resetting the cycle. Adapted from Bachtrog, D., 2013. Y-chromosome evolution: Emerging insights into processes of Y-chromosome degeneration. Nature Reviews Genetics 14, 113–124, with permission from Macmillan Publishers Ltd, © 2013.

Comparative genomics may help to discover SA genes and to determine whether they have been preferentially translocated to the sex chromosomes. Alternatively, they may act as landing sites for new SD genes in the origin of proto-sex chromosomes. Additionally, transcriptomic analyses may reveal differences in gene expression between the sexes, thereby increasing the power to identify SA genes. More empirical testing of SA effects, for example by manipulating inheritance of sex chromosomes through one sex, will be required to validate the role of SA in sex chromosome evolution.

The advent of the omics era has led to the development of a wide array of tools that may help to further disentangle evolutionary processes of sex chromosomes. Neo-sex chromosomes have proven useful in discerning which processes contribute to sex chromosome degeneration (e.g., Zhou and Bachtrog, 2012b; Zhou *et al.*, 2012). Comparisons between neo-sex chromosomes of various ages will reveal the extent to which the various processes outlined above contribute to the degeneration of sex chromosomes and may elucidate the interplay between degeneration and dosage compensation (Zhou and Bachtrog, 2012a).

The mechanisms of progressive suppression of recombination are not yet well understood. The discovery of novel proto-sex chromosomes and neo-sex chromosomes that still display recombination may allow for more in-depth analysis of how recombination becomes suppressed. Multi-factorial systems such as that of the housefly may be very promising in this respect (Feldmeyer *et al.*, 2008; Scott *et al.*, 2014).

In conclusion, insights in sex chromosome evolution have been much progressed over recent years. However, there still is an emphasis on theoretical approaches, while testing these theories has proven difficult. With the omics era in full bloom,

the field of sex chromosome evolution promises to be an exciting field of research in which novel insights can readily be gained.

See also: Gene Origin, Sex Chromosomes and. Recombination and Selection. Sex Determination. Sex, Evolution and Maintenance of. Sexual Conflict

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Sex Determination

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Glossary

Anisogamy Form of sexual reproduction where the gametes differ in size or structure. The most familiar type of anisogamy is oogamy where females produce a large non-motile egg and males produce small motile sperm.

Dosage-dependent sex determination Sex determination system where the transcription level of certain genes determines whether an individual develops as female or male.

Gonochoristic Having only one of the two possible sexes in any individual organism. In contrast to hermaphroditic where any single individual can function as both male and female.

Hermaphrodites Individuals capable of producing both oocytes and spermatocytes, either as (1) 'simultaneous hermaphrodites' producing both types of gametes at the same time, or as (2) 'sequential hermaphrodites' which can only produce one type of gamete at a given time, but switch sex at some point during their lifetime.

Heterogametic Individual that produces gametes with different sex chromosome complements.

Master switch Gene that acts as the initial trigger of female or male development. Sometimes also referred to as feminizers (causing female development) or masculinizers (causing male development).

Mating types A mechanism that determines the compatibility of individuals in a population to reproduce with one another. Mating types are common in fungi, and the number of different mating types in a population may range from two to many hundreds.

Sex chromosome (X and Y, Z and W) In heterogametic sex determination system, the pair of chromosomes that are responsible for sex determination. In male heterogametic species the male will have an XY complement and females an XX complement. In female heterogametic species the male will have a ZZ complement and females a ZW complement.

Sex determination cascade A process where the transcription of a specific allele or the transcription level of a gene regulates a series of genes downstream culminating in the genes that are responsible for the development of an individual as either female or male.

The prevailing view of reproduction is that of a female and male mating to produce offspring. This is indeed the case in most animals, some plants and even some unicellular organisms. But does reproduction always work this way? How did the sexes originate? And how is an individual's sex determined? These simple but fundamental questions are key to our understanding of evolution across the tree of life.

A Brief History of the Sexes

Sexual reproduction is an ancient feature of eukaryote life, yet the sexes as we currently recognize them are relative latecomers in the evolution of sex (Beukeboom and Perrin, 2014). The ability to reproduce through sex (fusion of haploid gametes) evolved in the common ancestor of all eukaryotes, but did not involve separate sexes; each individual was able to exchange genetic material with any other of the same species, as is still the case in many modern-day unicellular eukaryotes (Lahr *et al.*, 2011). However, throughout the course of evolution this changed in certain organisms: some, for example many fungi, evolved tens or even hundreds of 'mating types,' where certain genotypes become incompatible with others (Perrin, 2012). In others 'anisogamy' evolved: here individuals produce not one but two different types of gametes, a large type – 'oocytes' in low frequency and a more prevalent smaller type – 'spermatocytes.' Anisogamy is thought to evolve because an individual with limited resources has two options to maximize their reproductive success: either by maximizing the number of gametes they produce, or by maximizing the survival probability of each of their gametes (Parker *et al.*, 1972). In many cases an individual can produce both these types simultaneously and either self-fertilize or mate with others to exchange gametes ('hermaphroditism'), yet some – for example, most animals and some plants – specialized on producing one or the other, giving rise to the sexes as we currently recognize them (Jarne and Auld, 2006; Bawa, 1980).

How Sex Is Determined?

But how is an individual's sex determined? The answer to this question is far more complex than you might think. Sex-determining mechanisms are surprisingly diverse and evolutionary biologists have only just started to understand what causes this variation (Beukeboom and Perrin, 2014). Here we will start by describing some of this variation and how it is distributed across different organisms. We only focus on sex determination in species with true separate sexes, not those with mating types, and primarily focus on sex determination in multicellular organisms.

Genetic Sex Determination

Perhaps the most familiar sex determination systems are those that employ 'heterogametic' sex determination where a species

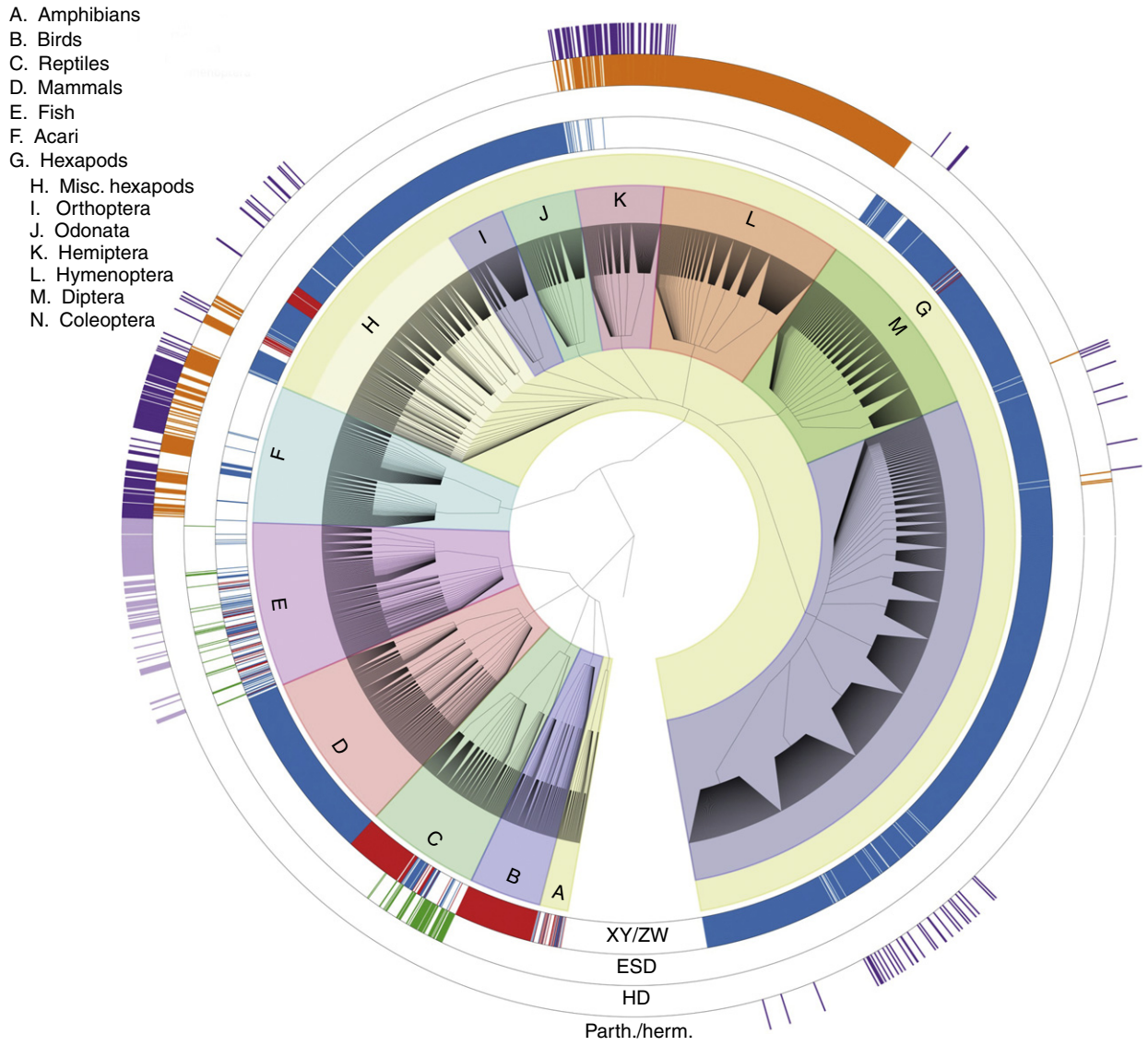


Figure 1 The phylogenetic distribution of sex-determining systems across animals. The tree structure is based on taxonomy and each tip represents all species in genus. The underlying data consist of 13 700 records representing 3886 genera from the tree of sex database. The XY/ZW ring is colored blue for XY and red for ZW taxa. The environmental sex determination (ESD) and haplodiploidy (HD) rings indicate the presence or absence of environmental and haplodiploid sex determination systems, respectively. The Parth./Herm. ring is colored dark purple for parthenogenic taxa and light purple for hermaphroditic taxa. Data reproduced from the Tree of Sex Consortium, 2014. Tree of Sex: A database of sexual systems. *Scientific Data*, 1.

has two alternative versions of a sex-determining region. Whether an individual is homozygous or heterozygous for this region will determine if it develops as a female or male. The sex-determining region can be a single gene, a portion of a chromosome, or even an entire chromosome; regardless, the chromosome that carries the sex-determining region is called the 'sex chromosome.' Heterogametic sex determination systems can be divided by whether the female or male is heterogametic.

In many species, including humans, the male is heterogametic and carries an X and Y sex chromosome while females are homogametic and carry two copies of the X chromosome. XY sex determination is the most common form of heterogametic sex determination and is found in amphibians, non-avian reptiles, mammals, and many invertebrates (Figure 1). In other groups it is the female that is heterogametic and the female possesses a Z and W chromosome while males carry two copies of the Z chromosome. ZW sex determination is found in birds, some amphibians and non-avian reptiles, and several groups of invertebrates most notably the butterflies (Figure 1). In both of these systems, the sex chromosome found in only one sex (Y or W) often degenerates and contains few genes in comparison to the X or Z chromosome. The taxonomically widespread observation of XO and ZO sex determination systems indicates that these sex-limited chromosome can be completely lost creating systems where the

heterogametic sex has one copy of the sex chromosome and the homogametic sex has two copies (The Tree of Sex Consortium, 2014). Finally, in some bryophytes and algae only the gametophytes (haploid life stage) have separate sexes and sex is determined by which version of the sex chromosome they carry. The gametophyte develops as a female if it carries a U sex chromosome and develops as a male if it carries a V sex chromosome. While sporophytes (diploid life stage) carry both sex chromosomes and do not have separate sexes (Bull, 1983).

In some fish, plants, and copepods there are multiple genes that can act as the 'master switch' for sex determination. In these polygenic sex determination systems there are more than two distinct versions of the sex chromosomes. For instance in the platyfish *Xiphophorus maculatus* which was ancestrally XY there are three types of sex chromosomes X, X^*, and Y, where X^* is a version of the X chromosome that carries a dominant feminizing mutation. This creates a system where there are multiple types of females, including the normal XX females, but also X^*X and X^*X^* and even X^*Y females, with the dominant female determining mutation (Volff and Schartl, 2001).

Generally the genotype of the offspring determines their sex. Yet a few species exhibit monogenic sex determination where the mother's genotype determines whether she will produce broods of all female or male offspring. One such example is the sciarid fly *Sciara coprophila*, where the sex of offspring produced by a female depends on the presence or absence of an inversion (X') on one of her X chromosomes (Sánchez, 2010).

Environmental Sex Determination

In many unicellular and some multicellular species, males and females have identical genomes and their sex is determined by environmental factors. Environmental sex determination (ESD) is common among unicellular eukaryotes (Beukeboom and Perrin, 2014). Among multicellular organism ESD is found primarily among non-avian reptiles, amphibians, and some fish (Bachtrog *et al.*, 2014). Temperature appears to be the most common determinant of sex under ESD. However, there are other factors too, like social environment. Many fish are sequential hermaphrodites, where they start their life as one sex, but change sex later in development. In the anemone fish (*Amphiprion akallopisos)*, which live in social groups with one dominant breeding pair as well a several subordinate males, sex change occurs when the dominant female dies and the largest male in the group becomes the dominant female (Fricke and Fricke, 1977). ESD systems are more often evolutionary labile than genetic sex determination (GSD): closely related species differ in their sex-determining mechanism and shifts can include changes in the threshold temperature or transitions between ESD and GSD. In some cases there is even variation within species: the lizard *Bassiana duperreyi* determines sex through GSD (XX-XY) but at low temperatures some of the XX females develop as males (Shine *et al.*, 2002).

Other Sex Determining Mechanisms

Among invertebrates the most common alternative sex determining mechanism is haplodiploidy (HD; Normark, 2003; Bull, 1983). Here, males develop from unfertilized eggs and have only a single copy of each gene ('haploid'), while females develop from fertilized eggs and have two copies of each gene ('diploid'). In haplodiploid organisms sex determination is therefore dependent on the fertilization of eggs, which is often thought to be under the control of mothers (Beukeboom and Perrin, 2014). HD has evolved repeatedly across insects, mites, nematodes, and rotifers and has been estimated to occur in around 12% of all animals (Normark, 2003; Jarne and Auld, 2006; The Tree of Sex Consortium, 2014). Another alternative reproductive system that shows similarity with HD is Paternal Genome Elimination (PGE) (Bull, 1983; Burt and Trivers, 2009). Here, both sexes develop from fertilized eggs, yet in males all genes inherited from the father are lost at some point during development. PGE occurs in thousands of species across insects, springtails, and mites. The loss of paternal genes occurs either early in development such that these males are haploid throughout development, or later such that males retain their father's genes in all cells but their sperm (Normark, 2003; Gardner and Ross, 2014). As both sexes develop from fertilized eggs, fertilization cannot serve as the trigger for sex determination, and it is currently unclear what sex-determining factor does. Both HD and PGE tend to occur in closely related species and are only found in terrestrial invertebrates. They are conspicuously absent from tetrapods, plants, and marine invertebrates (Normark, 2003; Normark and Ross, 2014).

Finally there are cytoplasmic sex determination systems where either an intracellular bacterial parasite (e.g., *Wolbachia*) or the genotype of the mitochondria determines the sex of offspring (Beukeboom and Perrin, 2014). Such cytoplasmic elements are only transmitted to the next generation by females and as such have an interest in manipulating the sex determination of their host.

Evolution of Different Sex Determining Systems

The variability of sex-determining mechanisms among eukaryotes is startling. But what evolutionary forces are responsible for transitions between different sex determination systems? Moreover, why are certain groups of plants and animals exceptionally variable in the way they determine sex?

Some of the difference in sex determination can simply be explained by the fact that they evolved independently. For example, separate sexes evolved independently in plants and in animals, so any similarities in sex-determining mechanisms – for example, differentiated sex chromosomes – are examples of convergent evolution (Beukeboom and Perrin, 2014). However, not all diversity is the result of the independent evolution of separate sexes: for example, insects – one of the most diverse group of animals in terms of their sex determination – ancestrally have separate sexes (Normark, 2003). So which evolutionary forces cause this variation? And what sex-determining systems are favored under different conditions?

Sex Ratio Selection

The first important selective force is sex ratio selection, in other words, what is the optimal offspring sex ratio an individual

can produce? In most sexually reproducing organisms the answer is an equal proportion of sons and daughters. The reason for this is that if one sex is more common than the other, the rarer sex has a higher fitness and will increase in frequency until equality arises (Fisher, 1930). Selection for balanced sex ratios is probably a primary explanation for why GSD is so prevalent across life – it is the only SD mechanism that guarantees an equal proportion of males and females (Beukeboom and Perrin, 2014). If this is the case, how can alternative SD systems evolve? One reason is that the sex ratio of an individual's offspring is selectively neutral as long as the population sex ratio is unbiased. So, mutations that alter offspring sex or local fluctuations in temperature under ESD are not selected against until they become so prevalent that they change population sex ratios. In fact ESD can be favorable if the environmental cue that determines sex differentially affects the fitness of males and females. Under that scenario selection favors sex-determining mechanisms that match each sex to its best environment (Bull, 1983). Finally there are conditions under which balanced sex ratios are selected against: for example, when related males compete for matings with their sisters a female-biased sex ratio is preferred (Hamilton, 1967). This would select for SD systems that allow mothers to bias their sex ratio. Several authors have suggested that HD and PGE might have evolved because of such sex ratio selection (Bull, 1983; Beukeboom and Perrin, 2014; Burt and Trivers, 2009; Gardner and Ross, 2014).

Sexual Conflict

The two sexes are defined by their difference in gamete size, however sexual dimorphism generally extends to other traits including differences in morphology and behavior. Sexual conflict occurs when the sexes have different fitness optima for such traits. One form of sexual conflict that is thought to be especially important in the evolution of sex determination systems is sexual antagonistic selection, which occurs anytime that a gene has multiple alleles that have different fitness in males and females. The presence of sexual antagonistic selection increases the probability of a transitions in the sex-determining gene, sex determination method, or sex chromosome pair (Van Doorn and Kirkpatrick, 2007). For instance, in an XY system, if a masculinizing mutation occurs near an autosomal allele that is associated with higher fitness in males, then this may give rise to a new Y chromosome that may fix in the population.

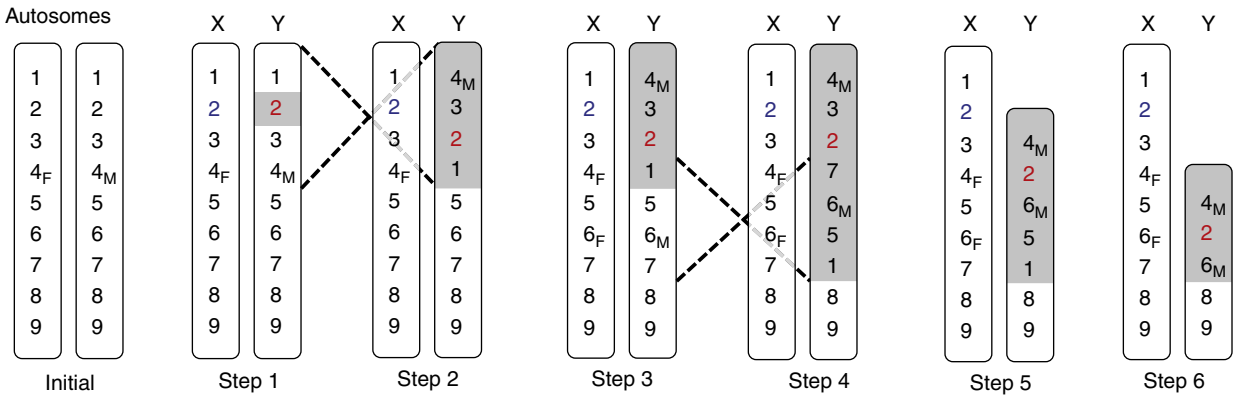
Endosymbiotic Bacteria

A large number invertebrates harbor bacteria that live inside their cells (Buchner, 1965). In some cases these bacteria are mutualists and essential for the survival of their host, often thought they are parasites that evolved ways to increase their frequency without providing any benefits. Many (in particular those in the genera *Wolbachia* and *Cardinium*) do so by manipulating their hosts into producing more female offspring. This benefits the bacteria because only females, not males transmit them to their offspring. By far the best example comes from the woodlice *Armadillidium vulgare*. In those individuals that are uninfected sex is determined genetically by a ZZ/ZW system, but infection with *Wolbachia* lead to the feminization of ZZ males into functional females, or one of two types of intersexes: (1) individuals with female physiology but some external characteristics of males, or (2) individuals that are physiologically male but are sterile (Juchault *et al.*, 1992).

Evolution of Sex Chromosomes

The canonical view of sex chromosome evolution begins with autosomes that gain a sex-determining gene (Westergaard, 1958). Upon the evolution of a sex-determining gene, the Y chromosome is expected to begin a process of degeneration because of its reduced population size (between one male and one female, there is only one copy of a Y chromosome and 3 copies of X chromosomes) and reduced recombination that sometimes evolves between the X and Y chromosomes; both factors reduce the effective population size of the Y chromosome and reduces efficacy of selection, which, in turn, can lead to its degeneration. Selection to reduce recombination may be a byproduct of sexual antagonism (SA), where different SA alleles provide higher fitness in males and females; for example, reduced recombination between the sex-determining region and a SA locus can lead to associations between a male-benefit (female-detriment) allele and the Y chromosome, and thereby restrict the allele's benefits to males and not expose females to its costs. Cessation of recombination often occurs through inversions that isolate increasingly large stretches of the Y chromosome (Charlesworth *et al.*, 2005). In chromosomal regions where recombination is suppressed genes are quickly lost until only a core set of genes essential to the male fertility and viability remain (Bachtrog, 2008) (**Figure 2**). Suppression of recombination between the X and Y chromosome of humans occurred in 5 steps over 200–300 million years (Hughes *et al.*, 2012). This process only affects the sex-limited chromosome; the X is still able to recombine in a normal fashion in females; this process of degeneration is expected to be largely the same in ZW systems, applying to the W chromosome. Sex chromosomes that have gone through this process are described as heteromorphic and the sex-limited chromosome as degenerate. However, not all organisms follow this pattern; for instance the emu as well as some amphibians and fish appear to maintain homomorphic sex chromosomes for long periods. It is likely that different explanations for the retention of homomorphic sex chromosomes apply to these groups. In the case of the emu sex specific gene regulation may allow the resolution of SA (but see Charlesworth *et al.* (2014) for an alternative explanation). In contrast very low levels of intermittent recombination may be responsible for the lack of degeneration seen in amphibians (Perrin, 2009; Vicoso *et al.*, 2013).

While heteromorphic sex chromosomes are usually quite stable, homomorphic sex chromosome often exhibit rapid changes in which chromosomal pair determine sex, and even between XY and ZW systems. The simplest type of turnover involves the translocation of the sex-determining gene onto an autosome creating a new sex chromosome pair. However, masculinizing or feminizing mutations of genes on autosome can create new sex chromosomes or cause transitions between



Initial: No sex chromosomes are present; sex is determined by environmental conditions.

Step 1: A masculinizing mutation occurs at gene 2 creating an XY sex determination system.

Step 2: Sexual antagonistic selection favors an inversion of genes 1 to 4 on the Y chromosome. This keeps the allele of gene 4 that is best for males with the male sex determining region, and expands the portion of the Y chromosome that cannot recombine.

Step 3: A new mutation at gene 6 creates sexual antagonistic selection at this gene.

Step 4: Sexual antagonistic selection favors an inversion of genes 1, 5, 6, and 7 on the Y chromosome further reducing the amount of the Y chromosome that can recombine.

Step 5-6: The region of the Y chromosome that cannot recombine begins to lose genes that are not essential to male fitness.

Figure 2 The process of sex chromosome differentiation. The progression from undifferentiated autosomes to heteromorphic sex chromosomes. The sex-determining locus is gene 2. The female allele is in blue and the male allele is in red. Alleles that have different fitness in males and females are indicated with a subscript where M indicates an allele with high fitness in males and F high fitness in females. The gray shading indicates the region that is non-recombining.

XY and ZW systems. Masculinizing mutations can change the 'master switch' in XY systems or transitions from ZW to XY. Likewise, feminizing mutations can change the master switch in ZW systems or transitions from XY to ZW. Whether these translocation, feminizing mutations, or masculinizing mutations fix in a population is likely determined by presence of SA (Van Doorn and Kirkpatrick, 2007). For instance if a feminizing mutation occurs near a gene with an allele that is associated with higher fitness in females (but lower in males) then the new sex-determining region is more likely to fix in the population.

Sex Ratio Distorters

GSD typically leads to equal sex ratios as a result of meiosis, where each copy of a gene (e.g., the sex-determining locus on a mammalian Y chromosome) has a 50% of being present in a particular gamete. This is not always the case though: we now know of genes that can 'cheat' the fair raffle of meiosis to ensure they are included in the gametes at a higher rate. These 'meiotic drive alleles' can thus rapidly spread through populations and if they are located on autosomes, they have little effect. However if located on a sex chromosome they can have profound effects, often threatening to wipe out populations through the demise of one of the sexes. Such 'sex ratio distorters' can therefore select for the evolution of new sex-determining loci that can override the ancestral locus linked to the driver, thereby restoring an equal sex ratio. The occurrence of autosomal feminizers and masculiners able to override the normal XY sex determination in house flies (*Musca domestica*) have been hypothesized to result through this mechanism, though there is as yet no direct evidence of driving sex chromosomes resulting in novel SD loci.

Molecular Mechanism of Sex Determination

Over the last few decades scientists have unraveled the molecular mechanisms of sex determination for a number of model organisms. This research has lead to some general insights: First of all, the underlying molecular mechanisms can vary dramatically between species that on the surface appear to have very similar sex determination systems (Bachtrog *et al.*, 2014). On the other hand, however, some of the same genes are involved in sex determination across all metazoan and shared among species with very different sex-determining systems (Beukeboom and Perrin, 2014; Smith *et al.*, 1999). Genes involved in sex determination can generally be divided into those that determine the initial switch (the master switch) and those further downstream in the 'sex determination cascade,' which are responsible for organizing and maintaining sexual differentiation. A general pattern that has emerged from genetic studies is that those 'master switch' genes show high turnover between closely related species while those genes further down the cascade tend to be more conserved (Beukeboom and Perrin, 2014). The reason for this pattern is not fully understood, though they might arise from the fact that mutations in the switch genes tend to result in a change in sex ratio, while mutations further down the cascade will lead to more detrimental effects, for example the production of sterile intersex offspring. The sex-determining switch can be a large chromosomal region, a single gene, or even a single nucleotide polymorphism. It can act either by its presence or absence or by the amount of gene product that is present ('dosage' above or below a threshold).

Here we briefly review the molecular sex-determining mechanism of a number of model organisms and how these examples allow us to make inference across the tree of life.

Insects

Most of what we know about the molecular mechanism of sex determination in insects comes from studies on fruitflies in the genus *Drosophila*. *Drosophila* have an XY sex determination system. Sex is determined by a 'dosage-dependent sex determination' factor on the X chromosome (XSEs, see **Figure 3**): those individuals with two copies of the X chromosome develop as females while those with just one copy develop as males. Expression of the X-linked XSEs above the dosage threshold promotes the transcription of another gene called transformer (TRA), which in turn causes the alternative splicing of the gene double sex (DSX) (**Figure 3**; Maine *et al.*, 1985). Double sex is responsible for organizing sexual differentiation across all insects studied to date, while the other genes are more taxonomically restricted. Other flies that seemingly have the same sex determination system as *Drosophila* (XY) do not determine sex through dosage but through a dominant Y-link gene (M) that blocks the transcription of transformer and leads to the male-specific splice form of DSX (**Figure 3**). Unlike most insects butterflies determine sex through a female-heterogametic sex determination system (either ZW or Z0). The molecular mechanisms of sex determination in butterflies has been studied primarily in the silkworm (*Bombyx mori*, **Figure 3**) and like other insects, DSX determines sexual differentiation. However, unlike other insects the master switch is not a protein, instead, a small non-coding RNA molecule located on the W chromosome and acts as a dominant 'feminizer' (Kiuchi *et al.*, 2014). Finally in many insects sex is determined through HD where males develop from unfertilized haploid eggs (Normark, 2003). The mechanisms by which sex determination is accomplished under HD are as yet only known for bees, ants, and wasps (Hymenoptera) (Schmieder *et al.*, 2012). Perhaps surprisingly much of the sex-determining cascade is identical to that found in *Drosophila* (**Figure 3**; Beukeboom and Perrin, 2014). In bees the master switch is the complementary sex determiner gene (CSD). Many different alleles at this locus segregate in a population and those individuals with two different alleles ('heterozygous' individuals) develop as females, while those with just one, either because they are haploid or because they have two copies of the same allele, develop as males (Beukeboom and Perrin, 2014). Although CSD is found across hundreds of hymenoptera, it is absent from some parasitoid wasps and there is no evidence for CSD in any of the other haplodiploid groups of insects or mites.

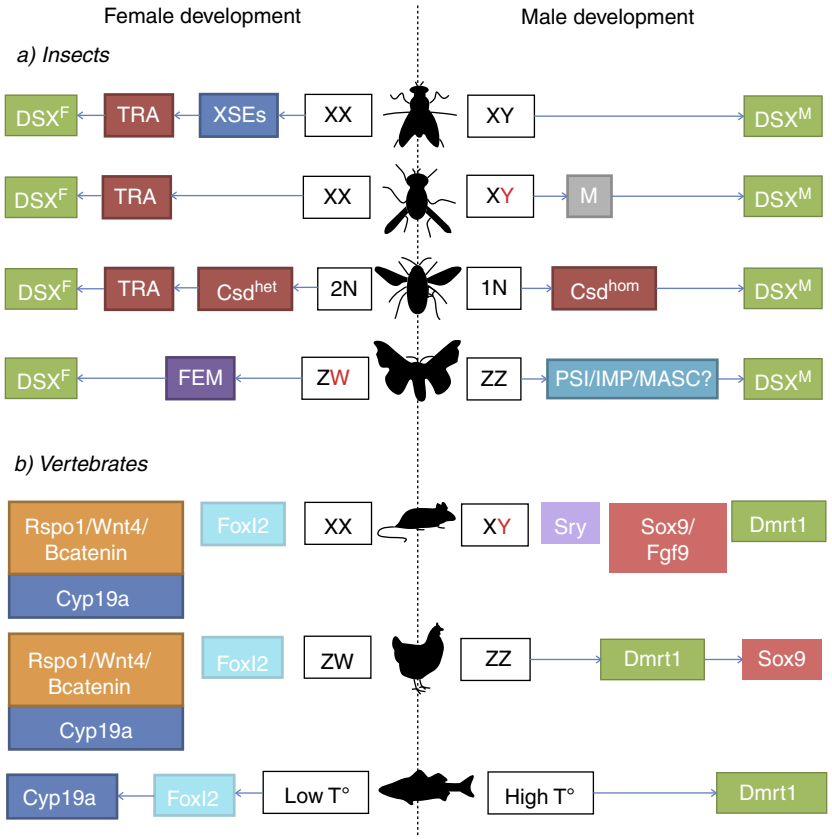


Figure 3 The molecular pathways of sex determination in a range of model organisms. The top half of the panel depicts examples of a number of well-studied insect model with different sex-determining pathways (from top to bottom: the common fruit fly: *Drosophila melanogaster*, the Mediterranean fruit fly: *Ceratitis capitata*, the buff-tailed bumblebee: *Bombus terrestris*, and the silkworm: *Bombyx mori*), while the bottom half shows such examples for vertebrates (from top to bottom: *Mus musculus, Gallus gallus domesticus*, and *Dicentrarchus labrax*). The solid white box indicates the 'master switch,' while the other colored boxes depict sex-determining genes further down in the cascade. Genes with a shared evolutionary history are depicted in the same color. Based on various references (Beukeboom and Perrin, 2014; Schmieder *et al.*, 2012; Kiuchi *et al.*, 2014).

Vertebrates

Across vertebrates sexual differentiation is regulated by Dmrt1 (Smith *et al.*, 1999), which simultaneously turns on male development, while suppressing female development (**Figure 3**). Dmrt1 is a transcription factor that belongs to the same gene family as *doublesex*, but it is its presence or absence, not its alternative splicing, that causes sex differentiation (Beukeboom and Perrin, 2014). While Dmrt1 is pivotal for vertebrate sex determination, it does not usually act as the primary master switch, which – like in insects – is much more variable across species. In mammals the master switch is a dominant masculinizing gene called Sry that is located on the Y chromosome (**Figure 3**; Foster and Graves, 1994). The identity of the master switch in birds remains controversial, though most evidence points to Dmrt1 fulfilling this role (Smith *et al.*, 2009; Beukeboom and Perrin, 2014). Dmrt1 in birds is Z-linked and the dosage of Dmrt1 is thought to directly determine male development. (**Figure 3**) In vertebrates with ESD, like some reptiles and fish, much of the downstream sex determination cascade is similar to that of species with genetic sex determination. Temperature-dependent sex determination is often mediated by the temperature-dependent expression of aromatase (Cyp19a), an enzyme that is involved in the synthesis of estrogens. Finally in some vertebrates, especially telost fish, sex is not controlled by a single master regulator but is instead a quantitative threshold trait with either a male or female outcome, which is determined by multiple regions in the genome (Bachtrog *et al.*, 2014).

Flowering Plants

Among flowering plants, separate sexes (dioecy) are rare (about 6% of species (Renner and Ricklefs, 1995)), and have evolved relatively recently from hermaphroditic ancestors. The transition from hermaphroditism to separate sexes is generally thought to involve two separate mutations: one suppressing male function and one suppressing female function. Indeed sex is determined by two separate genes in papaya, one of the few plants for which the sex-determining cascade has been deciphered. Like most dioecious plants papaya has a male heterogametic (XY) sex determination system and sex is determined by an X-linked feminizer and a Y-linked masculinizer (Beukeboom and Perrin, 2014).

Why Are Some Groups so Variable?

From an evolutionary viewpoint, one of the most intriguing questions is why some groups of plants and animals (e.g., reptiles, fish) are exceptionally variable in the sex-determining systems, while others display hardly any variation (e.g., birds, mammals). One possibility is that certain SD systems are more labile than others. Generally, GSD is thought to be more stable than environmental SD. For example in reptiles transitions from ESD to GSD occur frequently, but there are no clear examples of transitions the other way. And even among GSD systems, those with highly differentiated sex chromosome (XY, ZW), are thought to be more stable than those where the sex chromosomes are monomorphic, or those that have lost one of the sex chromosomes (X0 or Z0). Finally, HD is thought to serve as an 'evolutionary trap'; no haplodiploid lineage has re-evolved an SD system with diploid males (Bull, 1983).

Outstanding Questions

Some aspects of sex determination – such as the evolution of sex chromosomes and the molecular mechanism of sex determination in model organism – are now well understood. However many unresolved questions remain. Many of these involve the large-scale phylogenetic distribution of different sex determination systems. For example, why is male heterogamety more common than female heterogamety? Why does HD only occur in certain groups of invertebrates? Why do sex-limited chromosome degenerate in some groups but not others? Finally an important challenge is to understand how a species' sex determination mechanism can impact other aspects of their biology and evolution, such as rates of speciation (Orr, 1997), and the evolution of male ornaments (Kirkpatrick and Hall, 2004). Solving these challenging questions will require the concerted effort of scientist from a wide range of disciplines as well as a broad taxonomic outlook.

See also: Sex and Recombination in Snails. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth. Sex, Evolution and Maintenance of. Sexual Dimorphism

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Sex, Evolution and Maintenance of

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Introduction

The ability to reproduce is one of the most fundamental aspects defining life, yet reproduction is achieved through a panoply of mechanisms. Reproduction can involve different levels of recombination and genetic exchange between individuals, ranging from clonal reproduction to meiotic parthenogenesis, self-fertilization or mating between relatives, to sexual reproduction with outcrossing. Definitions of sex therefore often depend on the context. A broad definition is the joining of genetic material from two individuals to form offspring that combine genes from both of them. If defined in this way, sex is almost universal as it includes horizontal gene transfers observed in prokaryotes and some eukaryotes, as well as other types of 'parasexual' genetic exchange between individuals. A second definition of sex, the one adopted here, refers to the formation of haploid gametes through meiosis, followed by the fusion of these gametes (syngamy).

The evolution of sex combines two different topics; the origin and the maintenance of sex. **The origin of sex** is highly speculative as it happened during the early history of life on Earth, when the first self-replicating RNA/DNA molecules appeared. The single evolutionary origin of meiotic sex would have occurred during this time, some 1.5 billion years ago. There are different opinions concerning the mechanisms that would have favored sex at its origin. For example, meiotic sex could have been selected as a mechanism for DNA repair (Bernstein *et al.*, 1985). However, a more broadly accepted view is that sex at its origin was favored through the same mechanism that currently maintains it: it allows selection to work efficiently. How exactly sex could facilitate selection is explained below. Importantly, even small benefits conferred by sex at its origin may have been enough. This is because direct costs associated with sex at this time would have been small since sex occurred between isogametic cells. By contrast, sex has to generate strong benefits to be 'maintained' under anisogamy and biparental reproduction, the typical situation in metazoans.

Sex is indeed associated with significant direct costs in metazoans. In species where males provide little or no resources to their offspring, females pay the full cost of reproduction, yet only provide half of each sexually produced offspring's genes. This generates a transmission disadvantage relative to asexual reproduction, which is two-fold in species that invest equally in both sexes (formalized by Maynard Smith (1978) and Williams (1975)). Even in cases where males do contribute resources to their offspring, sex is typically still costly because it requires attracting mates and eventually mating. These behaviors may be costly and increase risk of predation or of infection with a sexually transmittable disease. Sex can further cause reproductive failure if individuals fail to find a mating partner. **The paradox of sex** – the fact that it is associated with considerable costs but maintained in the vast majority of organisms – thus stems in great part from a metazoan-centered view.

Fitness effects of sex – the costs and benefits it generates – can be expressed within populations (short-term consequences) or at the lineage level (long-term consequences), affecting the rate of adaptation, diversification, or extinction. Within populations, sex can affect fitness directly or indirectly. Direct fitness effects of sex are usually negative, for example, the cost of males or costs related to mating. This means that benefits of sex most likely stem from either indirect effects on fitness or from long-term consequences. Long-term consequences alone are probably insufficient to explain the maintenance of sex, given the considerable direct costs of sex (see Section Lineage-Level Selection For Sex). Indirect effects on fitness arise when sex breaks up associations between alleles under selection (see Section Short-Term Benefits of Sex). The intuitive idea that sex is good for the species was accepted until the 1970s, when it was realized that there has to be a gene-level advantage for sex and that this advantage has to be strong enough to fully outweigh all the costs (Williams, 1975; Maynard Smith, 1978). Therefore, there is an ongoing search for strong short-term or individual-level benefits to explain the maintenance of sex.

Short-Term Benefits of Sex

Sex generates indirect effects on fitness when it breaks up associations between alleles under selection. Much effort has been invested into identifying situations where breaking up such associations generates short-term fitness benefits. Before examining situations where this might be the case, it is useful to consider that for sex to have any indirect effect on fitness (positive or negative), associations between selected alleles at the same or at different loci need to exist in a population. Without **such linkage disequilibrium (LD)**, sex has no effect (**Figure 1**).

Generating indirect benefits from breaking up LD requires two mechanisms: (1) a mechanism that generates continuous directional selection, for example, mutation or unceasing changes in selection pressures. This is necessary to maintain additive variance for fitness – in the absence of such variance, there is no possibility for adaptation (and sex can thus not facilitate it). (2) A mechanism that generates associations between genes with opposite ($-$ and $+$) fitness effects. If associations were between genes of identical fitness effects, recombination would reduce the variance in fitness and thereby slow down adaptation (**Figure 2**).

Identifying the mechanisms that generate continuous directional selection is largely an empirical challenge, and these mechanisms most likely vary among different organisms. A general understanding of the advantage of sex therefore requires understanding why associations between genes with

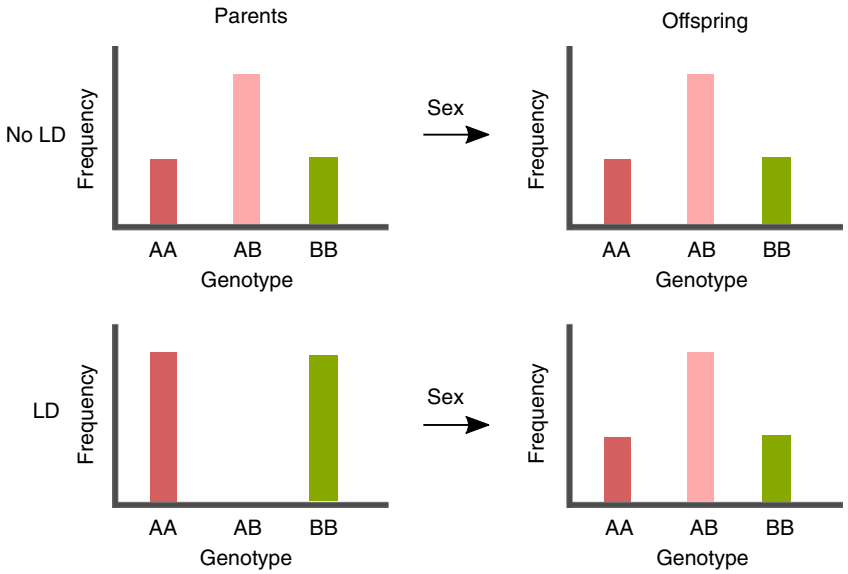


Figure 1 Linkage Disequilibrium (LD). Under LD, there is a nonrandom association of alleles at different loci. If there is no LD in a population, sex will have no effect. If there is LD, sex can generate new genotypes in the offspring that were not present in the parental population. Note that LD concerns the frequency of genotypes in the population, not the frequency of individual alleles.

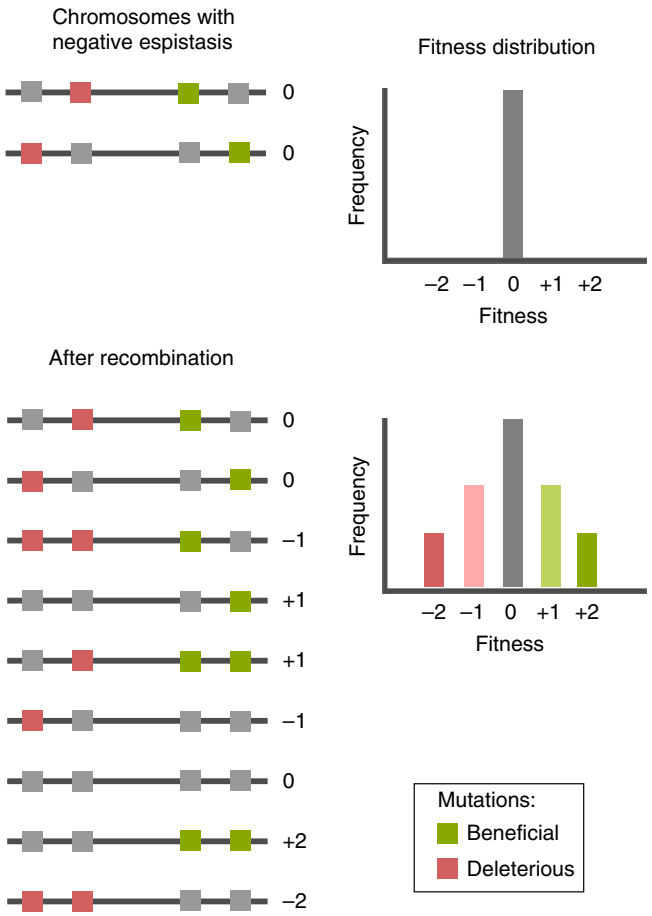


Figure 2 Negative epistasis. In the presence of negative epistasis, interactions between alleles reduce or reinforce fitness effects. Under negative epistasis, sex can generate benefits by breaking up LD and thereby increasing fitness variance, which will improve the response to selection.

opposite fitness effects should be predominant. The four best studied mechanisms that could generate such associations are:

Epistasis

If the effect of a locus on fitness depends on other loci (i.e., if there is epistasis), selection systematically generates LD. If epistasis is generally negative, sex would be favored because it would increase fitness variance in the next generation (Barton, 1995). Because fitness variance increases, recombination provides a benefit by increasing the response to selection. In addition to this benefit, recombination also generates a cost. This cost stems from the fact that LD was generated by selection, meaning that recombination breaks up good gene combinations and generates worse ones. This 'recombination load' causes an immediate reduction of mean fitness, which has to be compensated by the benefit of the increased response to selection. As a consequence, for recombination with negative epistasis to generate a net benefit, epistasis cannot be strongly negative or the costs due to the recombination load would be too high (Otto and Feldman, 1997). The current empirical evidence indicates that epistasis is generally not negative (Elena and Lenski, 1997; De Visser and Elena, 2007). This implies that negative epistasis is unlikely to be a main driver in the evolution and maintenance of sex.

Temporal Changes in Selection

Epistasis can generate a short-term advantage for recombination when it fluctuates over time, with different allele combinations being favored during different time intervals (Maynard Smith, 1971; Charlesworth, 1976; Barton, 1995). The evolution of recombination requires that these time intervals are quite short (a few generations), which is perhaps most likely if fluctuating epistasis is caused by interactions between coevolving species (Peters and Lively, 1999; Gandon and Otto, 2007).

Much research effort has been dedicated to the study of coevolution, especially between hosts and parasites. In the context of Red Queen dynamics, a parasite would adapt to the most common host genotype, because it can then infect many hosts. In this situation, a rare host genotype would be favored, causing its frequency to increase until it becomes common (negative frequency-dependent selection). Parasites should then shift to infect this newly common genotype, reducing its fitness in the current generation. There is accumulating evidence for Red Queen dynamics in natural populations and from experimental evolution (e.g., Lively, 1987; Decaestecker *et al.*, 2007; Morran *et al.*, 2011). However, whether these dynamics contribute to the maintenance of sex in many species remains unknown. For example, negative frequency-dependent selection does not require sex but can also work in asexual species consisting of a genetically diverse assemblage of clones. In that case, negative frequency-dependent fitness of different clones would act to maintain clonal diversity rather than sex. Furthermore, there are several examples where parasites appear to not contribute to the maintenance of sex (e.g., Parker, 1994; Hanley *et al.*, 1995; Elzinga *et al.*, 2012).

Migration and Spatial Changes in Selection

Spatial variation in selection can generate locally adapted allele associations with locally maladapted associations introduced by migration. Sex can then provide a benefit because it breaks such maladaptive allele associations, provided migration rates are high enough to regularly introduce locally maladapted associations, yet low enough to not constrain local adaptation via gene flow (Agrawal, 2009; Lenormand and Otto, 2000; Pylkov *et al.*, 1998). These theoretical predictions were supported by results from an experimental evolution approach in cyclically parthenogenetic rotifers. By controlling migration rates between similar and different rearing environments, Becks and Agrawal (2010) showed that higher rates of sex are maintained under migration between different environments.

However, potential benefits for sex under spatially heterogeneous selection depend on the specific conditions. For example, selected alleles should be dominant under conditions where they are beneficial, and recessive under conditions where they are maladaptive (Agrawal, 2009). Furthermore, depending on the correlation in selection on different loci across populations, migration can generate either positive or negative linkage disequilibria across loci (Lenormand and Otto, 2000).

Drift and the Hill–Robertson Effect

In finite populations, negative LD between loci under selection is generated by the combined effects of drift and selection. Drift generates all possible types of LD between loci under selection: associations between beneficial alleles, associations between deleterious alleles, and associations between alleles with opposite fitness effects. Selection acts efficiently on the first two categories, given the big fitness differences they generate. This means that most cases of LD that persist after selection are due to associations between alleles with opposite fitness effects (Hill and Robertson, 1966). This so called 'Hill–Robertson effect' favors sex because breaking up associations between alleles with opposite fitness effects increases the variance in fitness (Felsenstein, 1974; see also **Figure 2**).

Since natural populations (at least of macroorganisms) are typically within the size range where Hill–Robertson effects can generate benefits for sex, drift generates perhaps the most broadly applicable benefits for sex. However, it remains unknown to what degree such benefits can compensate for the direct costs associated with sex.

In summary, breaking up associations between alleles at different loci can provide an advantage for sexual over asexual reproduction when these associations hamper adaptation, as is often the case within finite populations, or when selection varies over time or space. Whether such indirect benefits of sex may be sufficient to outweigh the direct costs remains unknown. Furthermore, for obtaining benefits from reducing associations between loci, even very rare events of sex are sufficient. The presented models do therefore not explain the prevalence of obligate sex with high recombination rates (Hurst and Peck, 1996). There is currently no LD-based hypothesis that can account for obligate sex, the most widespread form of reproduction among metazoans.

Short-term benefits of sex not related to linkage disequilibria

A specific form of spatial variation in selection is at the root of some of the classical, ecology-based models proposing benefits to sex, which include the '**Tangled Bank**' model (e.g., Ghiselin, 1974; Maynard Smith, 1978; Bell, 1982; Song *et al.*, 2011). These models posit that there is genetically based variation among individuals for exploiting different niches. As a consequence, genetically identical individuals should experience more intense competition than genetically different individuals. Here, sex could provide benefits because sexually produced offspring would display more genetic variation than asexually produced ones. The 'Tangled Bank' concept thus proposes an advantage for sex in environments that are saturated, as sex would reduce sibling competition in this case.

Some recent extensions of the 'Tangled Bank' (Song *et al.*, 2011) work in similar ways to negative frequency-dependent selection under host–parasite coevolution. Here, the availability of resources depends on the frequency at which these resources are exploited and replaced. If different genotypes exploit different resources, rare genotypes will be favored because they use a resource that is temporarily abundant.

Lineage-Level Selection for Sex

As explained above, meiotic sex has evolved once and has been maintained in the vast majority of lineages on the tree of life. This means that current asexual lineages derive secondarily from sexual ancestors. It has been argued that only those sexual lineages that cannot give rise to new asexuals (due to genetic or developmental constraints) persist in the long-term (Williams, 1975; Nunney, 1989). Sexual lineages without constraints would be driven to extinction by the asexuals they generate.

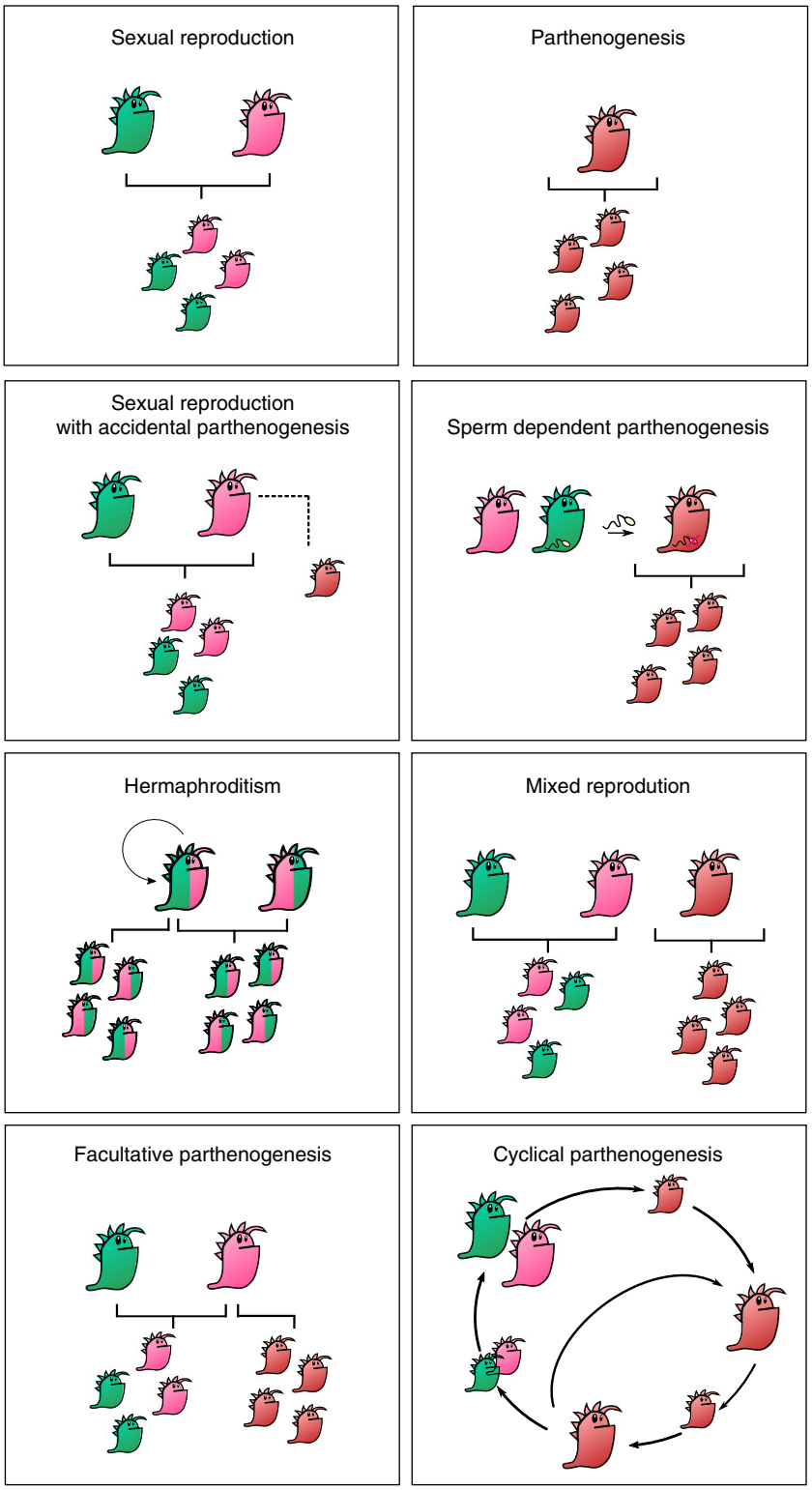
Several hypotheses further propose long-term disadvantages to asexual reproduction. For example, **'Muller's ratchet'** describes the pattern where small populations of asexual lineages tend to accumulate deleterious mutations over time (Muller, 1964). Clones can be lost from small populations as a consequence of drift, including the clones with the fewest deleterious mutations. New deleterious mutations are introduced during replication, such that the average number of deleterious mutations per clone in an asexual population can only increase over time, in a ratchet-like manner (hence the term **Muller's ratchet**). Small sexual populations also lose genotypes as a consequence of drift. However, via recombination and mixis, sex can regenerate mutation-free genotypes and thereby avoid the ratchet.

The **'lottery model'** compares sexual and asexual reproduction to different strategies when buying lottery tickets (Williams, 1975). Asexuality corresponds to buying many tickets with the same number, while sexual reproduction corresponds to buying tickets with different numbers. The expected payoff of the two strategies is similar, but the payoff variance should be greater for asexuals, with the consequence

Figure 3 Overview of the different reproductive modes described in the text. **Sexual reproduction** with or without spontaneous parthenogenesis. In sexually reproducing species, offspring contain the genetic material of the father and mother. Sexual reproduction involves meiosis and recombination, both of which may also be present at some level in alternative modes of reproduction. In some cases, spontaneous parthenogenesis (also called tychoparthenogenesis or accidental parthenogenesis) occurs under sexual reproduction when virgin females are able to produce viable offspring. All known cases of spontaneous parthenogenesis are meiotic. It is widespread among invertebrates and has also been documented in some vertebrate species, especially in species kept in zoos. Spontaneous parthenogenesis is sometimes called 'facultative parthenogenesis' although hatching success under spontaneous parthenogenesis, rarely higher than 1% and often as low as 0.1%, is typically more than an order of magnitude lower than under facultative parthenogenesis, where the majority of unfertilized eggs hatch. **Sperm-dependent parthenogenesis**: it (also called gynogenesis, pseudogamy or sperm parasitism) is a parthenogenesis in which the egg needs to be activated by sperm for embryogenesis to start. Thus as under other types of parthenogenesis, there is no paternal contribution (genetic or cytoplasmic) to the offspring. Sperm-dependent parthenogens derive from sexual ancestors where egg activation is already sperm-dependent, possibly because egg–sperm interactions may function as a control mechanism preventing the unwarranted development of eggs prior to fertilization. Sperm-dependent parthenogenetic lineages must coexist with their 'sperm donors,' usually males (or hermaphroditic individuals) from a related sexual species or population. Although rare, sperm-dependent parthenogenesis has been reported in a wide variety of animal taxa, including vertebrate and invertebrate taxa. Mating systems like these are potentially unstable, since they depend on the sperm from an individual who cannot gain paternity in return. **Cyclical parthenogenesis:** it is the alternation between a generation of sexual reproduction and one or more generations of asexual reproduction in a single population. During the parthenogenetic generations, females only produce daughters, except for the last generation, preceding the sexual generation. Prior to the sexual generation, females produce sons in addition to daughters. These parthenogenetically produced males and females mate, and the female offspring from these crosses are the first parthenogenetic generation of the next cycle. The parthenogenetic generations are generally mitotically produced (i.e., clonal). The parthenogenetic production of males is achieved via different developmental processes in different species, depending on their sex determination mechanism. In species with environmental sex determination (e.g., in water fleas) male differentiation is induced by specific abiotic conditions. In species with haplodiploid sex determination (e.g., in rotifers or cynipids) females start laying haploid eggs, while in species with other genetic sex determination systems, male development involves complex processes of sex chromosome elimination (as Wilson *et al.*, 1997 described for aphids). **Obligate Parthenogenesis:** parthenogenesis in animals often refers to the production of daughters without genetic contributions from males ('female-producing parthenogenesis' or thelytoky). Under obligately parthenogenesis all individuals only reproduce via parthenogenesis: the ability to produce offspring with genetic contributions from males has been lost. Both obligate meiotic and mitotic asexual reproduction falls under this definition. **Mixed reproduction:** in mixed reproduction a population consists of both sexually and asexually reproducing individuals. Crucially, each individual reproduces either sexually or asexually, and is not able to alternate between reproductive modes, making it functionally distinctive from both cyclical and facultative parthenogenesis. **Hermaphroditism:** these are organisms that combine male and female functions within the same individual. In some cases, hermaphrodites are able to fertilize their own eggs, making them functionally asexual, even though the fusion of gametes still occurs. **Facultative parthenogenesis:** in lineages with facultative parthenogenesis, reproduction can be through biparental sex and through female-producing parthenogenesis. Facultatively parthenogenetic females can flexibly shift between the two reproductive modes and parthenogenesis is generally meiotic.

that asexual lineages should face higher extinction rates than sexual ones.

Sexual and asexual lineages are also expected to differ in their long-term rate of adaptation, i.e., the 'classical' explanation for the benefits of sex. According to the **Fisher–Muller hypothesis**, sexual lineages adapt faster, because beneficial mutations occurring in different individuals can be combined in one (Fisher, 1930; Muller, 1932). In an asexual



lineage, the same beneficial mutations must be fixed sequentially. If different beneficial mutations appear simultaneously in different individuals, competition among such mutations ('**clonal interference**,' Muller, 1932; Gerrish and Lenski, 1998) can slow the rate of adaptation (and theoretical fitness optima may never be reached).

Although intuitively appealing, long-term mechanisms are unlikely to explain the maintenance of sex in the bulk of species. For example, they do not apply under very large population sizes, which are characteristic of many microorganisms. This is because all combinations of beneficial and deleterious mutations appear at their expected frequencies in infinite populations, and recombination confers no advantage. Furthermore, such arguments cannot explain the maintenance of sex in lineages characterized by sexual and asexual reproduction (i.e., in lineages with **facultative parthenogenesis**, **cyclical parthenogenesis,** or **mixed reproduction**): here, sex must confer benefits on a sufficiently short timescale so that its direct costs can be outweighed.

The Diversity and Taxonomic Distribution of Reproductive Modes

The level of sex can vary continuously among reproductive modes, from its complete lack in mitotic forms of parthenogenesis to obligate sex between unrelated individuals (Figure 3). **Female-producing parthenogenesis** ('thelytoky') occurs in different forms – it can be cyclical, facultative, accidental, or obligate. **Cyclical parthenogenesis** (also called heterogony) is a type of life cycle in which a sexual generation (bisexual or hermaphroditic) alternates with one or more generations of parthenogenetic reproduction. Six large animal groups are characterized by this life cycle: trematodes (a parasitic class of flatworms), rotifers, cladocerans (water fleas such as *Daphnia*), aphids (including adelgids, and phylloxerids), cecidomyiids (gall midges), and cynipids (gall wasps). Parthenogenesis typically predominates under favorable conditions; deteriorating or stressful conditions (e.g., linked to seasonality, resource depletion and/or crowding) trigger the production of males and sexual females. Cyclical parthenogens frequently generate strains characterized by **obligate parthenogenesis** in which the sexual cycle can no longer be induced. This is well documented for several strains of aphids and water fleas (Dedryver *et al.*, 2013; Tucker and Ackerman, 2013; Neiman *et al.*, 2014).

Similar to cyclical parthenogenesis, **facultative parthenogenesis** characterizes lineages that can use both biparental sex and female-producing parthenogenesis to generate offspring. In contrast to cyclical parthenogens, facultatively parthenogenetic females can flexibly shift between the two reproductive modes and parthenogenesis is generally meiotic while it is mitotic (i.e., clonal) in cyclical parthenogens. The efficiency of parthenogenesis and sexual reproduction (number of offspring produced) is comparable under facultative parthenogenesis, distinguishing it from **spontaneous parthenogenesis** in sexual species. However, survival rates are typically higher for sexually than parthenogenetically produced offspring such that, given the option, females will prefer to produce sexual rather than parthenogenetic offspring. Facultative parthenogenesis occurs and may be widespread in some insect groups such as phasmids, mayflies, or termites, but is most likely rare in other animal groups. More frequent is **mixed reproduction** (species with sexual and parthenogenetic strains) however females in each strain are obligately sexual or obligately parthenogenetic.

In summary, most types of female-producing parthenogenesis would avoid certain indirect and direct costs associated with sexual reproduction, including the breaking up of coadapted gene complexes, the production of sons as well as costs involved in mate finding and copulation. In this context female-producing parthenogenesis is often used interchangeably with asexuality, although parthenogenesis does not necessarily generate clones. Many forms of parthenogenesis involve meiosis whereby ploidy levels (reduced during meiosis) are maintained between generations via specific cell regulatory or developmental mechanisms that act before, during or after the meiotic divisions (Suomalainen *et al.*, 1987).

Among animals, female-producing parthenogenesis has been estimated to occur in approximately 1 in a 1000 species (Vrijenhoek, 1998). However, this estimate is largely based on vertebrates and ignores several species-rich groups with large proportions of parthenogenetic lineages (e.g., hymenopterans and mites). True proportions of lineages capable of parthenogenesis may be up to an order of magnitude higher. The incidence of parthenogenesis varies widely among groups; classic examples of the extremes are mammals and birds without any parthenogenetic species, and cyclical parthenogens where parthenogenetic generations are part of an every species' live cycle.

Evolution of Parthenogenesis from Sexual Ancestors

In species that are not cyclical or facultative parthenogens, the evolution of a transition to parthenogenesis is most likely complex, requiring the acquisition of multiple novel adaptations (such as diploid instead of haploid gametes and spontaneous gamete development without sperm contribution (Neiman *et al.* 2014)). In addition to mutations, at least three different mechanisms can generate new parthenogenetic lineages from bisexual ancestors. First, **hybridization** between two sexual species has generated many described parthenogenetic lineages, and notably all but one known vertebrate parthenogen (Avise, 2008). The overall frequency of hybrid species among invertebrate parthenogens remains to be estimated. The cause of the association between parthenogenesis and hybridization remains largely unknown but may have different origins in different taxa. In some cases hybridization per se induces parthenogenesis. Under the 'balance hypothesis' (Moritz *et al.*, 1989) parthenogenesis via hybridization can only arise when the genomes of parental species are divergent enough to disrupt meiosis in hybrids, yet not so divergent as to compromise hybrid viability or fertility. In other cases, it has been hypothesized that parthenogens of hybrid origin have better competitive abilities relative to sexual sister species than non-hybrid parthenogens (Innes and Hebert, 1988).

Second, in some species, parthenogenesis is induced by **infection with endosymbionts** such as the bacteria *Wolbachia*

and *Cardinium* (reviewed in Duron *et al.*, 2008). Thus far, parthenogenesis induction by endosymbionts has only been experimentally confirmed in species with haplodiploid sex determination (notably in wasps, thrips and mites; reviewed in Neiman *et al.*, 2014). However, there are at least two species with other sex determination systems (the springtail *Folsomia candida* and the hemipteran *Aspidiotus nerii* (Pike and Kingcombe, 2009; Provencher *et al.*, 2005)) with a strong correlation between parthenogenesis and endosymbiont infection.

Finally, in some parthenogenetic lineages, females produce males that, by mating with females of related sexual lineages, generate new parthenogenetic lineages. This process is referred to as '**contagious parthenogenesis**' because gene flow from parthenogenetic into sexual lineages could allow for the spread of parthenogenesis-causing elements in a contagious fashion (Jaenike and Selander, 1979). In some cyclical parthenogens (especially *Daphnia* and aphids), the spread of obligate parthenogenesis is indeed at least partly mediated by such gene flow. However, despite the high potential of this contagious mechanism to generate parthenogenetic lineages, its incidence in natural populations is unknown, and may be limited since the geographic distribution ranges of sexual and parthenogenetic relatives are often distinct.

Sometimes **polyploidy** is hypothesized to cause parthenogenesis because it is more widespread among parthenogenetic as compared to sexual animals (Otto and Whitton, 2000). It is unclear whether polyploidy per se can induce parthenogenesis or whether the evolution of parthenogenesis and polyploidy are generally independent events. Some polyploid parthenogens derive from diploid ones via rare fertilization of parthenogenetic eggs but it is not known how widespread this mechanism is. There is also a lack of broad estimates of polyploidy incidence among parthenogens. Estimates based on small numbers of taxa are unreliable as the incidence of polyploidy varies widely among groups. For example, while the parthenogenetic beetles in the weevil family are generally polyploid, the numerous parthenogenetic hymenopteran and mite lineages are diploid. In cases where parthenogens are polyploid, polyploidy is likely to play a major role in the persistence of sex versus parthenogenesis as it can affect ecology and life-history traits (Otto and Whitton, 2000) and delays the expression of recessive deleterious alleles (Archetti, 2010).

Empirical Evidence for Benefits of Sex

Three different empirical approaches can be used to identify benefits of sex, each with specific advantages and disadvantages. The first set of approaches measures fundamental parameters used in evolutionary models predicting benefits of sex: epistasis (reviewed in De Visser and Elena, 2007) or rates of genomic mutations and the distributions of their fitness effects (e.g., Haag-Liautard *et al.*, 2007; Lynch *et al.*, 2008; Ossowski *et al.*, 2010).

A second set of approaches relies on experimental evolution to test whether mechanisms predicted to favor sex in theoretical studies apply to real organisms. These approaches have shown, for example, that sex speeds up adaptation to new environments in microorganisms (Colegrave, 2002; Poon and Chao, 2004; Goddard *et al.*, 2005; Grimberg and Zeyl, 2005; Cooper, 2007) and that higher rates of sex are maintained during adaptation in cyclical parthenogens and facultatively selfing macroorganisms (Morran *et al.*, 2009; Becks and Agrawal, 2012). These studies thus provide a 'proof-of-principle' that theoretically predicted mechanisms can favor sex given the appropriate conditions. However, these conditions may not be realized in natural populations, such that experimental evolution does not provide insights into the maintenance of sex in natural populations. Indeed, it is impossible to know whether any benefit to sex detected under artificial conditions (that may include controlled migration rates, specific population densities or sizes) would outweigh its immediate costs expressed under natural conditions.

Finally, a third empirical approach involves field studies and comparisons of asexual and related sexual lineages. While such studies provide insights into mechanisms important in natural populations, they generally remain correlational. Furthermore, when benefits of sex are identified in natural populations, it is often difficult to disentangle through which mechanisms such benefits are generated.

One of the best-documented consequences of sexual reproduction in natural populations is that it facilitates the purging of deleterious mutations. Thus, an increase of putatively deleterious (i.e., coding) mutations under asexual reproduction has been shown in a number of studies, both in animals (e.g., molluscs (Johnson and Howard, 2007; Neiman *et al.*, 2010), stick insects (Henry *et al.*, 2012), *Daphnia* (Paland and Lynch, 2006; but see Tucker and Ackerman, 2013)) and plants (e.g., *Oenothera* primroses; Hollister *et al.*, 2014). The extent to which the accumulation of such coding mutations results in negative phenotypic effects remains unknown. Furthermore, deleterious mutation accumulation would generate lineage-level (long-term) selection for sex, which, as explained above is insufficient to maintain sex in most cases.

Host–parasite coevolutionary dynamics can drive the constantly changing conditions required to generate persisting benefits for sex. Accordingly, some of the strongest evidence for benefits of sex in natural populations stems from host–parasite dynamics. In natural populations of New Zealand freshwater snails (*Potamopyrgus antipodarum*), sexually reproducing snails are favored in lakes and microhabitats within lakes with low prevalence of trematode parasites while asexual snails tend to occur in lakes or microhabitats where parasites are rare (Lively, 1987; King *et al.*, 2011). Whether parasites are the main driver to maintain sex in natural populations remains however unclear, as similar evidence lacks for other systems of co-occurring sexual and asexual lineages or even shows benefits for asexuals (e.g., Parker, 1994; Hanley *et al.*, 1995; Elzinga *et al.*, 2012).

Correlational evidence from natural populations is further consistent with Tangled-Bank related mechanisms favoring sex. Relative to asexual mite species, sexual species occupy higher trophic levels and occur in habitats where resource availability is more limited (Fischer *et al.*, 2014). Furthermore, high proportions of sexually reproducing mites are found in locations with low population densities, suggesting that sexual reproduction is favored under resource-limiting conditions (Maraun *et al.*, 2012).

Conclusion

Sophisticated theoretical approaches have generated insights into the mechanisms through which sex could favor adaptation. However, it remains unknown whether any of the identified mechanisms (or all of them combined) is able to generate sufficiently strong selection for sex to be maintained in natural populations, that is, fully compensate the costs expressed under these conditions.

Although many of the predicted benefits of sex have at least some empirical support, the benefits and costs of sex might vary among species. For example, physiological and developmental constraints on asexual reproduction, levels of ecological differentiation within a species and different life-history traits can all affect the relative costs and benefits of sex (Meirmans *et al.*, 2012). Consequently, the maintenance of sex in natural populations has most likely strong lineage-specific components. The implications are that there might be no single universal theory that can explain sexual reproduction in all systems (West *et al.*, 1999).

See also: Mating Systems, A Brief History of. Recombination and Molecular Evolution. Recombination and Selection. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth. Sex Determination

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Sexual Conflict

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Glossary

Anisogamy Reproduction resulting from the production of differently sized gametes by males and females (i.e., smaller sperm produced by males and larger eggs produced by females).

Interlocus sexual conflict A specific form of sexual conflict that occurs when the optimal outcome of sexual interactions is different for males and females. Interlocus sexual conflict selects for adaptations in each sex that increase their own fitness even if they are harmful to their mates. This conflict can lead to open-ended cycles of sexually antagonistic coevolution.

Intralocus sexual conflict A specific form of sexual conflict that occurs when alleles are beneficial when expressed in one sex but are costly when expressed in the other (i.e., 'sexually antagonistic alleles'). Intralocus sexual conflict can prevent both sexes from maximizing their fitness, as an adaptation in one sex reduces the level of adaptation in the other.

Locus The physical location of a specific gene or DNA sequence in a genome (plural: loci).

Sexual conflict An evolutionary conflict that results from the divergent reproductive interests of males and females (i.e., when males and females maximize their fitness using different strategies).

Sexual dimorphism The existence of phenotypic differences (e.g., physical and/or behavioral) between males and females.

Sexual selection A form of selection that acts on an individual's ability to reproduce. Sexual selection can occur when individuals of one sex (usually males) compete with one another for mating/reproductive opportunities (i.e., intrasexual selection, or 'male competition'), or when members of the other sex (usually females) choose mates based on specific physical or behavioral attributes (i.e., intersexual selection, or 'female choice').

Sexually antagonistic alleles Alleles (i.e., variants of genes at a specific locus) that have opposite effects on fitness when expressed in males and females.

Sexually antagonistic coevolution An evolutionary process that occurs between males and females where an adaptation that benefits one sex harms the other sex, and vice versa. Sexually antagonistic coevolution is caused by interlocus sexual conflict, and it results in each sex evolving and counter-evolving new strategies to maximize their fitness. Sexually antagonistic coevolution is often referred to as a coevolutionary 'arms race' between the sexes.

Speciation The evolutionary process by which new biological species are formed.

Introduction: What Is Sexual Conflict?

Early evolutionary views of reproduction, dating back as far as Darwin (1871), assumed that sexual interactions were mutually beneficial to both males and females. This harmonious view was associated with the assumption that many mating systems were strictly monogamous; under these conditions, the total number of offspring that an individual can produce is inherently linked with their mating partner, and any increase in the reproductive success of one partner will directly benefit the other. However, we now know that strict monogamy is extremely rare; in non-monogamous mating systems, partnerships are more temporary and the numbers of offspring produced by each partner are no longer necessarily equal. This can cause the reproductive strategies of males and females to diverge, creating conflict between the sexes.

Sexual conflict occurs when males and females have conflicting reproductive interests. The underlying cause of this conflict is anisogamy, i.e., the production of differently sized gametes by males and females. While male gametes (sperm) tend to be numerous, small, and relatively inexpensive, female gametes (eggs) tend to be fewer in number, larger, and relatively expensive. This causes a male's reproductive success (i.e., the number of offspring he sires) to be primarily limited by his number of successful fertilizations, whereas a female's reproductive success is limited by her number of eggs. Bateman (1948) demonstrated that this imbalance in gamete investment can lead to conflict over mating frequency. He found that the reproductive success of *Drosophila melanogaster* males increased rapidly with each additional mating, while female reproductive success did not. The optimal mating rate for males is thus higher than that for females. Parker (1979) was the first to show that, under these conditions, a trait increasing male mating success can spread in a population even if it is directly harmful to females. These two observations form the foundation of sexual conflict theory – males and females maximize their reproductive success in different ways, and the strategies that benefit one sex are often harmful to the other.

The field of sexual conflict has exploded since Parker's seminal work, and sexual conflict is now recognized as a ubiquitous and important force shaping the evolution of sexual species. As our understanding of the consequences of divergent sex roles has increased, we now recognize that sexual conflict can manifest in two fundamentally different ways. The first form of sexual conflict recognized by Parker is now referred to as 'interlocus' sexual conflict. This conflict occurs when the optimal outcome of sexual interactions differs for males and females, such that each sex evolves adaptations that increase their own fitness

(i.e., move them closer to their sex-specific optimum), but are harmful to their mates (Rice and Holland, 1997). In contrast, 'intralocus' sexual conflict occurs when a trait that is expressed in both sexes has opposite effects on male and female fitness, such that genetic variation that is beneficial for males is costly when expressed in females, and vice versa (reviewed in Bonduriansky and Chenoweth, 2009; Lande, 1980).

Both inter- and intralocus sexual conflict result from the divergent reproductive roles that males and females have in non-monogamous mating systems, and both are thus inherently linked with sexual selection. Sexual selection on males can lead to the evolution of competitive male traits that increase their reproductive success while harming their mates (interlocus sexual conflict), and it can also cause the sexes to maximize their fitness in different ways, such that alleles or traits that are beneficial when expressed in one sex are costly when expressed in the other (intralocus sexual conflict). Below, we describe both forms of sexual conflict in greater detail, provide examples for each, and discuss the evolutionary consequences of these conflicts.

Interlocus Sexual Conflict

Why Harm Your Mate?

It is somewhat paradoxical that selection could favor traits in either sex that are harmful to their mates, when each sex is directly dependent on the other to reproduce. Indeed, in the situation of strict monogamy, any male behavior or trait that harms his mate would directly lower his own fitness and likely not persist. However, when a male mates with multiple females, each female contributes less to his overall reproductive success, and an adaptation that increases male mating success can still be beneficial to males even if it is somewhat harmful to females. For example, a trait that doubles male mating success is expected to persist in a population as long as it lowers female reproductive success by less than 50%, because these 'harmful' males will still receive a net advantage in terms of offspring number. Thus, selection does not favor male harm itself, but it instead favors traits that increase male reproductive success, with female harm occurring as a pleiotropic by-product. Moreover, it is worth noting that sexual conflict can still occur even in a monogamous mating context. For example, males often invest heavily in strategies to prevent females from engaging in extra-pair matings; while this may be successful at ensuring female fidelity, it may also prevent males from securing additional matings for themselves if this investment is costly (Hosken *et al.*, 2009). Functional monogamy, therefore, does not always equal the absence of sexual conflict.

Interlocus Sexual Conflict: An Evolutionary Battle of the Sexes

As we describe above, interlocus sexual conflict occurs when there is conflict over the outcome of male–female interactions, with different outcomes maximizing reproductive success for each sex. This can occur any time males and females interact, and such conflicts often involve traits like mating frequency and duration, fertilization, reproductive rates, and parental investment/care. At the genetic level, interlocus sexual conflict reflects the fact that this conflict occurs between different genes at different locations in the genome (loci). Consider the example of mating frequency, in which male reproductive success is maximized by a higher mating rate than is optimal for females. Under these conditions, a 'persistence' allele may arise at a gene locus involved in male mating success, enabling males carrying this allele to mate more often. If the overall mating rate in the population increases to the point that it is detrimental to females, a 'resistance' allele may arise in a different gene locus involved in female resistance to male mating attempts. This resistance allele may then be met by another persistence allele, which leads to another resistance allele, and so on. This ongoing conflict between persistence and resistance alleles at different gene loci manifests as overt battles between males and females. An adaptation in males that increases their ability to mate females will shift the mating rate in the population closer toward the male optimum (benefiting males) and further away from the female optimum (harming females). Females may then counter-evolve an adaptation that mitigates this harm by making females resistant to male mating attempts, shifting mating frequency back toward the female optimum (Figure 1).

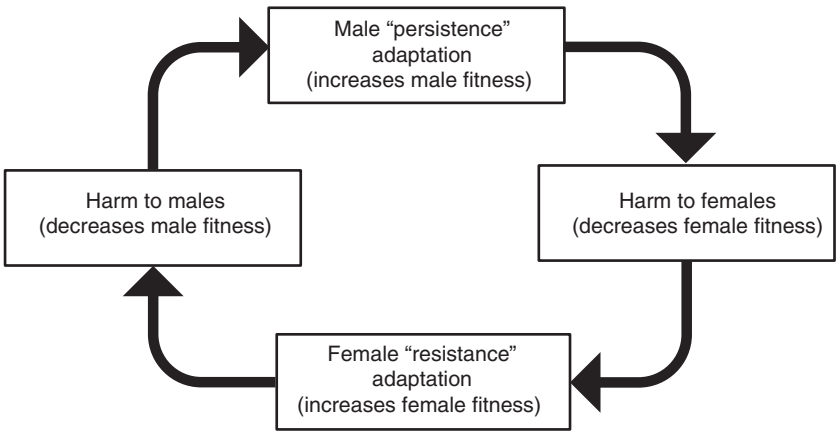

Figure 1 Sexually antagonistic coevolution can lead to an ongoing arms race between males and females as each sex evolves and counter-evolves new adaptations to maximize their respective fitness.

Interlocus Sexual Conflict and Sexually Antagonistic Coevolution

As the previous example suggests, interlocus sexual conflict can lead to ongoing, open-ended cycles of coevolution between the sexes, with each sex evolving and counter-evolving new strategies to maximize their fitness. This sexually antagonistic coevolution is an intraspecific coevolutionary 'arms race' between males and females that can involve any male–female interaction. The sexual arms race models developed by Parker (2006, 1979) assume that the costs of 'armament' (physical or behavioral) and the potential values of winning the arms race differ between the sexes. If females have a low arms level, any male mutation that is sufficient to overcome the female trait will be favored. Females can then counter-evolve by increasing their arms level, and this ongoing escalation of arms will continue until one sex reaches the point where evolving larger armaments is more costly than the value of winning. This 'losing' sex will then be selected to reduce its armaments to zero, because it will have higher total fitness by losing the race, but not paying any arms costs, than if it pays the costs necessary to win. The 'winning' sex can then continue to win the race with smaller armaments (and lower costs), so it will be selected to reduce its arms to just above the level necessary to win. However, in so doing, the 'losing' sex can then increase its armaments at a lower cost, potentially triggering another round of adaptation and counter-adaptation. This cycle can repeat indefinitely.

The ongoing nature of this coevolution makes it difficult to detect empirically, because at any given time, one sex could be 'ahead' of the other in this arms race, or the two sexes could be relatively 'even,' depending where they are in the cycle. However, a number of different approaches have been used to effectively demonstrate sexually antagonistic coevolution. Phylogenetic and experimental comparisons between closely related species of water striders have revealed intraspecific antagonistic coevolution involving mating behaviors and male grasping/female anti-grasping structures (Arnqvist and Rowe, 2002a, 2002b; Rowe and Arnqvist, 2002). Experimental evolution studies in the fruit fly, *D. melanogaster*, have indirectly demonstrated sexually antagonistic coevolution by showing that males evolve to be more harmful when females are unable to coevolve (Rice, 1996), and less harmful when reared under continuous monogamy (Holland and Rice, 1999).

Sexually antagonistic coevolution leads to a diverse array of behavioral, morphological, and physiological adaptations that can potentially influence any interaction between males and females. However, conflicts over the amount of parental investment or parental care may not be as strongly associated with harmful adaptations and sexually antagonistic coevolution as conflicts over mating (Lessells, 2006). These inconsistent outcomes likely result from differences in the benefits, costs, and feasibility of manipulating or resisting manipulation. Theory suggests that the benefits of manipulating parental investment are smaller, and the costs higher, compared to the benefits and costs of manipulating mating. Sexually antagonistic coevolution involving parental investment may consequently occur less frequently than antagonistic coevolution involving other forms of sexual conflict.

Can Interlocus Sexual Conflict Lead to Speciation?

Interlocus sexual conflict is now recognized as a dominant driver of evolutionary change within and between species. At the population level, these open-ended cycles of coevolution between males and females follow independent trajectories, indicating that interlocus sexual conflict may lead to divergence between populations, and can potentially act as an 'engine of speciation' (Gavrilets, 2000; Gavrilets and Waxman, 2002; Parker and Partridge, 1998; Rice, 1998; Rice *et al.*, 2005). Much of the work supporting this hypothesis is theoretical, but empirical support is mixed. A comparative study found that insect groups with strong interlocus sexual conflict speciated at a rate four times higher than groups without sexual conflict (Arnqvist *et al.*, 2000). Similarly, an experimental evolution study in dung flies found that large populations with stronger sexual conflict diverged more than small populations with weaker conflict (Martin and Hosken, 2003), but comparable experimental evolution studies in other species have failed to detect such a relationship (Gay *et al.*, 2009; Wigby and Chapman, 2006). Although interlocus sexual conflict can lead to population divergence and speciation, this may only occur when certain conditions are met and conflict is unconstrained in populations (Gavrilets, 2014).

Intralocus Sexual Conflict

Intralocus Sexual Conflict: A Genetic Tug-of-War Between the Sexes

Intralocus sexual conflict occurs when alleles for a shared trait are beneficial when expressed in one sex, but are detrimental when expressed in the other sex. Unlike interlocus sexual conflict, which occurs between alleles at two different loci, intralocus sexual conflict involves only a single locus, with alleles at this locus having opposite effects when expressed in each sex. Given that males and females are both members of the same species, how can such a genetic conflict exist? Intralocus sexual conflict results from the different selective pressures that males and females experience when their reproductive roles diverge, as is the case in non-monogamous mating systems. Although these different selective pressures create different optimal phenotypes for males and females, both sexes share largely the same genome; broadly speaking, each male and each female possess the same autosomes, and only differ with respect to the number and types of sex chromosomes. Therefore, the majority of gene loci are present in both sexes, and an allele that makes for a 'good' male (one with high fitness, compared to other males) may not make for a 'good' female, and vice versa. In other words, intralocus sexual conflict results in a genetic 'tug-of-war' between males and females over the optimal expression of these sexually antagonistic alleles, and it can ultimately lead to a negative genetic correlation between male and female fitness (**Figure 2**).

Hypothetically speaking, what would such a conflict look like? Imagine a population of early evolving humans, in which both males and females utilize the same skeleton for largely the same tasks (e.g., anchoring and protecting organs, calcium

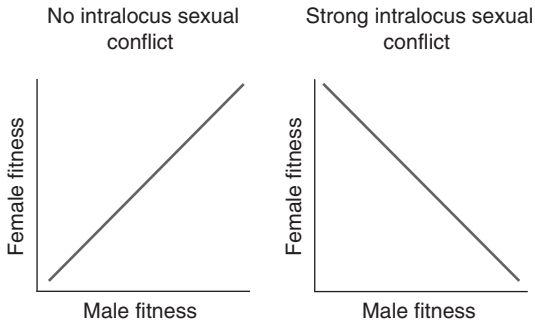

Figure 2 In the absence of intralocus sexual conflict (left), we would expect a positive genetic correlation between male and female fitness, with genetic variation influencing male and female fitness in the same way. In contrast, when there is strong intralocus sexual conflict in a population (right), we would expect a negative genetic correlation between male and female fitness, such that genetic variation that increases male fitness decreases females fitness, and vice versa.

storage, and locomotion). However, females have an additional functional constraint: childbirth. The head of the child must be able to pass through the pubic arch of females during childbirth, otherwise the life of the infant, the mother, or both, may be lost. Now suppose that a new mutation enters this population, one that widens hips to a small degree. The fate of this new mutation will depend on its effect in the individual in which it is expressed. If this mutation is present in a female, she will likely have a slightly easier time during childbirth, and the decreased risk of death (for both mother and infant) will increase her fitness relative to females that do not possess the mutation. This change in hip width might reduce her locomotor ability by decreasing speed and/or endurance, but if the benefit in reduced mortality during childbirth is larger than these costs, the net effect of this mutation in females will be positive, and natural selection will increase its frequency in the population. The daughters of this female will inherit this mutation and will have a similar increase in childbirth survival (and decrease in locomotor ability). Since the skeleton is a shared trait that is expressed in both sexes, the sons of this female will also possess wider hips. Unfortunately, males do not give birth, so they will receive none of the benefits of having wider hips but will suffer all of the locomotor costs. Thus, when expressed in males, the net effect of this mutation will be negative, and natural selection will decrease its frequency in the population. While this example is purely hypothetical, it is exactly the sort of relationship (a selective advantage in one sex, and a selective disadvantage in the other) that drives the genetic tug-of-war seen in intralocus sexual conflict. But, aside from theoretical and hypothetical arguments, what evidence is there that intralocus sexual conflict actually exists?

Empirical Evidence for Intralocus Sexual Conflict

Intralocus sexual conflict has been most extensively studied in laboratory populations of the fruit fly, *D. melanogaster*. Chippindale *et al.* (2001) provided the first direct evidence for intralocus sexual conflict by experimentally cloning multiple haploid genomes (i.e., a single copy of all major chromosomes) and measuring the fitness effects of these genotypes when expressed in males and females (a method referred to as 'hemiclonal analysis'). As predicted by intralocus sexual conflict, there were significant interactions between genotype and the sex that it was expressed in. Specifically, haploid genotypes that created males with high fitness also created females with low fitness, and vice versa. Follow-up work using similar techniques demonstrated that the majority of this intralocus sexual conflict was attributable to the X chromosome (Gibson *et al.*, 2002). Hemiclonal analysis in *D. melanogaster* was later used to demonstrate a fundamental consequence of intralocus sexual conflict: a negative relationship between the fitness of parents and their opposite-sex offspring. In this study, higher fitness mothers produced lower fitness sons, and higher fitness fathers produced lower fitness daughters (Pischedda and Chippindale, 2006). Additional evidence for intralocus sexual conflict in *D. melanogaster* comes from experimental evolution studies in which genomes evolved exclusively in males ('male-limited' genomes), removing any potential counter-selection on alleles when expressed in females (Prasad *et al.*, 2007; Rice, 1996). Males expressing these male-limited genomes exhibited increased fitness, but the same genomes substantially lowered fitness when expressed in females. Finally, a genomic analysis of intralocus sexual conflict estimated that at least 8% of the genes in *D. melanogaster* have sexually antagonistic effects on fitness (Innocenti and Morrow, 2010).

While experimentally tractable, laboratory studies of *D. melanogaster* do not provide sufficient evidence that intralocus sexual conflict is a widespread phenomenon. To that end, many other studies have shown that intralocus sexual conflict is present in a range of different species. For example, negative correlations for fitness between related males and females have been reported in populations of crickets (Fedorka and Mousseau, 2004), side-blotched lizards (Calsbeek and Sinervo, 2004), red deer (Foerster *et al.*, 2007), collared flycatchers (Brommer *et al.*, 2007), brown anole lizards (Calsbeek and Bonneaud, 2008), mountain goats (Mainguy *et al.*, 2009), Indian meal moths (Lewis *et al.*, 2011), bank voles (Mills *et al.*, 2012), and a number of different plant species (reviewed in Prasad and Bedhomme, 2006). It thus appears that intralocus sexual conflict is indeed widespread across sexually reproducing species.

Specific Traits Involved in Intralocus Sexual Conflict

Alongside attempts to demonstrate the action of intralocus sexual conflict, there have been a number of studies devoted to identifying specific traits that are involved in this conflict. For example, the same hemiclonal analysis used to provide evidence for intralocus sexual conflict (Chippindale *et al.*, 2001) was also used to identify locomotor activity as a trait at least partly responsible for the intralocus sexual conflict seen in *D. melanogaster* (Long and Rice, 2007). Specifically, genotypes that coded for relatively high rates of movement increased male fitness (possibly by increasing their contact rate with females), but decreased female fitness (possibly due to unnecessary energy expenditure).

Some of the most common traits involved in intralocus sexual conflict are those relating to overall body size or the size of different morphological structures. Body size is often genetically correlated between the sexes, but in many species

larger body sizes are favored in males and smaller body sizes are favored in females. This sexually antagonistic selection on body size has been reported in species like side-blotched lizards (Calsbeek and Sinervo, 2004), haplorhine primate species (Lindenfors and Tullberg, 1998), and humans (Stulp *et al.*, 2012). There are also several species in which large body size is favored in females and small body size is favored in males, including the mosquito *Aedes aegypti* (Bedhomme *et al.*, 2003) and collared flycatchers (Merilä *et al.*, 1997).

Sexual selection on males often creates a fitness advantage to males expressing large or elaborate structures, while the energetic and mechanical constraints associated with these traits are costly in females. For example, in carrion flies, males with elongated heads are favored by females and receive a fitness advantage, while females with elongated heads have lower fitness due to their reduced coordination, visual acuity, and movement (Bonduriansky and Rowe, 2003; Bonduriansky and Rowe, 2005). In Soay sheep, males with larger horns have a fitness advantage over males with smaller horns, while the opposite is true in females (Robinson *et al.*, 2006). Similar effects of wing length are seen in reed warblers, as wing length is positively associated with male fitness and negatively associated with female fitness (Tarka *et al.*, 2014). Alternatively, females may possess a complex trait that is necessary for reproduction but is not utilized in males (as in the human hip width example we describe above). This appears to be the case in the parasitoid fly *Emblemasoma auditrix*, as females use specialized 'ears' to locate hosts for oviposition, but males do not seem to use these costly structures for either mate location or predator avoidance (Lakes-Harlan *et al.*, 2014).

Although the genetic basis of many of the above traits is unknown, a specific allele has been identified in *D. melanogaster* that experiences intralocus sexual conflict. In this species, resistance to the pesticide DDT is caused by a single allele in the detoxification gene, *Cyp6g1* (Daborn *et al.*, 2002). In the absence of DDT, this allele has opposing fitness effects when expressed in males and females: it increases female fecundity (McCart and Buckling, 2005), but decreases male mating success (Smith *et al.*, 2011). Theory and experimental populations of *D. melanogaster* indicate that this sexually antagonistic selection is sufficient to maintain genetic variation at this locus (Rostant *et al.*, 2015).

Can Intralocus Sexual Conflict Be Resolved?

While there is ample evidence for intralocus sexual conflict across a range of species, this conflict is not expected to operate at every gene locus for two primary reasons. First, many gene loci are likely similarly selected in the sexes, in that alleles beneficial to a male will also be beneficial for a female (with the same being true for deleterious alleles). Secondly, unlike interlocus conflict, there are a number of genetic mechanisms that can potentially ameliorate intralocus sexual conflict (Stewart *et al.*, 2010). For example, if the conflict results from different optimal amounts of gene product, then natural selection could favor sex-specific regulatory elements (like androgen and/or estrogen receptors) that modify the amount of gene product produced in each sex, allowing each to move closer to their optimum. Alternatively, if the optimal amino acid sequence differs between the sexes, then gene duplication followed by sex-limited gene expression could restrict the expression of sexually antagonistic alleles to only the sex that they benefit (Rice, 1984). Day and Bonduriansky (2004) argued that selection should favor the evolution of genomic imprinting for sexually antagonistic alleles, such that maternally inherited alleles are silenced in sons, and paternally inherited alleles are silenced in daughters; this hypothesis is supported by evidence that sexually antagonistic alleles have reduced heritability to opposite-sex progeny (Bonduriansky and Rowe, 2005).

Although it appears that intralocus sexual conflict can be reduced, if not eliminated, the speed at which this resolution occurs remains an open question (Stewart *et al.*, 2010). Sexual dimorphism, in which males and females exhibit different phenotypes, is a likely outcome of resolved intralocus sexual conflict. However, many sexually dimorphic traits still show strong genetic correlations between the sexes (Bedhomme and Chippindale, 2007; Cox and Calsbeek, 2009), indicating that conflict has not been completely resolved. In addition, intralocus sexual conflict can continue to involve sexually dimorphic traits that have become genetically decoupled between the sexes. For example, male broad-horned flour beetles develop enlarged mandibles to use in male–male competition, and males with larger mandibles have higher fighting and mating success (Harano *et al.*, 2010). Female beetles do not have enlarged mandibles, and mandible size is uncorrelated between the sexes. However, males have evolved a modified body form with a larger thorax and smaller abdomen to support these large mandibles, and body size *is* correlated between the sexes. Small abdomens are costly to females, as abdomen size determines the number of eggs a female can carry. Thus, despite sexual dimorphism for mandible size in this species, intralocus sexual conflict over this trait persists because of its pleiotropic effects on body size.

Persistent intralocus sexual conflict should select for traits in males and females that minimize the associated costs. For example, females of several species, including side-blotched lizards (Calsbeek and Sinervo, 2004), anole lizards (Calsbeek and Bonneaud, 2008; Cox and Calsbeek, 2010), and *D. melanogaster* (Fuller and Mousseau, 2007) appear to bias the sex-ratio of their progeny depending on the fitness of their mate, producing more sons when mated to high fitness males and more daughters when mated to low fitness males. This 'cryptic female choice' is a behavioral adaptation by females that minimizes the fitness costs associated with intralocus sexual conflict (Cox and Calsbeek, 2010). Even if intralocus sexual conflict were completely resolved at a given point in time, each new mutation reopens the possibility of conflict, so the elimination of intralocus sexual conflict is unlikely to ever be absolute, and the tug-of-war will persist.

How Does Sexual Conflict Affect Populations?

Sexual conflict occurs between males and females (or, more specially, between alleles expressed in males and females), but it has consequences for fitness at the population level. Because both sexes are prevented from reaching their respective reproductive optima, sexual conflict lowers the overall reproductive output of a population, potentially interfering with adaptation. Interlocus sexual conflict has been shown to reduce the reproductive performance of whole populations

(Holland and Rice, 1999), indicating a strong cost to sex in non-monogamous species. Moreover, a combination of theoretical and experimental work suggests that male harm not only impedes female adaptation, it can also reduce the effectiveness of natural selection within a population (Long *et al.*, 2009). Intralocus sexual conflict can similarly lower the fitness of populations by preventing both males and females from maximizing their fitness. In support of this, both sexes exhibit decreased fitness in populations of *D. melanogaster* with higher levels of intralocus sexual conflict (Morrow *et al.*, 2008). The reduction in population fitness associated with sexual conflict has led to the hypothesis that sexual conflict may lead to an increased risk of extinction, but there is no clear relationship between the strength of sexual selection (and, by extension, sexual conflict) and the risk of species extinction in birds (Morrow and Pitcher, 2003) or mammals (Morrow and Fricke, 2004).

The impacts of sexual conflict on males, females, and the population as a whole demonstrate the substantial costs of divergent sex roles. Sexual conflict is unavoidable in non-monogamous mating systems, and will exist in populations whenever there are deviations from strict, lifelong monogamy. Sexual conflict is thus inherently linked to sexual selection, and both inter- and intralocus sexual conflict increase when sexual selection operates more strongly in populations (Cox and Calsbeek, 2009; Holland and Rice, 1999; Nandy *et al.*, 2013). Sexual conflict is thus a ubiquitous and powerful force driving diversity, and it directly influences the evolution of behavior, morphology, and reproduction in sexual species.

See also: Intraspecific Coevolutionary Arms Races. Life History Evolution: The Role of Mating Systems. Speciation, Sexual Conflict and

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Sexual Dimorphism

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Glossary

Allometry (*adj.* allometric) Departure from geometric similarity; disproportionate change in a given variable (often size of a body part) with some measure of body size. A relationship is allometric if the ratio of trait value to body size increases or decreases with body size.

Clade A group of species defined by descent from a single common ancestor and including all descendants from that ancestor.

Dioecy (*adj.* dioecious) The allocation of reproductive roles in a population such that all individuals are either male or female and possess only male or female reproductive organs. Individuals with the reproductive organs of only one sex are *gonochoric*, so dioecious species contain only gonochoric individuals.

Disruptive selection Selection against intermediate values and in favor of values both above and below the population mean.

Fecundity The number of eggs or offspring produced by a female.

Fertility When referring to individuals: the quality of being fertile; being able to produce offspring. When referring to populations: the average number of births per female within a given age class.

Fitness In evolutionary biology: an abstract concept that refers to the success of an individual in contributing genes to future generations. Fitness is determined by survivorship (viability), reproductive success (mating success, fertility, and fecundity), and generation time.

Genetic correlation Correlated variation in the breeding values of two or more traits caused by the additive effects of overlapping sets of genes (pleiotropy) or linkage. If traits are genetically correlated, selection on one trait causes a correlated response in the other.

Genomic imprinting An epigenetic mechanism whereby the expression of alleles at a given locus depends upon the parent of origin. For example, if the allele inherited from the father is imprinted (epigenetically silenced), only the allele inherited from the mother is expressed in the offspring.

Haplodiploidy A sex-determining system in which females develop from fertilized eggs and are diploid, whereas males develop from unfertilized eggs and are haploid.

Sex-linked gene A gene located on a sex chromosome.

Somatic Referring to body components (cells, tissues, organs, or traits) other than those that comprise the reproductive system.

What Is Sexual Dimorphism?

The term *dimorphism* denotes a trait that occurs in two distinct forms or morphs within a given species and traits that differ consistently between males and females are *sexual dimorphisms*. Sexually dimorphic traits may differ so radically between sexes that they can be reliably used to differentiate males from females. Color dimorphisms in many species of birds, lizards, and fishes are familiar examples of this (**Figure 1**). Other common examples include the presence of antlers or other weapons in only one sex (usually males), adaptations of one sex (usually females) for parental care (e.g., mammary glands in female mammals and brood pouches in many invertebrates), and externally apparent differences in genitalia (**Figure 2**). However, many sexual dimorphisms are not as extreme as this. Any trait that differs on average between sexes is considered sexually dimorphic, even if the trait distributions overlap considerably between sexes. Height in humans provides a familiar example of this type of sexual dimorphism (**Figure 3**).

Primary and Secondary Sexual Dimorphisms

Sexual dimorphisms have traditionally been categorized as either primary or secondary, depending upon what role they play in reproduction. This distinction goes back to Darwin's initial discussions of sexual differences in which he defined sexually dimorphic traits as primary if they were directly connected to reproduction and as secondary if they were not (Darwin, 1859, p. 150). He later clarified that primary sexual traits are components of the reproductive tract; specifically the organs and tissues that produce and release large, nutrient-rich macrogametes (eggs) in females and more numerous, smaller, and more motile microgametes (sperm) in males (Darwin, 1871). Secondary sexual traits are not essential for reproductive function in this strict sense, although they often increase mating or reproductive success. Familiar examples include sexually dimorphic breeding plumages in many birds (**Figure 1**), antlers in male deer and bright throat and belly patches in male lizards. In humans secondary sexual traits include, but are not limited to, enlargement of female breasts, increased male facial hair, deepening voice in males and differences between the sexes in body size, shape, muscle mass, and fat distribution (Mealey, 2000).

Why Do Sexual Dimorphisms Evolve?

Sexual dimorphisms evolve in response to persistent selection favoring different trait values in the two sexes (Lande, 1980; Slatkin, 1984; Reeve and Fairbairn, 2001; Blanckenhorn,

Figure 1 A pair of mallard ducks illustrate sexual dimorphism in color (*sexual dichromatism*) that clearly distinguishes the breeding male (bottom) from his mate (top). Photo by Derek Roff.

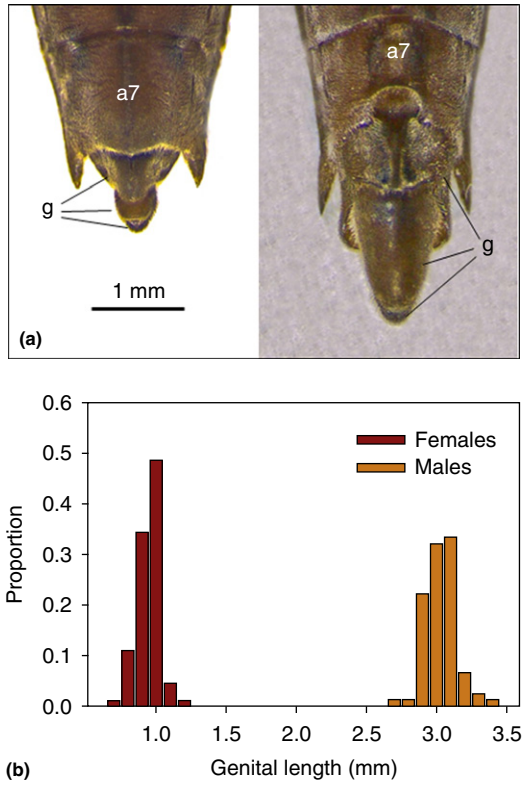

Figure 2 The terminal segments of an insect, the water strider *Aquarius remigis* (Hemiptera, Gerridae) exemplify dimorphisms that clearly distinguish the sexes. (a) Ventral views of a female (left) and a male (right) photographed on the same scale. a7: the seventh and final abdominal segment; g: the three genital segments. (b) Distribution of genital lengths for males and females from a single population. Genital length is measured at midline in ventral view.

2005). For example, in many sexually dichromatic species selection favors bright colors in males but more subdued and often cryptic coloration in females (**Figure 1**). The disparity in selection on the two sexes ultimately arises from the different ways they achieve reproductive success: males through fertilizing eggs and females through producing eggs or live young. Trait values that confer high fitness for male function often differ from those that convey high fitness for female function, resulting in disruptive selection favoring sexual dimorphisms.

Many of the most conspicuous and familiar secondary sexual dimorphisms have evolved in response to sexual selection. Sexual selection on males arising from both direct competition among males for mates (*intrasexual selection*) and female mate choice (*intersexual selection*) has been responsible for the exaggeration and diversification of many traits in males, including body size, weapons, and sexual display traits (Darwin, 1871; Andersson, 1994; Fairbairn *et al.*, 2007; Emlen, 2014). The large size and bulbous noses of male elephant seals (**Figure 4(a)**), the enlarged claw of male fiddler crabs (**Figure 4(b)**), and the colorful plumage of male mallard ducks (**Figure 1**) are classic examples of such traits. Sexual selection also influences secondary sexual traits in females, particularly in species where males invest substantially in mating or paternal care, but its effects are generally more muted than in males (Bonduriansky, 2001; Clutton-Brock, 2009).

Many traits traditionally classified as primary sexual traits are also subject to sexual selection (Eberhard, 1996, 2009; Birkhead and Moller, 1998; Birkhead, 2000; Hosken and Stockley, 2004; Leonard and Cordoba-Aguilar, 2010; Simmons and Fitzpatrick, 2012). For example, if fertilization is internal and females mate with more than one male during a given reproductive bout, competition among males extends to competition for fertilizations within female reproductive tracts. Sexual selection during this internal competition affects many aspects of the male reproductive tract, including the size and sperm-producing capacity of testes, the production and composition of accessory seminal fluids, and the size, shape, and function of the copulatory organ. Females, in turn, evolve adaptations that enable them to resist matings or to preferentially store and use the sperm of different males. The adaptations and counter-adaptations of male and female reproductive tract traits under this scenario provide classic

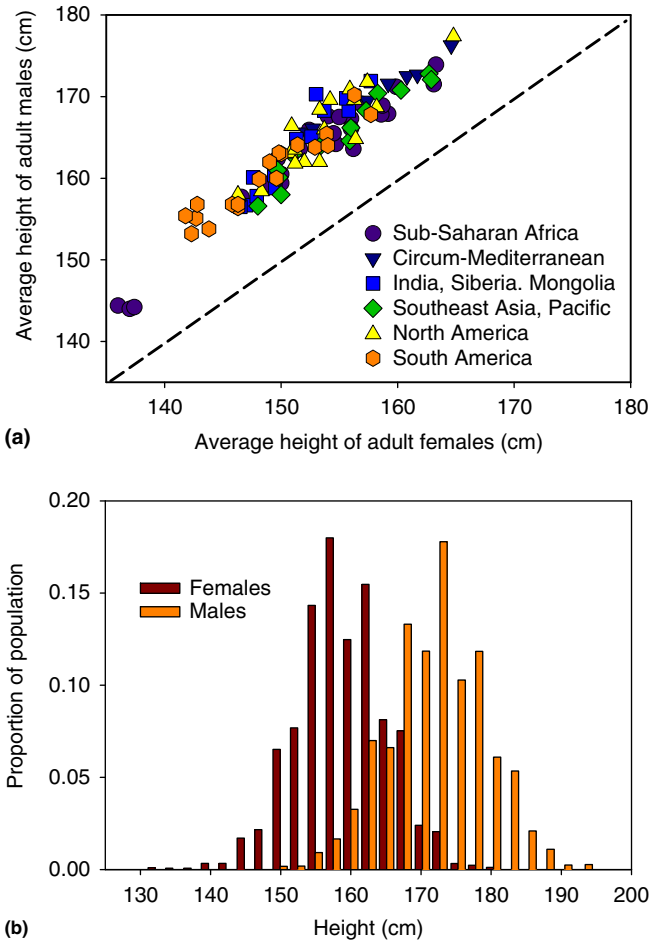

Figure 3 Height in humans is an example of a sexually dimorphic trait that shows extensive overlap between sexes. (a) A plot showing the mean male height versus mean female height for 97 human populations. The dashed diagonal line is the one-to-one line. The average height of males exceeds that of females within every population as well as overall (the overall means are 164 cm for males and 153 cm for females), but the ranges in population means overlap extensively (144–177 cm in males and 136–165 cm in females). Data from Holden, C., Mace, R., 1999. Sexual dimorphism in stature and women's work: A phylogenetic cross-cultural analysis. American Journal of Physical Anthropology 110, 27–45. (b) The distribution of height in adult males and females in the US population, 1960–62. The mean height of males is 172 cm and of females 159 cm, a difference of about 8%, which is typical of human populations. Data from the National Center for Health Statistics, 1966. Public Health Service Bulletin No. 1000, Series 11, No. 14, p.39.

examples of *interlocus sexual conflict* and *coevolutionary arms races* driven ultimately by sexual selection (Rice and Holland, 1997).

Sexual selection contributes to the evolution of many sexual dimorphisms, but it is far from the sole cause. Selection acting through variation in viability, fertility, and fecundity also acts differentially on each sex, tailoring the overall biology of each to maximize fitness through either male or female function (Clutton-Brock, 1988; Blanckenhorn, 2005; Ruckstuhl and Neuhaus, 2005; Fairbairn *et al.*, 2007; Fairbairn, 2013a). For example, adaptations for female reproductive function such as brood pouches, enlarged abdomens, thickened bodies, and modifications of genital openings for spawning eggs or to serve as ovipositors are common distinguishing features of females across the animal kingdom (Fairbairn, 2013a,b). Although these traits may be subject to sexual selection through male mate choice, the primary evolutionary driver is selection favoring female traits that confer high fecundity and parental success (Preziosi and Fairbairn, 1997; Prenter *et al.*, 1999; Blanckenhorn, 2005; Fairbairn, 2013a).

Many sexual dimorphisms in ecological and life-history traits also show little or no influence of sexual selection (Clutton-Brock, 1988; Shine, 1989; Ruckstuhl and Neuhaus, 2005; Barrett and Hough, 2013; Fairbairn, 2013a). For example, males and females often mature and die at different ages, have different diets, occupy different habitats during at least some phases of their lives, and differ in their propensity to disperse or migrate. These differences in ecology and life history are frequently associated with dimorphisms in size and shape and in the appendages or organs used for movement or feeding. Darwin attributed these types of secondary sexual differences to the 'different patterns of life' adopted by each sex as a consequence of their different roles in propagating the species (Darwin, 1871, p. 127). This explanation, now formalized as the 'dimorphic niche hypothesis,' has been well

Figure 4 Examples of secondary sexual dimorphisms that have evolved at least partly in response to sexual selection on males. (a) Male and female northern elephant seals (*Mirounga angustirostris*) show pronounced secondary sexual dimorphisms in body size and shape, and in the size and shape of the nose, which is greatly elongated in males. (b) Male (left) and female (right) fiddler crabs (*Uca pugilator*) differ in body size and color, and males have one greatly enlarged major claw. Photos by Derek Roff.

supported by both theoretical models and empirical studies (Slatkin, 1984; Hedrick and Temeles, 1989; Ruckstuhl and Neuhaus, 2005; Dudley, 2006; Bulte *et al.*, 2008; Fairbairn, 2013a,b). An alternative hypothesis, that ecological differences between the sexes evolve to reduce competition between them, is plausible, but the weight of evidence supports the dimorphic niche hypothesis, with reduction in intersexual competition a beneficial consequence of ecological segregation rather than the initial cause (*op. cit.*).

How Do Sexual Dimorphisms Evolve?

When selection favors different trait values in males and females, the evolutionary response to such selection is constrained by genetic correlations between the sexes that arise because the two sexes share all or almost all of their genome (Lande, 1980; Reeve and Fairbairn, 2001; Mank, 2009). The selection acts antagonistically on genes that influence the trait similarly in the two sexes, producing *intralocus sexual conflict* at those shared loci (Bonduriansky and Chenoweth, 2009). The most pervasive mechanism for mitigating this conflict is the evolution of sex-specific patterns of gene expression such that genes beneficial to females are preferentially expressed in females, whereas the opposite is true for genes beneficial to males (Connallon and Knowles, 2005). Transcriptomic studies have revealed sex-biased gene expression in thousands of genes on both autosomes and sex chromosomes and in both reproductive and somatic tissues (Mank, 2009; Mank *et al.*, 2013; Wright and Mank, 2013; Perry *et al.*, 2014). These sex-specific patterns can evolve through a number of processes including gene duplication, genomic imprinting, sex-specific posttranscriptional regulation (e.g., sex-specific patterns of gene splicing) and co-option of hormones from the sex-determining pathway as regulatory switches (Gallach and Betran, 2011; Wright and Mank, 2013). The latter has the potential to produce very rapid evolution of sexual dimorphisms and may account for rapid evolution of many androgen-dependent traits in response to sexual selection in vertebrates.

Many species lack sex chromosomes and instead use mechanisms such as haplodiploidy or environmental or social cues to determine sex (Beukeboom and Perrin, 2014). In these species sexual dimorphisms must arise solely from sex-specific patterns of autosomal gene expression. However, in species with chromosomal sex determination, genes located on sex chromosomes (*sex-linked* genes) are exclusive to one sex (e.g., genes on Y chromosomes occur only in males in species with XX/XY chromosomal systems) or occur in one sex more often than the other (e.g., genes in X chromosomes spend two-thirds of the time in females). Sex chromosomes thus permit the evolution of sex-limited or sex-biased genes as an additional mechanism for mitigating intralocus sexual conflict. A number of theoretical models have predicted that sex chromosomes should therefore be hotspots for genes subject to antagonistic selection on males and females (e.g., Charlesworth and Charlesworth, 1980; Rice, 1984, 1987; Charlesworth, 2002; Kirkpatrick and Hall, 2004; Connallon and Clark, 2010). In accordance with these predictions, the proportion of genes with sex-biased expression is higher on sex chromosomes than on autosomes and such genes are distributed on the homomorphic (e.g., X in XX/XY systems and Z in ZZ/ZW systems) and heteromorphic (Y or W) sex chromosomes as predicted by theory (Mank, 2009; Stewart *et al.*, 2010; Wright and Mank, 2013; Dean and Mank, 2014). However sex chromosomes typically comprise only 2–18% of the genome and sex-linkage seldom accounts for much more of the variance in sexually dimorphic traits than would be expected based on this proportion (Fairbairn and Roff, 2006; Husby *et al.*, 2013; Dean and Mank, 2014). Thus, although sex-linkage likely facilitates the evolution of sexual dimorphisms, sex-biased expression of autosomal genes accounts for

most of the genetic variation in dimorphic phenotypes even in species that have sex chromosomes.

The Prevalence and Distribution of Sexual Dimorphism in Animals

Variation among Animal Classes

Most animal species are dioecious (ca. 95%; Bachtrog *et al.*, 2014) and exhibit some form of externally apparent sexual dimorphism (Fairbairn, 2013a,b). The following discussion pertains only to dimorphisms in external morphology (hereafter sexual dimorphism in morphological traits (SDM)) because these are the most reliably reported in species descriptions. However, dimorphisms in life history, ecology, and behavior are also common and are often correlated with SDM. Species with SDM occur in at least 73 animal classes (**Figure 5**) and more than 96% of animal species are found in classes where SDM is common to universal.

Among the major animal lineages, SDM is most prevalent in the Ecdysozoa (**Figure 5**; Fairbairn, 2013a). This is the most speciose animal lineage and includes the massive phylum Arthropoda (insects, arachnids, crustaceans, and relatives), roundworms (Nemata), and six minor phyla. Morphological dimorphisms are common to universal in 80% of ecdysozoan classes and these classes contain 99.9% of ecdysozoan species. In the Deuterostomia, the major lineage composed of chordates, echinoderms, and hemichordates, SDM is common to universal in 52% of classes which contain 90% of species. The prevalence of SDM is notably lower in the Lophotrochozoa, the diverse lineage composed of 18 phyla of mainly shelly or vermiform animals, the most speciose of which are the molluscs (Mollusca), flatworms (Platyhelminthes), and segmented worms (Annelida). In this lineage SDM is common to universal in 43% of classes containing 71% of species. By far the lowest prevalence of SDM occurs in the eight phyla not included in these three major lineages, the largest of which are the jellies (Cnidaria), comb jellies (Ctenophora), and sponges (Porifera). In these phyla, SDM is common to universal in only 20% of classes which contain only 19% of species.

Why is SDM more prevalent in some types of animals than in others? Surveys across the animal kingdom suggest several predisposing factors (Ghiselin, 1974; Fairbairn, 2013a). Prevalence is clearly higher in animals that are active and mobile and in which mating requires close behavioral interactions, especially if these include sexual grasping and internal fertilization. If such species occur at high densities or aggregate for reproduction, there is ample opportunity for sexual selection and sexual conflict to drive the evolution of SDM in both primary and secondary sexual traits. Alternatively, if such species occur at low densities, selection for finding mates favors sexually distinct adaptations for sending and receiving sexual signals, as well as adaptations (most commonly in males) for long distance movement in search of mates. Regardless of density, sexual grasping and internal fertilization require sex-specific adaptations in genitalia and somatic traits and are invariably associated with SDM. High female fecundity is another predisposing factor for SDM. Many female animals produce large clutches of eggs or live young and have evolved large body sizes (relative to males), enlarged abdomens, or expanded cavities within their exoskeletons to accommodate these. Similarly, if eggs are brooded after oviposition, the brooding sex (usually females) typically has morphological adaptations for holding and protecting the brood.

Sexual dimorphisms reach their greatest extremes in species with dwarf males (**Figure 6**; Ghiselin, 1974; Vollrath, 1998;

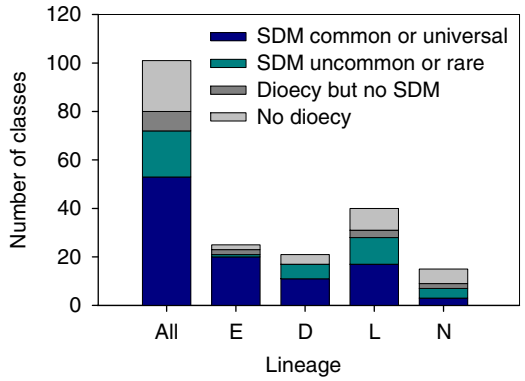

Figure 5 Prevalence of externally apparent sexual dimorphism in morphological traits (SDM) in animals. Each of 101 taxonomic classes has been scored for the presence or absence of dioecious species and for the frequency of SDM among dioecious species (absent, rare, uncommon, common, universal). Small Phyla that lack class designations are treated as single classes. Data are shown for all classes combined (left bar) and separately for the three major evolutionary lineages of bilaterian animals, Ecdysozoa (E), Deuterostomia (D), and Lophotrochozoa (L), and for the phyla not included in these lineages (N). Classification is based upon Roskov *et al.* (2014) and assignment of phyla and their component classes to evolutionary lineages is based upon Hickman *et al.* (2007) and Nielsen (2012). Recent taxonomic revisions cause slight disparities between the frequencies shown and those reported in Fairbairn (2013a,b).

Figure 6 A pair of golden orb spiders, *Nephila clavipes*, illustrate male dwarfism. The large female is in the middle of her mating web and a much smaller male can be seen above and to her right. Photo by Derek Roff.

Fairbairn, 2013a). Males are conventionally defined as dwarfs if they are less than ½ as long or ⅛ the mass of their mates and SDM at least this extreme has evolved independently in 23 animal classes distributed across 12 phyla (Fairbairn, 2013a). Male dwarfism is most common in parasitic and commensal species, an association that occurs in at least 13 classes and 8 phyla, but it also occurs in diverse clades of free-living species. In the latter, females are typically highly fecund but rare and widely dispersed, and males are adapted for long distance mate searching. The tiny males mature early and have short lives relative to their mates. In many species, they die after a single mating or live their reproductive lives attached to or inside the bodies of their mates. In the extreme, mature females outweigh their mates by several orders of magnitude and the sexes are so disparate morphologically that only trained zoologists can discern that they belong to the same species (Fairbairn, 2013a).

Variation among Traits

Some traits are more likely than others to show SDM (**Figure 7**; Fairbairn, 2013a). For example, gonadal dimorphisms are seldom visible externally, whereas dimorphisms in gonopores or external genitalia are common, especially in species that have internal fertilization. The traits most likely to show SDM are body size and shape. Females average larger than males in 42 classes, whereas males average larger in seven and both types of dimorphism occur with nearly equal frequency in two (Fairbairn, 2013a). Females tend to be the larger sex in species in which they are subject to strong fecundity selection favoring large size while sexual selection on male size is absent, weak, or favors smaller males (Roff, 1992; Fairbairn, 1997, 2013a; Blanckenhorn, 2000, 2005; Fairbairn *et al.*, 2007). Conversely, strong sexual selection favoring large males combined with weak fecundity selection on females (as occurs in many large mammals and birds) is the best predictor of male-larger dimorphisms (*op. cit.*).

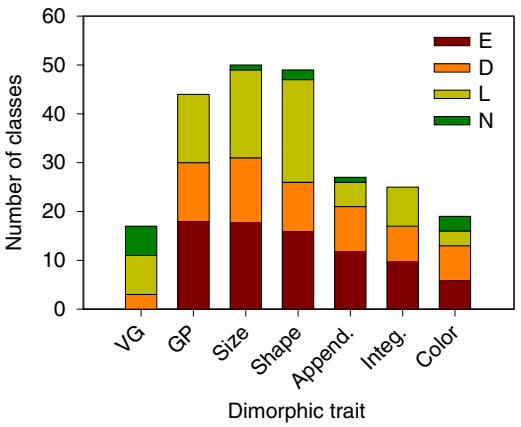

Figure 7 Prevalence of sexual dimorphism in morphological traits (SDM) for different trait types in animals. All animal classes containing dioecious species ($n=80$) have been scored for the presence or absence of SDM in externally visible gonads (VG), gonopores or external genitalia (GP), body size, body shape, appendages (Append.), integumental morphology (Integ.), and color or pigment (color). The few small Phyla that lack class designations are treated as single classes. The stacked bars indicate the number of classes in which each type of SDM has been observed within the three major evolutionary lineages of bilaterian animals, Ecdysozoa (E), Deuterostomia (D), and Lophotrochozoa (L), and in the phyla not included in these lineages (N). See text for further explanation of the trait types. Recent taxonomic revisions cause slight disparities between the frequencies shown and those reported in Fairbairn (2013a,b).

The magnitude of sexual dimorphism in body size often correlates with mean body size when comparisons are made among species within a given clade. Rensch (1959) was the first to describe this trend, noting that size dimorphism increases with mean size in clades where males are the larger sex but decreases as size increases if females are the larger sex. Although seemingly disparate, these two allometric trends (known as *Rensch's rule*) both derive from greater evolutionary divergence in male size than in female size within a given clade. Subsequent analyses have confirmed Rensch's rule in many clades where males average larger than females but in only a minority of clades where females are the larger sex (Abouheif and Fairbairn, 1997; Fairbairn, 1997; Blanckenhorn *et al.*, 2007; Fairbairn *et al.*, 2007; Webb and Freckleton, 2007). The evolutionary mechanisms underlying Rensch's rule have not been fully discerned, but a consistent association with sexual selection on male body size implicates sexual selection as an important driving factor.

Next to size, body shape is the trait most likely to differ between sexes (**Figure 7**). Most commonly females are thicker-bodied than males or have noticeable brood pouches or larger abdomens, all characteristics that adapt female body shape for the production and storage of eggs or live offspring. Conversely, male bodies are most commonly shaped to facilitate grasping and holding females during mating (e.g., the concave plastrons of male turtles and j-shaped tails of nematode males); for success in physical contests with other males (e.g., proportionally larger heads and upper bodies in many mammals; **Figure 4(a)**); or for efficient mate searching (thinner, more streamlined shapes) (Fairbairn, 2013a).

Appendages are much less likely to show SDM than body size and shape (**Figure 7**). This is mainly because many animals do not have appendages, especially among the Lophotrochozoa, where shelly and vermiform body forms predominate. However, where appendages exist, they are frequently modified in sex-specific ways (Fairbairn, 2013a). Some of these differences reflect differences in the dispersal or locomotory strategies of the two sexes (e.g., Roff, 1990), but most relate directly to sexual selection and reproduction. For example, male appendages are often modified for grasping females during mating or to serve as copulatory organs. They may also serve as weapons in contests between males, visual displays to attract females or both (**Figure 4(b)**). Appendages in one or both sexes may also be modified for sending and receiving chemical or acoustical signals (e.g., antennae in many moths, wings in male crickets) or for brooding eggs (e.g., ovigera in male sea spiders (class Pycnogonida)).

Integumental dimorphisms include all visible sexual differences in body covering other than color or pigment, including structures derived from epidermal tissues such as teeth, horns, claws, spines, hair, feathers, and scales. The functions of these dimorphisms often parallel those of appendage dimorphisms (Fairbairn, 2013a). For example,

teeth, spines, horns, and claws often function as weapons in male contests over mates; teeth and claws serve as grasping organs in mating interactions; and many hairs, bristles, feathers, scales, spines, and pits function in sexual displays or long distance sexual signaling. However, not all integumental dimorphisms are so readily interpreted: the functions of many remain obscure, especially in lesser-known species.

Color or pigment patterns are less likely to show SDM than other secondary sexual traits (**Figure 7**) primarily because the majority of animal species lack the ability to distinguish colors or resolve patterns (Kelber *et al.*, 2003; Land and Nilsson, 2012). In such species color and pigmentation cannot function as intraspecific signals, although they may still have ecological functions such as camouflage or thermoregulation. In rare cases SDM in color or pigment *sexual dichromatism*) is unrelated to intraspecific signaling, but most examples are attributed least partly to sexual selection (Badyaev and Hill, 2003; Kelber *et al.*, 2003; Fairbairn, 2013a). Most commonly, sexual selection favors conspicuous colors and patterns in males whereas females are less conspicuous, often to the extent of being cryptic or camouflaged (**Figures 1** and **4(b)**).

Sexual Dimorphisms in Plants

Dioecy is much less prevalent in plants than in animals. Approximately 96% of species within the kingdom Plantae are flowering seed plants (angiosperms; Roskov *et al.*, 2014) and only about 6% of angiosperm species are dioecious (Renner and Ricklefs, 1995; Barrett, 2002; Vamosi *et al.*, 2003). Among the remaining seed plants (gymnosperms) 50% of species are dioecious, including 37% of conifers (Givnish, 1980; Bateman *et al.*, 2011). Among spore-forming plants, dioecy is very rare in ferns (Jesson and Garnock-Jones, 2012) but relatively common in bryophytes (mosses, liverworts, and hornworts) where it occurs in 50–60% of species (Hedenas and Bisang, 2011; Jesson and Garnock-Jones, 2012; McDaniel *et al.*, 2013).

In most dioecious plants, only reproductive structures distinguish the sexes. These consist of primary reproductive organs (e.g., the stamens and pistils of angiosperm flowers) plus surrounding somatic tissues, often formed from modified stems or leaves (e.g., the calyx and corolla of angiosperms). Sexual dimorphisms in these somatic tissues are generally interpreted as adaptations in males for dispersing pollen or sperm and in females for capturing pollen or sperm and for protecting and provisioning embryos (Givnish, 1980; Eckhart, 1999; Geber, 1999; Barrett and Hough, 2013; McDaniel *et al.*, 2013). These are the predominant secondary sexual dimorphisms in plants, and are most pronounced in species that depend on wind or water for pollen dispersal but produce large seeds or fruit that are dispersed by animals (Givnish, 1980; Renner and Ricklefs, 1995; Vamosi *et al.*, 2003; Biernaskie, 2010; Bateman *et al.*, 2011).

The secondary sexual trait most likely to differ between male and female reproductive structures is size. Flower size dimorphisms occur in more than 80% of diecious angiosperm species, with male flowers somewhat more likely to be larger than the reverse (Eckhart, 1999). In contrast, in dioecious gymnosperms the female reproductive organs (strobili) are larger and only the female strobili develop into the large, long-lived cones typical of conifers and cycads. In angiosperms, the number of flowers and the sizes of inflorescences (flower clusters) may also differ between sexes, with males more commonly having more flowers or larger flower clusters than females. In a few families, differences in flower shape and in the position or orientation of the flowers on the plant have also been described (Eckhart, 1999; Barrett and Hough, 2013).

Other than reproductive structures, the most prevalent SDM in plants is overall size. In both angiosperms and gymnosperms, males tend to be larger than females in large, long-lived, woody species (e.g., trees and shrubs), whereas females tend to be larger in small, short-lived, herbaceous species (Obeso, 2002; Barrett and Hough, 2013). Size dimorphism is most extreme in pleurocarpous mosses (class Bryophyta) where, in about a third of species, dwarf males develop from spores that land on the stems or leaves of females and remain attached to the female throughout their lives (Hedenas and Bisang, 2011).

Sexual dimorphisms in other aspects of plant vegetative morphology are much less common but have been noted in at least a few species. Examples include leaf size (females more commonly larger), leaf shape, stem size (females more commonly thicker), and branching architecture (males usually more branched) (Dawson and Geber, 1999; Kavanagh *et al.*, 2011; Barrett and Hough, 2013). As in animals, male and female plants also often differ in ecological and life-history traits (Dudley, 2006; Geber *et al.*, 1999; Barrett and Hough, 2013). The sexes are sometimes partially segregated by habitat, with females more restricted to sites with more water or nutrients, and males often exceed females in their capacity for clonal reproduction, reproduce at an earlier age, and senesce earlier than females. These differences reflect sex-specific trade-offs between growth and reproduction and, as in animals, are interpreted as adaptations for maximizing fitness through male or female reproductive functions.

See also: Epigenetic Inheritance. Intraspecific Coevolutionary Arms Races. Life History Evolution: The Role of Mating Systems. Mate Choice and Sexually Selected Traits. Multivariate Quantitative Genetics. Natural Selection, Introduction to. Polyandry and Female Postcopulatory Choice. Sex Chromosome Evolution: Birth, Maturation, Decay, and Rebirth. Sex Determination. Sexual Conflict. Sexual Selection, Theory of. Sperm Competition

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Sexual Networks

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Glossary

Assortativity Broadly defined as the correlation between attributes of a focal individual and those of its sexual partner(s).
Bateman gradient The slope of the univariate regression of reproductive success (number of offspring) over mating success (number of sexual partners).
Degree Number of links that an individual node has with other members of the population. Links represent categories of social interactions, such as sexual interactions.
Mating system Spatio-temporal variation of sexual partners across members of one sex within a population.
Node Focal element of a network, usually individuals but can also characterize groups of individuals or gametes.
Operational sex ratio The ratio of males to females in a population that are available for reproduction at a particular point in time.
Polyandry A mating system characterized by females mating with more than one male.

Introduction

In sexually reproducing organisms, a large source of variation in individual fitness and a key determinant of the genetic structure and viability of a population arises through the differential ability to outcompete members of the same sex over reproductive opportunities. Sexual selection is the selective process that promotes those traits which confer an advantage in such competition between members of one sex over access to the gametes of the opposite sex (Darwin, 1871; Andersson, 1994). Resolving the way sexual selection operates and drives adaptive evolution hinges on understanding the mechanisms that govern patterns of variation in individual reproductive success. While the original view of sexual selection was largely restricted to competition over mating opportunities (i.e., premating sexual selection) in males, increasing evidence indicates that sexual selection can also target females and in males often continues after mating (postmating sexual selection), through episodes of sperm competition (Parker, 1970; Parker and Pizzari, 2010) and cryptic female choice (Thornhill, 1983; Eberhard, 1996), whenever females mate with multiple males (polyandry). Intrasexual variation in reproductive success is therefore determined by the outcome of often complex social interactions within and between sexes. Patterns of spatial and temporal variation in the structure of such webs of sexual interactions mean that standard selection approaches based on population-level measures may offer inadequate estimates of sexual selection and a misleading view of the underlying mechanisms and episodes (McDonald *et al.*, 2013). Sexual networks is an emerging quantitative tool designed to enable a more sensitive measure of sexual selection by capturing fine-grained patterns of variation in the structure of the web of sexual interactions within populations.

Sexual Networks

A sexual network is a collection of nodes (male and/or female individuals) connected by edges that may represent any behavior of interest, such as copulations and male–male competitive interactions (**Figure 1**). Originally developed as a tool to characterize social interactions more generally (Scott, 2000; Whitehead, 2008), social network analysis (SNA) as applied to sexual reproduction is an approach that seeks to characterize how interactions between individuals create population-level patterns, and has been used extensively to study the dynamics of infectious disease transfer in sexually reproducing populations (Gupta *et al.*, 1989; Liljeros *et al.*, 2001; Schmid and Kretzschmar, 2012). The more recent use of networks as descriptors in the context of sexual selection, not only represents a conceptual framework to visualize the pattern of interactions between individuals, but also provides a variety of quantitative network analytical tools that can measure patterns of variation in inter- and intrasexual interactions (Croft *et al.*, 2008; Krause *et al.*, 2014, 2007; Whitehead, 2008). By building up the social and competitive structure of populations as an emergent property from individual interactions, sexual networks enable the identification of unique competitive environments for individuals, potentially revealing detail beyond population-level measures of social structure, such as population density and operational sex ratio (Aronsen *et al.*, 2013; De Jong *et al.*, 2009; Kokko *et al.*, 2012; Krupa and Sih, 1993; Wacker *et al.*, 2013). Sexual

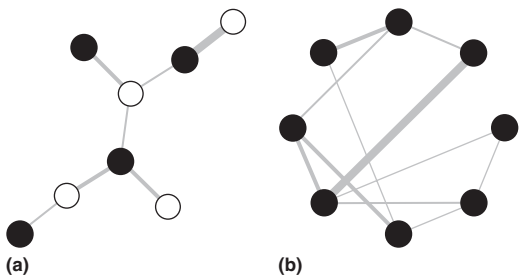

Figure 1 Hypothetical sexual networks (a) nodes represent males (black) and females (white), edges between nodes can represent any sexual interaction including courtship or mating; (b) nodes represent males and edges between nodes can represent any sexual competition, for example, sperm competition or male–male contests. Thickness of edges can represent the frequency or intensity of the interaction.

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networks can then be used to understand the implications of the pattern of sexual interactions in shaping sexual selection and the evolutionary trajectories of sexual populations. For this reason, an increasing number of studies have begun to study different aspects of mating systems and sexual selection, characterizing sexual networks across a range of organisms. While the concept of 'mating networks' has been used by anthropologists and primatologists for decades (e.g., Wobst, 1976), recent examples include the harvestman (*Serracutisoma proximum*) (Muniz *et al.*, 2015), the Asian red palm weevil (*Rhynchophorus ferrugineus*) (Inghilesi *et al.*, 2015), forked fungus beetles (*Bolitotherus cornutus*) (Formica *et al.*, 2011), the house finch (*Carpodacus mexicanus*) (Oh and Badyaev, 2010), white-throated sparrows (*Zonotrichia albicollis*) (Formica and Tuttle, 2009), and blue tits (*Cyanistes caeruleus*) (Schlicht *et al.*, 2014). We discuss some of these studies below.

Sexual Selection and Competitive Structure

Phenotypic Assortment

A key goal in sexual selection research is to understand how and why sexual selection favors particular traits, disfavors others and why such patterns vary within and across populations. To understand trait evolution at the population level we need to understand the relationship between traits and relative reproductive success. This is because the relationship between a trait and fitness at the level of the population predicts changes in the trait mean value across selective episodes. There are a number of techniques to quantify both the opportunity of sexual selection and the actual strength of sexual selection operating on specific phenotypic characters at the population level. One such population-level approach is based on selection gradients, which are estimated by regressing fitness on trait values (both standardised at the level of the population) as:

$$\omega_i = \beta z_i + \varepsilon \quad [1]$$

where ω_i and z_i are relative fitness and trait value for the ith individual respectively, β is the slope of the regression of relative fitness on the trait (i.e., the selection gradient) and ε represents the residual error. Using this approach we can investigate selection across both pre- and postmating selection episodes. However, this approach assumes that competition within populations occurs at random with respect to individual phenotypes, an assumption that has been undermined by research revealing that populations usually display structure such that interactions are local and nonrandom (Cornwallis and Uller, 2010). For example, when restricted dispersal results in an increased similarity between neighboring individuals, leading to greater similarity/relatedness between locally competing conspecifics (e.g., Duncan *et al.*, 2010; Johannesen and Lubin, 2001). In addition to limited dispersal, structure may arise as a property of a preference of individuals to interact with conspecifics of certain phenotypes (e.g., Formica *et al.*, 2011; Formica and Tuttle, 2009; O'Ha, Blaus 1985; Oh and Badyaev, 2010). The structure resulting from these mechanisms may have strong implications for the patterns of sexual selection at the population level. Imagine a population

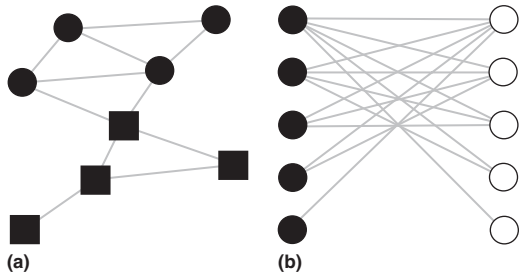

Figure 2 (a) An intrasexual network of competing males displaying positive phenotypic assortment where node shapes represent two distinct phenotypes and edges represent competitive interactions. (b) A sexual network displaying negative degree assortment where nodes represent males (black) and females (white) and edges between nodes can represent copulations.

where individuals are segregated into multiple subgroups and groups are structured such that more similar individuals are found within the same group (i.e., small individuals compete with small competitors and large with large competitors; **Figure 2(a)**). Within each group, individual males compete for mates and those individuals that are larger attain higher reproductive success than their local group competitors (i.e., competition is local and there is positive sexual selection on male size at the local scale). If groups also vary in their mean reproductive success, i.e., groups with on average larger males produce more offspring, then measuring selection at the population level may reveal a positive relationship between body size and relative fitness. However, if groups are equally productive regardless of average group male body size (e.g., because female fecundity is similar across groups), the covariance between trait and fitness may be strong at the local level, but – due to population structure – this covariance may be obscured at the population level. One way to understand how such local structure contributes to population-level selection on traits is to incorporate local variation in competitive environments using multilevel selection techniques. These are extensions of analyses provided in eqn [1] (Heisler and Damuth, 1987; Nunney, 1985; Wolf *et al.*, 1999; Okasha, 2004) and can be used to measure selection in structured populations, providing estimates of selection operating on a focal trait (e.g., tail length) controlling the local competitive environment (e.g., mean tail length of competitors), therefore quantifying the effect of nonrandom competitive structure on the operation of sexual selection. Crucially, these approaches rely on determining functionally competitive groups, i.e., the local competitive environments of individual competitors. Using sexual networks built up by competitive interactions provides a quantitative framework to delineate the local competitive group for each focal individual, which can inform selection analyses across both pre- and postmating episodes of selection (McDonald *et al.*, 2013; McDonald and Pizzari, 2014).

Sperm Competition Intensity

Two key axes in which nonrandom interactions may affect the operation of sexual selection are: (1) assortative phenotypic interactions such as assortative mating (Jiang, 2013) and competition by phenotypes as mentioned above (**Figure 2(a)**),

and (2) patterns of variation in the intensity of competition across individuals. The intensity of competition describes the magnitude of competition, in terms of the number of competitors, with which an individual competes. Individuals are likely to vary in the number of sexual competitors both across and within populations, and such variation may affect the operation of sexual selection, potentially changing its strength and direction on phenotypic traits (Krupa and Sih, 1993). For example, in the myobatrachid frog (*Crinia georgiana*) among-population variation in breeding male density correlates with variation in testes size, suggesting a role for local competitor density to influence between-group patterns of selection on males due to increased risk and/or intensity of sperm competition (Dziminski *et al.*, 2010). When variation in such intensity is spread nonrandomly across phenotypes in the population (e.g., more attractive males must compete with more competitors), this may result in reduced or increased reproductive success for males of a given phenotype, potentially accentuating or abating sexual selection on, and the evolution of, those phenotypes. Such patterns are particularly relevant in the case of postmating sperm competition. In this context, variation in competitor density is determined by the number of males and\or ejaculates with which a male competes across his female mating partners. The polyandry of a male's sexual partners therefore impacts on his chances to fertilize his partners' ova and thus ultimately on his reproductive success. For example, Muniz *et al.* (2015) studied variation in female polyandry in a population of harvestman (*S. proximum*). In this species, males tend to adhere to two alternate mating tactics, larger males that tend to guard territories and females ('territorials'), and smaller males that do not hold territories but furtively mate with females present in other male territories (Muniz *et al.*, 2015). This work suggests that the way females of differing polyandry are distributed nonrandomly across male partners playing such alternative mating tactics, may result in a mating tactics-specific intensity of sperm competition (Muniz *et al.*, 2015), and potentially modulate the reproductive outcomes of different reproductive strategies. More generally, when males that copulate with many females also mate with the most polyandrous females, they may face on average higher sperm competition and this will weaken sexual selection on male mating success (Sih *et al.*, 2009; McDonald *et al.*, 2013). In networks, this would be described as positive assortment by degree between males and females and would be expected to reduce selection on males to increase mating success. Similarly, populations may display negative assortment by degree, which may be expected to increase selection on male mating success (i.e., Bateman gradients) (**Figure 2(b)**). Sexual network analysis provides a variety of metrics to quantify variation in assortment by degree and test hypotheses regarding its effects on the operation of sexual selection, and these methods continue to be refined (McDonald and Pizzari, 2014).

Determinants of Competitive Structure

Ecology

In addition to delineating competitive environments, by providing a mean to quantify population-level patterns of structure of sexual competition, sexual network analysis can also shed light on the forces that generate a given competitive structure.

The mating system of a population describes the pattern of mating behavior across individuals within that population (Klug, 2001; Shuster, 2009; Shuster and Wade, 2003). Research has highlighted an immense variety in animal mating systems between and within species, ranging from strict monogamy to high levels of both male and female promiscuity (Andersson, 1994; Clutton-Brock, 1989; Emlen and Oring, 1977; Shuster, 2009; Shuster and Wade, 2003; Thornhill and Alcock, 1983). Because mating systems describe the distribution of sexual partners across individuals, they also largely determine variation in reproductive success and are therefore expected to be intimately linked to the operation of sexual selection (Emlen and Oring, 1977; Shuster and Wade, 2003). A predominant paradigm is that spatial and temporal variation in ecological factors dictate opportunities for partner monopolization and as such modulate local patterns in sexual interactions (Clark *et al.*, 1997; Crook, 1965; Emlen and Oring, 1977; Endler and Houde, 1995; Kelly *et al.*, 1999; Lack, 1968; Mück *et al.*, 2013; Orians, 1969; Reichard *et al.*, 2009; Reynolds, 1996; Reynolds *et al.*, 1993). Network analytical techniques may provide a useful tool to determine how individual traits and ecological variables interact to shape nonrandom interactions in sexual populations. For example, several studies have assessed whether genetic relatedness, social familiarity or male territoriality determines social network structure (Atton *et al.*, 2014; Hirsch *et al.*, 2013; Wolf *et al.*, 2007; Wolf and Trillmich, 2008). More recently, Schlicht *et al.* (2014) used spatially explicit networks (rather than networks built on interactions per se) to investigate the relative importance of spatial versus nonspatial factors in determining the rate of paternity in blue tits. Such approaches could be used to understand how individual traits and resource ecology together determine population-level interaction patterns such as assortative mating between phenotypes. For example, whether shared dietary preferences result in associations between individuals due to resource distribution. Understanding how such ecological variables determine patterns of assortative mating may also have implications for population differentiation and speciation (McDonald and Pizzari, 2014).

Individual Strategies

Beyond group-level descriptions of interaction structure, network analysis also allows the characterization of an individual's placement within networks (Newman, 2010). The position of individuals within a network may have important implications for their reproductive success. For example, if females within a male–female courtship network are peripheral and interact with few other individuals they may have relatively poor information on the distribution of male quality throughout the population, whereas those females more central to the network may be at liberty to make more informed decisions with regards to mate choice (Sih *et al.*, 2009). Similarly, metrics of individual network positions may represent reproductive strategies and behaviors. For example, using social networks in the house finch, Oh and Badyaev (2010)

showed that males that move between different social groups were able to modify their socio-competitive environment such that they increased their relative attractiveness to prospective mates compared to rivals. Such behaviors would be overlooked without the use of network analysis.

Potential Applications

The potential for sexual networks to help understand eco-evolutionary processes is only beginning to be explored. This potential raises interesting opportunities for the applications of sexual networks in other contexts. For example, in epidemiological research examining the spread of sexually transmitted infections, researchers have understood how sexual networks may provide information for targeted interventions to reduce disease spread (Robinson *et al.*, 2012). One potentially important application of the tools described in this work is in the context of biological control strategies of pest species (Inghilesi *et al.*, 2015). Biological control strategies often involve techniques that interfere with sexual interactions of a pest population, with the aim of significantly reducing the productivity of the population, for example, the introduction of sterile males into the population (Alphey *et al.*, 2010). By providing an important tool to characterize the patterns of sexual interactions, sexual networks may facilitate our understanding of the pattern of male–male competition.

Conclusion

The study of sexual networks in the context of sexual selection is still in its infancy and represents an emerging field in evolutionary ecology. The harnessing of quantitative tools developed by social network theory may provide evolutionary biologists with an additional arsenal to complement more traditional sexual selection research techniques and further refine our understanding of sexual selection. The potential to apply sexual network analysis to the study of sexual selection is expected to increase substantially in the near future, thanks to the increasing availability of large datasets with sufficiently detailed information on mating behavior and reproductive success, that is being catalyzed by rapid advances in animal tracking technology (Krause *et al.*, 2011) and the advent of increasingly more efficient molecular tools to assign parentage, measure genetic relatedness and population structure.

See also: Mate Choice and Sexually Selected Traits. Polyandry and Female Postcopulatory Choice. Sperm Competition

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Sexual Selection, Theory of

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Glossary

Anisogamy The situation in which one sex produces small, mobile gametes while the other sex produces large, immobile gametes.

Bateman gradient (β_{ss}) The least-squares regression of reproductive success on mating success. The standardized Bateman gradient (β'_{ss}) is the least-squares regression of relative reproductive on relative mating success.

Correlation A covariance standardized to have a minimum value of -1 and a maximum value of 1. The equation for the correlation coefficient is: $r_{x,y} = \frac{\mathrm{cov}(x,y)}{\sqrt{\sigma_x^2 \sigma_y^2}}$, where σ_x^2 and σ_y^2 are the variances in x and y.

Covariance A statistical concept that summarizes the extent to which two variables are non-independent. A positive covariance indicate that high values of one variable are associated with high values of the other variable, whereas a negative covariance indicates that high values of one variable are associated with low values of the other variable. The equation for an unbiased estimate of a covariance from a sample is: $\mathrm{cov}(x,y) = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})$, where n is the number of individuals in the sample.

Environmental potential for polygamy The extent to which the ecological setting permits individuals of one sex to monopolize access to individuals of the other sex in the context of mating.

Fitness (or absolute fitness) The number of offspring produced by an individual or the average number of offspring produced by individuals with a particular genotype or phenotype.

Genetic mating system A summary of the mating interactions among individuals in a population that result in the production of zygotes. Genetic mating systems include monogamy, where each individual mates with only one individual of the opposite sex; polygyny, where successful males mate multiple times but each female mates at most once; polyandry, where females mate with multiple males but each male mates once; and polygynandry, where both sexes have multiple mates.

Intersexual selection Sexual selection arising from mate choice. Members of one sex are predisposed to mate with individuals of the other sex with particular attributes, so the most attractive individuals tend to have more mates than less attractive individuals. The term 'mate choice' is unfortunate in that it implies a conscious decision-making process, which is unnecessary for sexual selection to operate. It is enough for individuals simply to respond more strongly in a mating context to certain stimuli. The organisms needn't 'think' about which choice is the best.

Intrasexual selection Sexual selection arising as a consequence of direct competition among members of the same sex. For instance, males might fight each other in ritualized combat for access to females or males might race each other to the females. In either case, the winner would typically mate with the female, although even in these systems females could still exert some choice.

Mating differential (m) The covariance between trait values and relative mating success. If the trait values are standardized to have a variance of one, then the mating differential is called the standardized mating differential (m').

Mating success The number of other individuals with whom a focal individual produces offspring during a time period of interest.

Operational sex ratio The ratio of males ready to mate to females ready to mate in a local breeding population. The operational sex ratio is often expressed as the fraction of receptive adults that are male, with a value of 0.5 indicating that half the adults ready to mate are male and half are female.

Opportunity for selection (I) The variance in relative fitness.

Opportunity for sexual selection (I_s) The variance in relative mating success.

Relative fitness An individual's absolute fitness divided by the mean absolute fitness in the population.

Relative mating success An individual's actual mating success divided by the mean mating success in the population for individuals of the same sex.

Reproductive success The number of biological offspring produced by an individual during some specified time frame.

Selection differential (s) The covariance between trait values and relative fitness. The selection differential is a major determinant of the response to selection, which is described by the breeder's equation: $R = h^2 s$. The response to selection (R) is the product of the heritability of a trait (h^2) and the selection differential (s). The heritability is the proportion of the variance in trait values that is due to additive genetic effects. If the trait values are standardized to have a variance of one, then the selection differential is in units of phenotypic standard deviations and is called the standardized selection differential (s').

Sexual selection Variation in fitness arising from competition for access to mates or fertilization opportunities.

Social mating system The behavioral interactions among adult individuals in a breeding population. Examples of social mating systems include monogamy, in which individuals form reciprocal pair-bonds involving one male and one female, and promiscuity, in which individuals mate with multiple partners without forming any long-term bonds. Other social mating systems also exist, but they are beyond the scope of our discussion.

Introduction

'Sexual selection' is often defined as variation in 'fitness' that arises from competition for access to mates or fertilization opportunities (Andersson, 1994; Jones and Ratterman, 2009). Fitness here is defined in the Darwinian sense as the total number of offspring produced by an individual during its lifetime, and in the sexual selection literature fitness is often also called 'reproductive success.' In Charles Darwin's original formulation of sexual selection (Darwin, 1859, 1871), he correctly realized that competition for mates could occur as a consequence of either direct contests among rival males, a process now called 'intrasexual selection,' or choice of members of one sex as mates by individuals of the other sex, or 'intersexual selection.'

It is instructive to consider why sexual selection theory is important and what puzzles this theory has been developed to solve. Some historical context will illustrate part of the problem. When Darwin first proposed the idea of sexual selection (Darwin, 1859, 1871), it was not embraced by the scientific community (see Andersson, 1994 for a review). Even Darwin (1871) believed that sexual selection only occurred in species with sufficiently developed intellectual faculties to have a sense of aesthetics. His critics argued that no nonhuman species were intelligent enough to care about something as frivolous as the beauty of a prospective mate's plumage, for example, and sexual selection was consequently pushed aside from mainstream evolutionary biology. Thus, for sexual selection to be a topic worthy of study, the field needed some plausible models for how it could operate even in species incapable of making rational decisions.

Perhaps the two biggest questions facing sexual selection theory were (and still are), first, why some lineages, such as peafowl, display dramatic evidence of sexual selection, while other lineages do not; and second, how mating preferences could originate and be maintained in populations. The first question resulted in the development of theory related to the strength of sexual selection, and the second led to the development of an impressive body of literature on how mating preferences could evolve as a consequence of understandable evolutionary processes. Other smaller issues arose along the way, but these two challenges form the core body of sexual selection theory.

The Intensity of Sexual Selection

Selection and Mating Patterns

An understanding of the intensity of sexual selection starts with the realization that sexual selection is one form of selection acting on natural populations of organisms, so general selection theory can be applied. Selection on a trait arises from the 'covariance' between trait values and fitness (Price, 1970; Lande and Arnold, 1983). This idea makes sense, because if, for instance, large males tend to have more offspring compared to small males, then there will be a positive covariance between male size and male fitness. The larger males will pass more genes to the next generation, and if their size happens to have a genetic basis, then the 'large size' trait will tend to increase in frequency in the population. In fact, one way to quantify the intensity of selection is by using the 'selection differential,' which is simply the covariance between the values of the trait of interest and 'relative fitness' (Falconer and Mackay, 1996).

If selection can be quantified by a selection differential, then an important question is how a selection differential arises in the context of sexual selection. In its simplest form, sexual selection arises from competition for access to mates, which means that the 'winners' of sexual selection have more mates than the 'losers' of the competition. If success in this competition depends on the trait values of the competitors, and it makes sense that it would, then individuals with trait values favored in the competition will have greater numbers of mates (i.e., greater mating success) compared to individuals with poor trait values. In this case, it does not matter whether the competition is due to direct combat among the competing individuals or choice by the opposite sex. Regardless of the exact manifestation, competition for mates can result in a covariance between trait values and mating success, a quantity known as the 'mating differential' (**Figure 1**; Jones, 2009).

A nonzero mating differential indicates that individuals with certain trait values have more mates than individuals with other trait values, but the mating differential by itself does not measure selection on the trait. Selection arises due to a nonzero selection differential, which measures the extent to which particular trait values covary with relative fitness, measured in terms of offspring numbers. Of course, individuals with more mates often have more total offspring, but not always. The last piece of the puzzle, then, is the relationship between number of mates (or mating success) and number of offspring (or reproductive success). This relationship is called the 'Bateman gradient' (**Figure 2**), as it was devised as an outgrowth of a classic experiment on mating patterns in *Drosophila melanogaster* conducted by A. J. Bateman in the 1940s (Bateman, 1948; Arnold and Duvall, 1994; Jones, 2009).

We can put all of this information together with a simple equation that describes the source of the selection differential due to competition for mates: $s' = \beta'_{ss} m'$ (Jones, 2009). In words, the standardized selection differential (s', with the prime sign merely indicating that the selection differential is standardized to be in units of phenotypic standard deviations) equals the product of the standardized Bateman gradient (β'_{ss}) and the standardized mating differential (m'). This way of viewing the selection differential is useful because it identifies the underlying processes giving rise to selection on traits as a consequence of mating competition. On the one hand, we need to understand the sources of the covariance between trait values and mating success, an issue embodied by the mating differential. On the other hand, we also need to understand how a mating differential is converted into a selection differential, a conversion facilitated by the Bateman gradient. Many extrinsic ecological factors and intrinsic biological constraints act together to affect the magnitudes of both mating differentials and the Bateman gradient, and some of the most important of these factors are discussed below.

The Maximum Strength of Precopulatory Sexual Selection

One other topic worth mentioning here is that the definition of the selection differential as a covariance makes it possible to

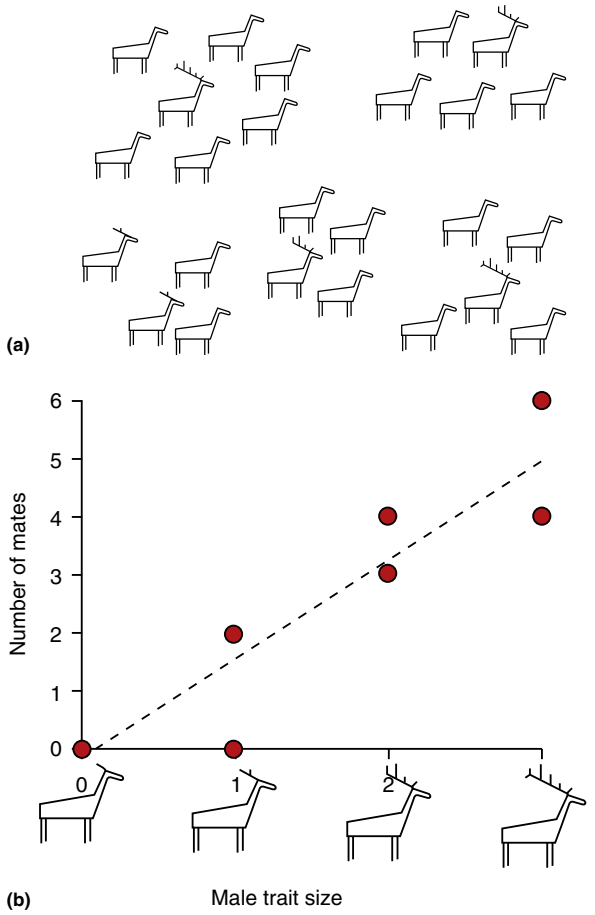

Figure 1 An example of how a mating differential can arise. In this hypothetical species of deer, which is much like the real red deer (*Cervus elaphus*), males with larger antlers can defend larger harems of females. In the top panel (a), males (with antlers) are shown associated with groups of females (without antlers). The males with larger antlers have larger groups of females, and the female mates with the closest male. The bottom panel (b) shows a graph of number of mates as a function of male antler size for the population in (a). A least-squares regression line is shown, and a positive slope means there must be a positive covariance between the two variables. Incidentally, the slope of a regression line is given by the covariance between the *x* and *y* variables (in this case this covariance is the mating differential) divided by the variance in the *x* variable. This graph thus indicates a positive mating differential on male antler size, a sexually selected trait in red deer (Kruuk *et al.*, 2002).

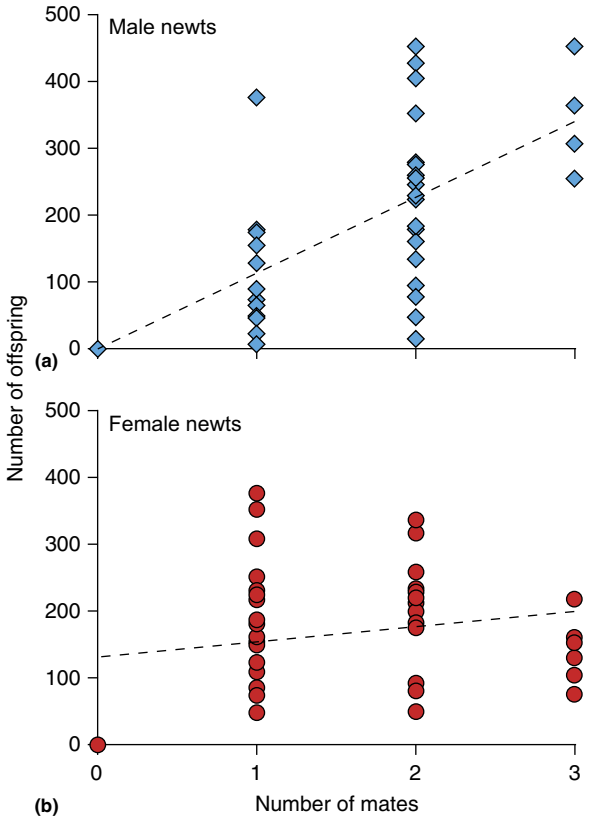

Figure 2 Bateman gradients for male and female rough-skinned newts (Jones *et al.*, 2002). Rough-skinned newts are sexually dimorphic. Males are larger than females, have nuptial pads that are absent on females, and have an enlarged tail crest. Males compete for access to receptive females and sometimes even wrestle females away from rival males. The Bateman gradient for males (a) is significantly positive, with males gaining on average an additional 74.6 offspring per mating event. The female Bateman gradient (b), however, is not significantly different from zero.

derive mathematically some limits on its magnitude. If, for instance, we assume that a phenotypic trait has been standardized to have a variance of one, then we can use the definition of the correlation to see that the selection differential cannot be larger than the square root of the variance in relative fitness (Crow, 1958; Arnold and Wade, 1984). The variance in relative fitness is called the 'opportunity for selection (I),' and a population with a larger opportunity for selection is mathematically capable of experiencing stronger selection than a population with a smaller opportunity for selection (Crow, 1958). A similar argument can be applied to the mating differential, and in this case the maximum value of a mating differential is equal to the square root of the variance in 'relative mating success' (Arnold and Wade, 1984; Jones, 2009). The variance in relative mating success is called the 'opportunity for sexual selection (I_s),' so a larger opportunity for sexual selection indicates a greater potential for a mating differential in a population (Wade, 1979; Arnold and Wade, 1984). If we consider the opportunity for sexual selection in the context of the equation $s' = \beta'_{ss} m'$ from above, then we see that the maximum value of a selection differential on any trait (in units of phenotypic standard deviations) due to mating competition in any population must be: $s'_{max} = \beta'_{ss}\sqrt{I_s}$ (Jones, 2009). This relationship can be useful whenever the mating differential is difficult to estimate directly.

Factors Affecting the Mating Differential and the Bateman Gradient

With the appreciation that sexual selection results in a selection differential on a trait and that this selection differential largely arises through the mating differential and the Bateman gradient, it is worth considering the various factors that can

affect these aspects of the reproductive ecology of a population. Some of these factors arise from intrinsic constraints on the organism, such as how often an individual is capable of mating or how many gametes can be produced, whereas other constraints are products of the ecological setting. Numerous such constraints exist in natural populations, so only the most important ones are considered here.

Anisogamy

We will see why in a moment, but sexual selection usually acts more strongly on males than on females in most taxa. Discussions related to the source of this pattern usually start with the idea of anisogamy, a term that refers to the observation that one sex produces small, relatively mobile gametes (i.e., sperm), whereas the other sex produces larger, relatively immobile gametes (i.e., eggs). Why anisogamy evolves is beyond the scope of our discussion, but anisogamy frequently does evolve and it leads to a situation in which the smaller gametes tend to be more numerous in a population than the larger gametes because smaller gametes are less costly to produce on a per-gamete basis (Parker *et al.*, 1972; Maynard Smith, 1978). The larger gametes, being relatively rare, are thus a limiting resource for reproduction, which means that only a subset of the sperm will find an egg to fertilize. If the individual sperm cells differ with respect to traits that affect fertilization success (such as swimming speed), then only the sperm with the most effective traits will pass their cargo of genes to the next generation. Thus, ignoring for the moment all of the complications that might arise from the attributes of the organisms carrying the gametes, sperm will tend to compete for eggs. Because the male, by definition, is the sex producing the smaller gametes, males also tend to be the sex that experiences a stronger intensity of sexual selection. As we will see, however, anisogamy is important, especially heuristically, but many other factors are involved in determining the intensity of sexual selection, and in some extreme cases sexual selection can act more strongly on females than on males. Thus, we still have a mystery: if, in almost all species with separate sexes, males produce relatively numerous, small sperm and females produce relatively rare, large eggs, then why do species differ from one another in terms of the intensity of sexual selection? Clearly, mitigating factors must be involved, and the next several sections explore these factors.

The environmental potential for polygamy

A breakthrough in sexual selection research came with the realization that many of the ideas related to mating competition could be expressed in economic terms. Even in biological systems, the economics of mating competition can instructively be considered in the context of supply and demand. This thread of logic stretches all the way back to Darwin (1871), who realized that an inequality of the sex ratio could result in strong sexual selection, and was taken up by some of the most important evolutionary thinkers of the twentieth century, such as George Williams (1975) and Robert Trivers (1972). Perhaps the most important paper in this vein, however, was one published by Stephen Emlen and Lewis Oring in 1977, which formalized the notion of the 'environmental potential for polygamy.'

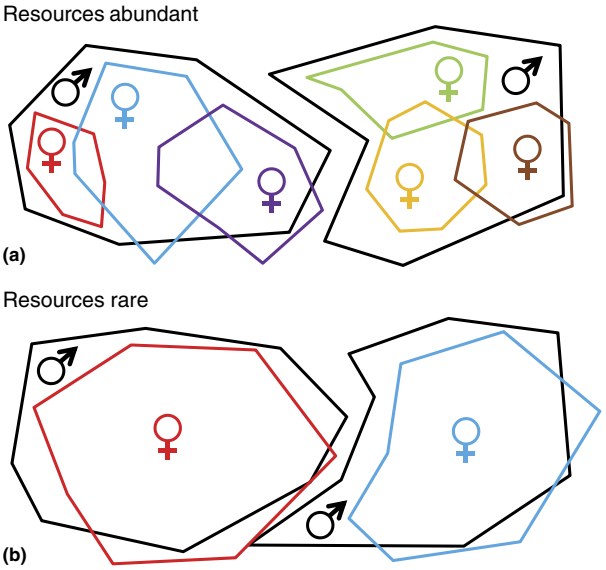

Figure 3 An example of how the environmental potential for polygamy can affect the mating system. In this hypothetical example, males defend territories (black polygons). Females have home ranges indicated by the colored polygons. When resources are abundant (a), female home ranges can be small, and a male's territory may encompass many female home ranges. If females mate on their home range, then each territorial male can expect high mating success. When resources are rare (b), however, female home ranges are larger and each male territory encompasses only one female home range. In this case, territorial males will experience reduced mating success on average, the mating system will tend toward monogamy, and the strength of sexual selection will generally be reduced. This figure provides one example of how the environment can affect mating systems, but any environmental factor that alters access to mates can have an impact on the environmental potential for polygamy (Emlen and Oring, 1977).

The environmental potential for polygamy refers to the ecological factors that make it possible for some individuals to monopolize access to members of the opposite sex for reproduction. For instance, if females are spatially clustered, then one male may be able to defend a territory encompassing many females (**Figure 3**). If the females also prefer to mate on the territory, then the defending male may experience considerably higher reproductive success compared to males without territories. This sort of territorial behavior would be sexually selected in this context. Any ecological factor that affects the temporal or spatial clumping of potential mates or the defensibility of other resources related to reproduction can, in principle, affect the environmental potential for polygamy, which in turn impacts the intensity of sexual selection (**Figure 4**; Emlen and Oring, 1977).

The operational sex ratio and the potential reproductive rates of the sexes

The concept behind the role of the 'operational sex ratio' in sexual selection is simple: if more individuals of one sex are ready to mate than individuals of the other sex, then the sex in abundance will be forced to compete for the sex in short supply. This concept, also formalized by Emlen and Oring

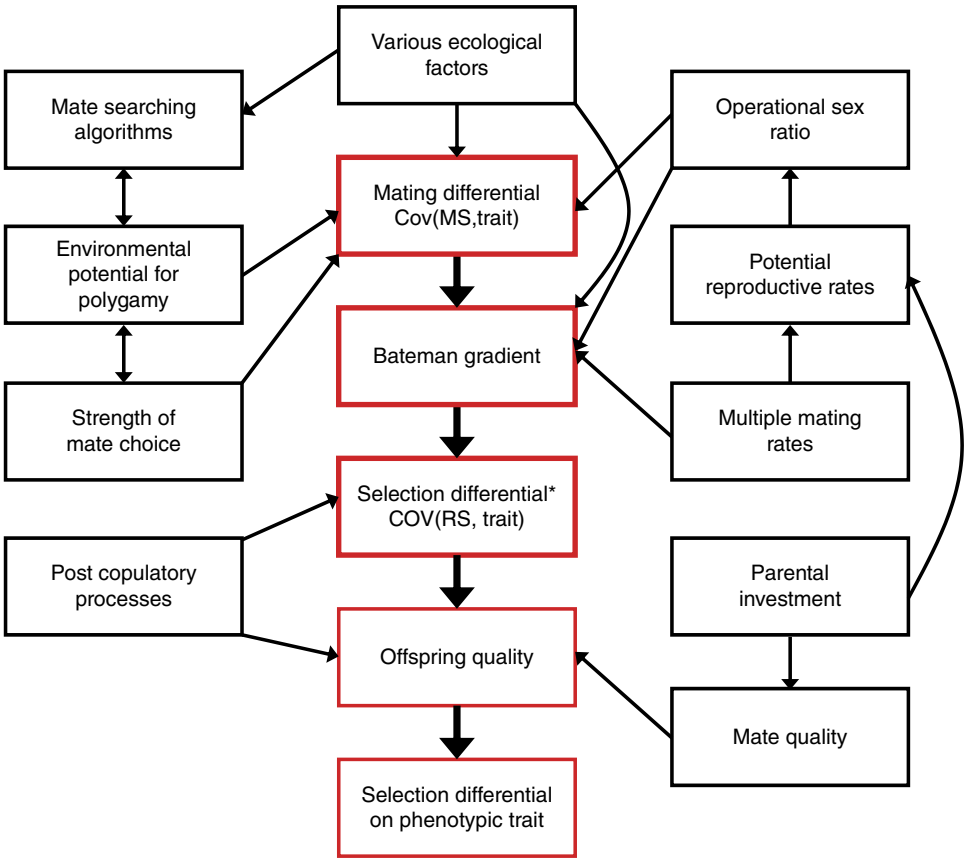

Figure 4 A 'reproductive ecology web' showing interactions among some of the important variables related to the intensity of sexual selection. Some arrows are certainly missing, and other factors probably could be included. At the center of this figure, in red, are the boxes that form the path to fitness from the mating differential, through the Bateman gradient to the selection differential on sexually selected traits. The selection differential with the asterisk represents selection due to mating success and number of offspring. If post-copulatory processes occur or mate quality alters offspring quality, then the true selection differential could be modified by these effects. All of the other behavioral and ecological factors interact in various ways to affect both the mating differential and the Bateman gradient. Reproduced from Jones, A.G., Ratterman, N.L., 2009. Mate choice and sexual selection: What have we learned since Darwin? Proceedings of the National Academy of Sciences USA 106, 10001–10008.

(1977), is a close outgrowth of the economics of mating dynamics. The operational sex ratio is defined as the ratio of receptive males to receptive females in the population, and many ecological factors can affect this ratio. The most obvious factor is the adult sex ratio, but even when the adult sex ratio is even, complications such as parental care, limited periods of receptivity, and the need to obtain a territory can alter the operational sex ratio (Figure 4).

One very important determinant of the operational sex ratio is the 'potential reproductive rate' (Clutton-Brock and Parker, 1992). If the two sexes differ dramatically with respect to their potential reproductive rates, defined as the maximum expected reproductive rate of the members of a sex given unconstrained access to potential mates, then, all else being equal, the sex with the higher potential reproductive rate should compete for members of the sex with the lower potential reproductive rate. Thus, potential reproductive rates, acting in concert with many other ecological factors, are an important part of the pathway leading from the ecology of reproduction to the intensity of sexual selection (Figure 4).

Parental care and parental investment

Armed with the ideas presented in the previous sections, we can see why parental care has an overriding influence on the intensity of sexual selection. Parental care refers to virtually any effort that a parent expends on behalf of its offspring. Parental investment, on the other hand, has a very specific definition, and it is probably best defined by Trivers (1972) as "any investment by a parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring". Hence, parental investment is a limited resource (Williams, 1966; Trivers, 1972).

From a sexual selection standpoint, parental investment is important because it represents a cost for the parent and this cost reduces the rate at which individuals can produce offspring. Parental investment can take the form of provisioning eggs, for example, and when eggs become sufficiently costly they will be a limiting resource for reproduction. This situation leads to a higher potential reproductive rate for males than for females, a surplus of males in the operational sex ratio, and thus a higher intensity of sexual selection in males than in females.

The importance of parental investment is especially noticeable in the few species in which sexual selection acts more strongly on females than on males (**Figure 5**). Species falling into this category include some birds, such as spotted sandpipers, red-necked phalaropes and wattled jacanas (Oring, 1986); some fishes, such as the pipefishes (Berglund *et al.*, 1986); a number of insects (Gwynne and Simmons, 1990); and a smattering of other taxa (Clutton-Brock, 2007), most of which have not been well studied. All of the species with stronger sexual selection on females than on males thus far studied exhibit substantial male parental investment in offspring, such that male potential reproductive rates are depressed below those of females. This situation tends to result in a female-biased operational sex ratio, steeper Bateman gradients in females than in males, higher variances in mating and reproductive success in females than in males, and thus a situation conducive to strong selection differentials on female sexually selected traits.

Social and genetic mating systems

Another useful concept to organize thought on the intensity of sexual selection is the mating system. In the sexual selection literature, the mating system describes the mating interactions among reproductive individuals in a population (Andersson, 1994; Arnold and Duvall, 1994). The 'social mating system' refers to the behavioral interactions among individuals, such as pair-bonding or promiscuity, whereas the 'genetic mating system' describes whose gametes actually unite to form zygotes (Searcy and Yasukawa, 1995). Both sorts of mating systems are relevant to the sexual selection process, because mating systems are closely tied to the other factors that determine the strength of sexual selection. However, the genetic mating system is more closely tied to fitness, and the social mating system is important because it represents a major source of constraint for the genetic mating system. For instance, in a population characterized by perfect monogamy with an equal sex ratio, sexual selection will usually be quite weak, because every individual should have a mate. If everyone mates exactly once, then there simply is not enough variation in mating success to generate much sexual selection. Other genetic mating systems include polygyny, in which successful males mate multiple times but each female mates with only one male; polyandry, in which successful females mate multiple times but each male mates once; and polygynandry, in which both sexes have multiple mates. Any of these non-monogamous mating systems can generate very strong sexual selection, depending on the particulars of patterns of multiple mating (**Figure 6**).

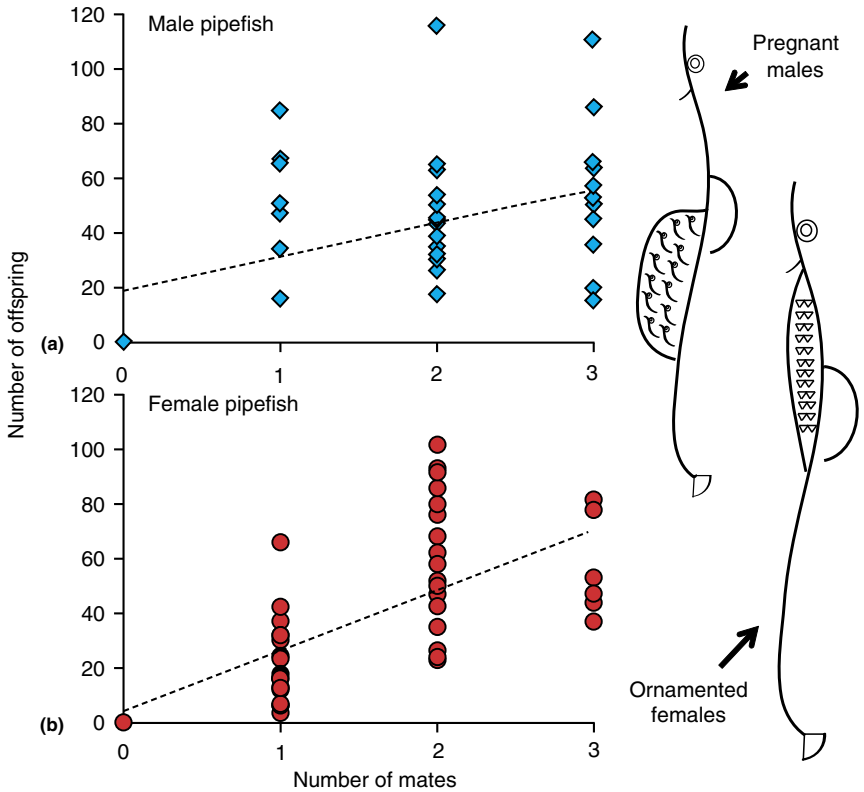

Figure 5 Bateman gradients in a sex-role-reversed pipefish. In this species of pipefish, *Syngnathus typhle*, males become pregnant and carry developing offspring in their pouches (as seen in the diagram of a pregnant male to the right). Male pregnancy depresses male potential reproductive rates and makes males a limiting resource for reproduction (Berglund *et al.*, 1989). Females compete for males, males are choosy, and females consequently evolve ornaments that are not present in males (see 'B'-shaped ornament in female diagram to the right). Bateman gradients for males (a) are less steep than those for females (b), a pattern that is to be expected for a species in which sexual selection acts more strongly on females than on males (Jones *et al.*, 2000).

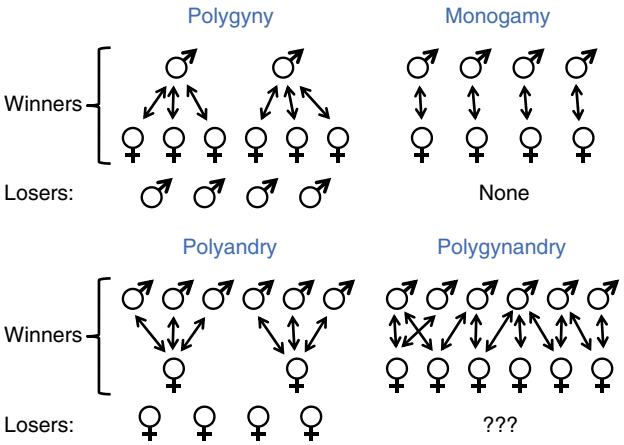

Figure 6 A graphical depiction of the four categories of genetic mating systems. Two-headed arrows indicate mating events among individuals. Assuming an equal sex ratio, polygyny will produce a situation in which successful males mate with multiple females and some males are left out of reproduction. If the males left out have different trait values than the males that succeed, then a nonzero mating differential will result. Conversely, polyandry will result in some females being left out, causing stronger sexual selection on females than on males. In monogamy with an equal sex ratio, selection should be weak because everyone will have a partner. Finally, in polygynandry almost any strength and direction of sexual selection is possible, depending on the relative patterns of multiple mating of the sexes.

The Evolution of Intra- and Intersexual Selection

The previous section dealt mainly with the question of the factors that produce a given intensity of sexual selection in a population. A separate, but related, question is why some organisms experience intrasexual selection, usually in the form of male–male contests, and other species are characterized by intersexual selection, or mate choice by one sex for members of the other sex. In the case of intersexual selection, the evolution of mating preferences has been a particularly challenging area of inquiry.

Intrasexual Selection

Called the Law of Battle by Darwin (1871), intrasexual selection makes a good deal of sense from an evolutionary standpoint, and Darwin's original explanation for it still remains valid (Andersson, 1994). In a population in which males can effectively defend females or resources, those males better at defending these reproductive resources will have more mates compared to the less competitive males. Sexual selection in such cases usually favors the evolution of weaponry or body size. Some excellent examples include the evolution of antlers in male deer (Clutton-Brock *et al.*, 1980) and the evolution of large male body size in elephant seals (Le Boeuf *et al.*, 1972). If all sexual selection were of the intrasexual variety, then the process of sexual selection would make a lot of sense, but even species with apparently strong intrasexual selection often have a large component of mate choice involved in their mating dynamics.

Intersexual Selection

The origin and maintenance of intersexual selection is perhaps the most enduring and baffling question in sexual selection theory, and progress in the field stalled for almost a hundred years waiting for a workable set of hypotheses to explain mating preference evolution (see Andersson, 1994 for a review). An important realization here is that the challenge is to explain the evolution of the preferences, not the traits upon which the preferences act, because once the preference evolves, the evolution of the preferred trait is entirely straightforward. In other words, if a preference exists and the trait upon which the preference acts is variable, then a mating differential on that trait will almost certainly arise due to mate choice. Darwin didn't even address the question of where preferences originate, and this topic did not become an active area of research until the 1970s and beyond when a number of hypotheses originated during a sort of sexual selection theory renaissance. Some of the most important models are discussed here (**Figure 7**). For simplicity in the discussion of these models, I will assume that females are choosy and males are the sexually selected sex, since most species have stronger sexual selection on males than on females. However, these same mechanisms can explain the evolution of male choice in species with strong sexual selection on females.

Sensory bias

The sensory bias hypothesis is based on the premise that organisms evolve to be attuned to certain sensory stimuli in order to thrive in their environments (Ryan, 1990; Endler and Basolo, 1998). For example, a species that eats orange fruit might be especially responsive to stimuli in the orange color range (Endler, 1983). If a male can mimic this sensory stimulus, then he can be more noticeable to females. Because the first step in mating is to be noticed by the members of the opposite sex, these noticeable males could experience heightened mating success relative to less noticeable males. In this way, a male sexually selected trait could be adaptive for the males even though the females derive no real benefit from mating with these males. The female preference, then, should not be seen as a behavior that evolved due to selection but rather as a by-product of the female evolving a sensory system that functions well in the current ecological setting.

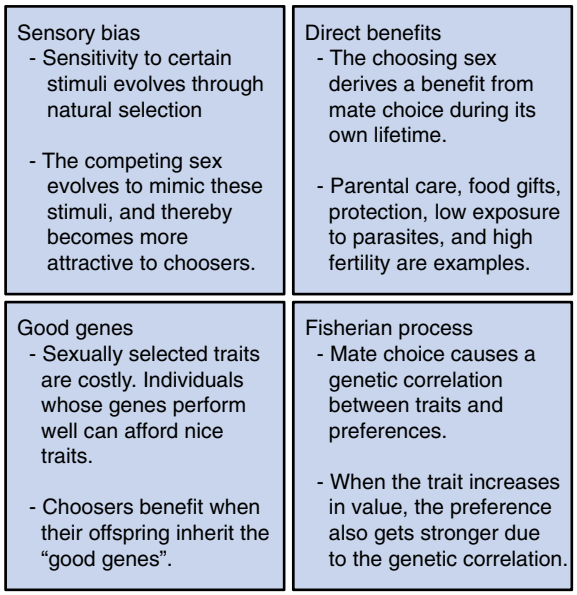

Figure 7 The four main models for the evolution of mating preferences. Some other ideas, such as genetic compatibility (Tregenza and Wedell, 2000) or chase-away sexual selection (Holland and Rice, 1998) are left out, but the four mechanisms shown here form the nucleus of theory on the evolution of mating preferences.

Direct benefits

Direct benefits models posit that mate choice is beneficial to females because choosing females are rewarded for their choice with a beneficial resource or outcome within their own lifetime (Williams, 1975; Kirkpatrick and Ryan, 1991). For instance, females sometimes choose males that provide excellent parental care, freeing the female from some of her caring duties and saving her energy for future reproduction. Other direct benefits include defense of foraging territories, food gifts for the female (i.e., nuptial gifts), and higher fertility. In some cases, the direct benefits may not be entirely obvious. For example, females may prefer males with well-developed ornaments because they have lower parasite loads, reducing the risk that females will become infected. Regardless of the exact nature of the direct benefit, the direct benefits model is accepted as an explanation for the evolution and maintenance of mate choice in a large number of taxa (Kirkpatrick and Ryan, 1991; Andersson, 1994).

Good genes

Good genes models are also known as indicator models, because the attractiveness of a male is a sign that he has genes that perform especially well in the current environment (Williams, 1966; Zahavi, 1975). The most intuitive good genes models assume that the sexually selected traits are condition dependent, such that males of better overall phenotypic quality can produce the most elaborate plumage or most potent pheromones or the most impressive manifestation of whatever the trait happens to be (Rowe and Houle, 1996). Good genes models can be rather involved (Mead and Arnold, 2004), so many of the nuances are beyond the scope of this article. However, the essence of the idea is that a female chooses a male with an elaborate trait because the trait signals that the male has a genetic constitution that will excel in the current environment. The benefit to the choosing female is that her offspring inherit the good genes and thus have higher fitness than if she had mated at random.

Good genes models, while they make sense, are controversial because they lead to several difficulties not present in some other models of preference evolution. The most significant problem is the 'paradox of the lek,' which observes that genetic variation is expected to disappear in the face of strong directional selection (Kirkpatrick and Ryan, 1991), so the good-genes benefit should quickly vanish. Another problem is that production of the trait must result in a substantial cost to its bearer or cheaters will evolve to express the trait even when they don't have good genes.

The Fisherian process

The final model of preference evolution we will discuss here is the 'Fisherian process' (Fisher, 1930), which in its most extreme case can be called 'runaway sexual selection' (Lande, 1981; Pomiankowski and Iwasa, 1993). The Fisherian process occurs as a consequence of the fact that mate choice results in a genetic association between preference genes and trait genes. In other words, when females with strong preferences mate with males with extreme traits, their offspring have both extreme traits and strong preferences. When this sort of genetic coupling of traits and preferences occurs, a change in trait values also results in a correlated change in preference values, even without any direct selection on the preference. Given appropriate starting conditions, this Fisherian process can result in a positive feedback loop in which traits get more extreme, resulting in stronger preferences, which in turn select for even more extreme traits, and so forth. In the most extraordinary cases, mathematical models show that this process can runaway to infinitely large trait values and infinitely strong preferences (Lande, 1981; Mead and Arnold, 2004). Of course, this theoretical outcome is not possible in living systems, because other constraints will eventually place limits on both the magnitude of traits and the strengths of preferences. Nevertheless, the kind of correlated selection described by the Fisherian process likely does play a role in some natural systems.

Summary

In summary, this article has discussed some of the most important aspects of sexual selection theory related to sexual selection based on mating competition. The intensity of sexual selection is determined by the mating differential, which summarizes the extent to which individuals with different trait values differ in mating success, and the genetic mating system, as embodied by the opportunity for sexual selection and the Bateman gradient. Many ecological factors are involved in determining the exact values of mating differentials and Bateman gradients in nature, but the field continues to move toward answers to these questions. In a population with a reasonable potential for sexual selection, then, we have to wonder why sexual selection takes its particular form. For instance, why are some taxa characterized by intrasexual selection and others experience intersexual selection? The answer

again lies in ecological circumstances, and a body of theoretical literature addresses the exciting issue of mating preference evolution, a topic that still has not been fully resolved.

Several additional complications are ignored here. For example, sexual selection can continue after mating in the forms of gamete competition or cryptic choice. Mate choice can also be based on genetic compatibility, a topic that was not covered here. Each topic addressed in this article further has many complications and nuances that were not discussed. Overall, the field of sexual selection has explained many aspects of mating behavior in nature and has generated many testable predictions. However, the field is still young and much work remains to be done.

See also: Mate Choice and Sexually Selected Traits. Natural Selection, Introduction to. Sexual Conflict. Sexual Networks. Speciation, Sexual Selection and

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Shifting Balance Theory, Sewall Wright and

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Glossary

Adaptive landscape This is the surface of mean fitness of a population as a function of its multidimensional genetic composition.

Additive effect Additive effect of a gene on the phenotype is the gene's effect averaged across all multi-locus genotypes and all environments.

Deme It is a local, randomly mating and interacting population of individuals.

Effective number It is a concept developed by Wright in order to relate the properties of individuals in real biological populations with those of the idealized individuals of evolutionary genetic theory. For example, real populations vary from one generation to the next in the numbers of breeding males and females; sometimes there are more breeding males than females or vice versa. Evolutionary genetic theory often tends to assume that the numbers of males and females are equal. Wright's concept of effective number translates the real world variation in male and female numbers into a smaller number, equivalent to an idealized theoretical population where the numbers of breeding males and females are always equal.

Epistasis It refers to the interactions between combinations of genes that affect the phenotype of an individual, such that the effect of a gene on a phenotype depends upon another gene or genes elsewhere in the genome.

Fitness peak It is an optimum on the adaptive landscape, which can have many optima of different absolute heights when there are interactions between genes, between genotypes or between genes and the environment.

Indirect genetic effect It is the effect of a gene in the genome of one individual on the phenotype of another individual. For example, some genes in the maternal genome are transcribed into messenger RNA that initiate and control early development of the offspring; these same genes may be carried in the offspring genome but may not be expressed at that time in development or may never be expressed, particularly if the offspring is a male.

Interdemic selection It is a process of selection that favors some local demes at the expense of others. In Wright's theory, demes of high average fitness send out more migrants than local demes of lower average fitness. In addition to Wright's interdemic selection by differential dispersal, interdemic selection could occur by the differential extinction of existing demes (where some demes persist longer than others) or differential colonization of new demes (where some demes have more successful colonizing propagules than others).

Island model migration It is the random movement or dispersal of individuals among demes.

Pleiotropy It occurs when a single gene affects two or more aspects of the phenotype.

Random genetic drift It is the local change in allele frequencies that occurs in populations with finite numbers of breeding adults owing to the sampling of gametes that occurs in the formation of zygotes for the next generation. Since this sampling is independent of the consequences of the alleles for fitness, alleles with harmful effects on fitness may increase in frequency by chance and conversely alleles with beneficial effects may decrease in frequency by chance.

In his studies of the deep pedigree of shorthorn cattle and his colony of inbred lines of guinea pigs, S. Wright observed a "… net-like relationship between the genome and complex characters" (Wright, 1968, p. 59). Moreover, just as Darwin used artificial selection as a model for his process of natural selection, Wright modeled his Shifting Balance Theory (SBT) of evolution in Nature after the processes used by breeders to transform longhorn cattle. From these empirical observations, he drew several broad generalizations about genes and the evolutionary process that he considered 'obvious.' These evolutionary genetic generalizations included (Wright, 1968, pp. 59–60): (1) the multiple factor hypothesis, namely, that most traits are affected by a large number of loci; (2) the principle of universal pleiotropy, wherein each gene replacement affects several different traits, so that a gene's different effects contribute differently to selective value; (3) the uniqueness of alleles in light of (2); (4) the dependence of dominance on genetic background and environment; (5) the universality of nonadditive gene interactions (epistasis); (6) the dependence of homology on the expression of similar chains of gene interactions under similar developmental conditions; and (7) the existence of multiple fitness peaks as a result of interaction effects of the most extreme sort with regard to overall selective value.

Wright's SBT (Wright, 1931, 1932) was founded on his empirical observations and the inferences he drew from them about the genetic system. Evidence from experiments and from modern genomic studies of gene expression and gene mapping support Wright's inference that nonlinear molecular interactions are the foundation of the relationship between genotype–phenotype (e.g., Lehner *et al.*, 2006; Szappanos *et al.*, 2011; cf. review in Mackay, 2014). These and other studies provide direct support for each of Wright's genetic principles. Nevertheless, the role of gene interactions (epistasis) in evolutionary genetics and the status of Wright's grand theory of evolution (SBT) remain controversial (Coyne *et al.*, 1997; Wade and Goodnight, 1998; Goodnight and Wade, 2000; Coyne *et al.*, 2000). In this article, the author will discuss the

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elements of Wright's theory, the evidence in support of it, and some of the reasons behind the controversy.

Wright's Shifting Balance Theory

Wright's SBT (Wright, 1931, 1968, 1977; Wade and Goodnight, 1991; Wade, 1992, 1996; Johnson, 2008) has three phases. Phase I is an 'exploratory phase,' where different gene combinations arise by chance in different local populations as a result of random drift. Phase I is necessary for discovering new adaptive gene combinations. In Phase II, favorable gene combinations spread to fixation within local demes by individual selection. In this phase, demes become more genetically differentiated from one another than they were in Phase I as different demes are attracted to different fitness peaks. Because of gene interactions, Wright conceived of the surface of mean fitness as a landscape with multiple possible fitness optima or 'adaptive peaks.' During Phases I and II, each deme in a metapopulation arrived at a local fitness peak that was generally different from the local fitness peaks of neighboring demes. At the end of this phase, the genetic differences among demes are large owing to the combined effects of drift and local or mass selection. And, each deme in a metapopulation resides on a fitness peak separated from other demes by valleys of lower mean fitness.

For Wright (1932, pp. 358–359) this was an intermediate stage in his SBT: "The problem of evolution as I see it is that of a mechanism by which the species may continually find its way from lower to higher peaks.… In order that this may occur, there must be some trial and error mechanism on a grand scale by which the species may explore the regions surrounding the small portion of the field which it occupies. To evolve, the species must not be under strict control of natural selection." In Phases I and II, each deme in a metapopulation is held to a fitness peak by individual selection while random genetic drift permits it to explore neighboring genotypic space. Together, Phases I and II are Wright's 'trial and error mechanism,' where each deme is a small experiment in adaptive evolution (Wade and Goodnight, 1998).

Phase III of the SBT is interdemic selection where migrants leave demes with higher mean fitness and move into demes of lower mean fitness. With local individual selection, the fittest individuals contribute more offspring to the deme in the next generation. Under interdemic selection, the fittest demes contribute more offspring to the metapopulation in the next generation. After a globally favorable gene combination arrives in a low fitness deme by migration, local selection, recombination, and recurrent migration act together to change its genetic background. Thus, the interdemic selection of Phase III favors the higher fitness peaks and is an essential component of Wright's SBT.

Gene Interactions Create a Multiplicity of Fitness Peaks

Wright argued that some gene combinations would have fitnesses equal to one another while, at the same time, other gene combinations would have higher or lower fitness. Wright accepted a relative constancy of genotypic selective values for adaptation to the external environment, but, for genes involved in interactions with other conspecifics, Wright believed that genotypic relative fitnesses would be a function of gene frequencies. The study of genes that affect the evolution of interactions between conspecifics is currently referred to as the study of Indirect Genetic Effects (IGEs; Wolf *et al.*, 1998; Bijma and Wade, 2008). Adapting to other conspecifics is different from adapting to abiotic environments because, when other conspecifics affect the quality of the environment and the fitness of an individual, the environment must be seen as having a genetic component. In this sense, an individual's phenotype or fitness is affected by the indirect action of genes in the genomes of other individuals. Moreover, the phenotype of the individual will change whenever the 'environment evolves.' With respect to Wright's genetic insight, interacting genotypic fitnesses are not only frequency dependent, but they also respond to inter-group or inter-deme selection in ways that genes with direct effects do not. Indeed, with random local mixing and no correlation between direct and indirect effects, IGEs do not respond to individual selection at all (Wade, 2016). The evolutionary consequences of such IGEs are more complicated than the simple genetic models of most evolutionary theory. As Feldman *et al.* (1983, p. 1009) concluded from their review of models of fertility selection with interacting males and females, "…the simplest interactions between individuals in the process of selection can produce evolutionary conclusions not expected from individual fitness models."

Wright (1931, 1932) introduced his concept of the 'adaptive landscape' or 'surface of selective value' to illustrate how the simultaneous action of mutation, migration, random genetic drift, and natural selection resulted in a multiplicity of selective peaks. "Selection, whether in mortality, mating or fecundity, applies to the organism as a whole and thus to the effects of the entire gene system rather than to single genes. A gene which is more favorable than its allelomorph in one combination may be less favorable in another. Even in the case of cumulative effects, there is generally an optimum grade of development of the character and a given plus gene will be favorably selected in combinations below the optimum but selected against in combinations above the optimum. Again the greater the number of unfixed genes in a population, the smaller must be the average effectiveness of selection for each one of them. The more intense the selection in one respect, the less effective it can be in others. The selection coefficient for a gene is thus in general a function of the entire system of gene frequencies" (Wright, 1931, p. 101). Here, Wright is clearly arguing that the selection coefficient of a gene, i.e., the gene's effect on fitness, changes as the genetic background changes, so that a gene favored by individual selection in one genetic background might be selected against in a different genetic background.

Despite the evidence from modern genomic studies (e.g., Lehner *et al.*, 2006; Szappanos *et al.*, 2011; cf. review in Mackay, 2014) that provide direct empirical support for each of Wright's genetic principles, the role of epistasis in evolutionary genetics remains controversial. Because the local genetic variation for most traits appears to be additive and because the additive genetic variance determines the

heritable response to natural and artificial selection, most of evolutionary genetic theory is framed by assigning genes a fixed, additive effect on fitness at their mutational origin. For example, Hill *et al.* (2008) argued on empirical and theoretical grounds that interactions at the level of genes do not generate much interaction at the level of the phenotypic variance. They concluded (Hill *et al.*, 2008, p. 9) that "…maintaining emphasis on utilizing additive variation by straightforward selection remains the best strategy." In a similar vein, Crow (2010) asserted that epistasis "…is unimportant in polygenic directional selection."

This view is sometimes called the 'gene's eye view' of evolution because it allows the adaptive process to be understood as the sequential substitution of single genes. Williams (1966, p. 56) provided one of the earliest and best expressions of the 'gene's eye view' of adaptation by natural selection:

> No matter how functionally dependent a gene may be, and no matter how complicated its interactions with other genes and environmental factors, it must always be true that a given gene substitution will have an arithmetic mean effect on fitness in any population. One allele can always be regarded as having a certain selection coefficient relative to another at the same locus at any given point in time. Such coefficients are numbers that can be treated algebraically, and conclusions inferred from one locus can be iterated over all loci. Adaptation can thus be attributed to the effect of selection acting independently at each locus.

From the 'gene's eye view,' genetically complex adaptations are assembled gradually, one gene at a time by natural selection. Interactions between genes, genes and environments, or between genotypes variations in finite populations are of little consequence because they do not significantly affect the relationship between a gene and its long-term average fitness effect.

Wright disagreed with this view as is evident in his writings on the "…inadequacy of the simple additive concept of gene effect" stating that, "…all genes that approach additivity in their effects on varying characters will be favorable in some combinations and unfavorable in others in terms of natural selective value (fitness) and, thus, exhibit interaction effects of **the most extreme sort** in the latter respect" (Wright, 1969, pp. 419–420; the author's emphasis in bold). In Wright's theory, the fitness of a genotype is not the sum of the properties of its component genes but rather an indivisible property of the entire genetic system. For this reason, Wright's SBT is different from standard Fisherian theory, where average gene effects are often treated as constants (Williams, 1966; Loewe and Hill, 2010) and the variations about the average are unimportant (Williams, 1966; Crow, 2010; Hill *et al.*, 2008).

In a metapopulation consisting of small, isolated demes connected by migration, Wright proposed that the gene combinations best for fitness would become fixed within local demes by drift or selection even if they conferred relatively poor mean fitness from a more global perspective. The joint influences of selection and random genetic drift would result in a metapopulation whose demes achieved a multiplicity of 'local fitness optima' or 'adaptive fitness peaks.' Because of interactions, Wright argued that the number of possible peaks increased geometrically with the number of genes (Wright, 1968). Without interactions between genes, between genes and environments, or between genotypes, there would be only a single peak and no need for a process like the SBT allowing for the evolution of demes from one fitness peak to a higher one.

Gene Interactions Limit Mass Selection

Importantly, gene interactions do more than simply create an opportunity for interdemic selection by creating a multiplicity of fitness peaks. As Wright argued, gene interactions change the sign of a gene's effect on fitness (Wade, 1992). With changes in sign, a gene can be favored by selection in one background but selected against it in another (Wade, 2001, 2002). Thus, interactions ultimately limit the effect of individual selection because the average effect of local selection includes positive and negative changes in gene frequency corresponding to demes in which a gene is favored and those in which it is selected against, respectively.

A multiplicity of fitness peaks and the conservative nature of local individual selection (which Wright called mass selection) prevented evolutionary innovation. In Wright's view, individual or 'mass selection' not only moved a deme toward the nearest fitness peak, it also held the population at that local optimum. If sufficiently small relative to the strength of selection (Wright, 1931), a deme might move by random drift across the valley of mean fitness from one fitness peak to an adjacent peak in gene frequency space. However, the relative mean fitness of the neighboring fitness peak was not guaranteed to be higher than the peak left behind nor was it guaranteed to be the globally highest fitness peak. Lande (1985) showed that the average time between peak shifts by random drift increases exponentially with the effective number of breeding adults in the local deme, so that doubling the local density more than doubles the time to move by chance from one fitness peak to another. The time necessary for a shift between fitness peaks by chance is so long that environmental changes that alter the landscape of mean fitness are likely to be more common (Whitlock, 1997). However, the 'nearest' peak was unlikely to be a higher fitness peak, let alone 'the highest' peak, given the myriad of possible optima afforded by gene interactions. For Wright, the question became: How could a species find the highest peak? He added interdemic selection to his SBT because it was a process that could provide an answer to this question.

Interdemic Selection

USDA breed histories showed that the selective effort of local breeders was not the primary force transforming the phenotypic characteristics of a breed. The transformation of a breed occurred when, by a combination of chance and local selection, a local farm produced an important combination of genes. Subsequently, demand by other breeders on other farms for that improved quality of animal led to the dispersion of the favorable gene combination out from the originating farm and into other farms. Interdemic selection in Wright's SBT was Nature's version of this process of breed transformation. Interdemic selection occurred as particularly good gene combinations arose within local demes, resulting in higher mean fitness and therefore higher local density. Individuals moved

out from demes of high mean fitness and into demes held at lower fitness peaks by local individual selection. Because more migrants left the demes with higher mean fitness, the globally good gene combinations were differentially exported from demes at higher fitness peaks into demes at lower fitness peaks. The mechanism of interdemic selection, also called selection among demes or group selection, was differential migration.

What feature of Nature could play the role of 'breeder demand' so that populations with higher fitness sent out more migrants than other populations? Wright (1931, 1932) proposed that it was the ecological mechanism of density-dependent migration. Individuals moved out from crowded areas of high density, where competition was intense, and into more sparsely populated areas of lower density with less competition for resources. In his classic book, *Animal Ecology* (Elton, 1927), the eminent ecologist, C. S. Elton had included a chapter on dispersal. He stated (Elton, 1927, p. 156): "… many animals migrate on a large scale in order to *get away from* a particular place rather than *go towards* anywhere in particular. That is to say, there are often very cogent reasons why a large section of the population should migrate somewhere else, the most common one being overpopulation." Wright's ecological idea was founded on the theoretical relationship between mean fitness, W, and population size, N. Mean absolute fitness, the net difference between births and deaths, proscribed the relationship between population numbers at time t, N_t, and population numbers at time $(t+1)$: $N_{t+1}=WN_t$. According to Wright's logic, good gene combinations caused high mean fitness, W; high mean fitness caused high population density, N; and, the competition that attended high density caused dispersal or migration. Wright discussed his argument connecting genes to ecology on many occasions with his colleague at the University of Chicago, Thomas Park, the founder of laboratory ecology, who was a close personal friend of C. S. Elton (pers. comm. from TP to MJW).

The addition of interdemic selection to Wright's theory has been as controversial as its foundation on gene interactions. It has been argued that random migration is sufficient to spread a good gene combination from one deme to another and that, once it has arrived in a deme, local individual selection is sufficient to insure its spread (Barton, 1992; Barton and Rouhani, 1993). As a consequence, some have concluded that the reliance on interdemic selection is a weakness of Wright's SBT (for both sides of this controversy, see: Coyne *et al.*, 1997; Wade and Goodnight, 1998; Goodnight and Wade, 2000; Coyne *et al.*, 2000).

Experimental Tests of Wright's Shifting Balance Theory

The most general, albeit qualitative, prediction about the evolutionary process that Wright drew from his SBT was that the rate of evolution would be faster in a metapopulation than in a large, randomly mating population at least for complex traits closely connected to fitness and involving gene interaction effects. Artificial interdemic selection experiments on 'additive' genetic traits without gene interactions (reviewed in López-Fanjul, 1989) have not shown any advantage of selection in metapopulations over selection in large randomly mating populations. As López-Fanjul (1989) concluded, "it is desirable that experiments of this kind [metapopulation selection] be carried out for epistatic traits in order to test Wright's hypothesis." Wade and Goodnight (1991) tested Wright's theory by focusing artificial interdemic selection on fitness itself, a key trait in Wright's SBT, by creating experimental and control metapopulations, each with 50 demes, where each deme was founded with 20 adult flour beetles of *Tribolium castaneum*. Importantly, the differences in mean deme fitness that developed as the offspring of each group of 20 beetles reproduced and developed were created by the beetles themselves and were not imposed by Wade and Goodnight (1991). That is, despite starting each deme with the same number of founders in the same amount of resource and holding them at the same environmental conditions, the beetles created a multiplicity of fitness peaks across the experimental metapopulations as one group of 20 adults was more or less productive than another.

In the experimental metapopulations, Wade and Goodnight (1991) converted the beetle-created variation in W, into interdemic selection by differential dispersion as prescribed by Wright's SBT. Here, each deme was assigned a 'relative demic fitness' value, W/W_{mean}, by dividing each W by the average (W_{mean}) of all 50 W values in the metapopulation so that highly productive demes, where $W>W_{\text{mean}}$, had higher relative demic fitnesses than less productive demes whose $W<W_{\text{mean}}$. Demes with relative fitness >1 contributed migrants to a migrant pool, while demes with relative fitness <1 received migrants from the migrant pool as per Wright's Phase III interdemic selection.

The control metapopulations were treated in the same way as the experimental metapopulations except that migration among demes was random with respect to demic fitness, W. Wade and Goodnight (1991) imposed the same amount of migration in the control as in the experimental metapopulations. The only difference was the pattern of migration, random in the case of the controls but biased toward demes of higher mean fitness in the experiments. The random migration among demes in the control metapopulations allowed a test of the prediction that random migration was sufficient to spread favorable gene combinations and that interdemic selection was not essential as Wright claimed.

In each of the three experimental metapopulations with interdemic selection, Wade and Goodnight (1991) observed a significant increase in mean fitness relative to the corresponding control metapopulation with the same level of random migration. This finding supports Wright's SBT as a process that can change mean deme fitness. Because the mean fitness of the control metapopulations with random migration was lower in all three cases, the results indicate that interdemic selection changed mean fitness where individual selection with random migration did not (for further discussion see Wade, 2016).

Nonrandom Phase III Migration and F_{ST}

Another aspect of the interdemic selection by differential dispersion (Phase III) of Wright's SBT has been controversial and

that is the contrasting rate of migration between Phases I and II. The exploratory period of Phase I is more effective with low migration rates than it is with high migration rates because Wright's own measure of genetic differentiation among demes, F_{ST}, is a declining function of the migration rate. Differently put, demes become much more genetically different from one another by random genetic drift when migration rates are very low than they do when migration rates are high. The opposite obtains in Phase III: exporting an adaptive peak from one deme to another is more effective when migrations rates out of the deme with high mean fitness and into the deme or demes of low mean fitness are high (Moore and Tonsor, 1994; Coyne *et al.*, 1997). As Lenormand (2002, p. 188) stated: "Gene flow plays a crucial role in shifting balance models; it has to be both low enough for a peak shift to occur (so that drift enables alleles to cross the 'valley') and high enough for a high-fitness peak to spread." Similarly, Gavrilets (1996, p. 1034) suggested that the migration conditions necessary for Wright's shifting balance process are restrictive because "… Migration should be neither too strong not [sic] too weak relative to selection."

Recent experimental studies of migration during Phase III have shed some light on this question (Wade, 2013). When migration deviates from the island model of migration the arithmetic mean migration rate is not the appropriate measure of the genetic mixing that attends dispersion between populations (Sved and Latter, 1977). Where the island model assumes random migration, migration in natural populations tends to deviate from random. Phase III migration is a particular case where migration deviates from the island model. Specifically, where migrants disperse at random among metapopulation demes under the island model, in Wright's Phase III, migrants disperse nonrandomly such that the variation among demes in migrants received (and therefore gene flow) exceeds random. This is necessary for the interdemic selection in the SBT because migrants differentially originate in demes of high mean fitness and disperse into demes of lower mean fitness. In fact, the interdemic selection differential in Phase III of Wright's process is a function of the degree to which the variation in migration at Phase III exceeds random expectation (Wade, 2013, eq. 6). This variation in excess of random 'lowers' the effective migration rate among demes so that the arithmetic mean rate of migration overestimates the effective migration rate and overestimates the effect of Phase III migration in reducing F_{ST}.

Wade (2013) used data from the experimental study of the SBT (Wade and Goodnight, 1991) to illustrate the degree to which Phase III migration increased the variance in migration rate and thereby lowered the effective migration rate. The protocol of Wade and Goodnight (1991) resulted in identical arithmetic mean migration rates in their control (island model migration) and their experiment (Phase III migration) metapopulations. However, the greater variance in Phase III migration resulted in a lower effective migration rate, which manifested itself in higher values of F_{ST} for a neutral genetic marker. Most estimates of migration in natural populations confound its random and differential components, providing estimates of Nm, the effective number of migrants, with unknown and unmeasured biases (Whitlock and McCauley, 1999).

Several Open Questions Remaining

The central question of Wright's SBT (Wade, 1992) remains unanswered: Does natural selection assemble adaptive gene combinations one gene at a time acting on the average 'additive' effects of single genes; does it select directly among different gene combinations; or is the adaptive process a combination of selection processes within and among demes as Wright suggested? Unfortunately, this question remains unanswered because the predictions from Wright's theory are not particularly specific.

Wright's SBT predicts that the additive effect of a gene on fitness should change in sign and in magnitude with genetic background, but it does not specify how different two backgrounds have to be in order to detect measurable changes. Recent studies indicate that epistasis dominates the genetic architecture of *Drosophila* quantitative traits (Huang *et al.*, 2012), accounting for the lack of replicability among gene discovery studies. But no studies to date quantify the variation in gene effect, either on a trait or on fitness, in relation to the variation in genetic background. The relationship between the genome-wide average among-deme genetic variance, F_{ST}, and the among-deme variance in gene effects might be linear or curvilinear, and the latter might be compensatory or synergistic. This unknown relationship ought to affect the rate of speciation with synergistic or compensatory relationships accelerating speciation. Moreover, there may be categories of genes whose effect changes greatly with small changes in background and other categories of genes whose effects change little or not at all with large changes in background. In short, there is a tremendous amount of additional genetic and ecological research that is necessary to determine the scope of Wright's SBT in adaptive evolution.

See also: Coevolutionary Fitness Landscapes. Evolutionary Genetics, History of. Synthetic Theory of Evolution, History of

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Social Effects

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Glossary

Altruism Any behavior that benefits a conspecific at a cost to the actor's own fitness.
Hamilton's rule A rule first derived by William Hamilton predicting that altruism should be favored by selection when fitness costs are outweighed by benefits to related individuals.
Indirect genetic effect The influence of the genes of one individual on the phenotypic expression of another.
Nonsocial selection The influence of an individual's phenotype on its own fitness.
Social selection The influence of the phenotype of one individual on the fitness of another.

Social interactions among conspecifics are ubiquitous in nature (Frank, 2007). Some species, such as the eusocial insects (ants, bees, wasps, and termites), form complex societies with differentiated castes and division of labor (Wilson, 1971). In these species, social interactions are obviously important for fitness. For example, a queen bee would be unable to reproduce without the cooperation of workers who forage and provide parental care for the colony. In other species, the importance of social interactions might be less obvious but no less important. For example, social competition among conspecifics for resources or mates is often both highly important for fitness and reliant on social interactions (West-Eberhard, 1979). Quorum sensing in bacteria, reproductive aggregation in slime molds, and chemical communication in plants serve as reminders that the importance of social interaction is not limited to animals but extends across all of life's kingdoms (West *et al.*, 2006; Frank, 2007; Karban, 2008).

Despite their importance in nature, social interactions were mostly ignored by evolutionary biologists for over 100 years after the publication of the *Origin of Species*. The founders of theoretical population genetics, Haldane, Fisher, and Wright, made occasional references to sociality but were largely concerned with other issues. A major breakthrough came in 1964 with the publication of Hamilton's theory of inclusive fitness, which demonstrated the evolutionary consequences of genes that influence the fitness of related individuals (Hamilton, 1964a,b). Specifically, Hamilton showed that alleles associated with altruistic behavior may evolve by natural selection when the cost to the actor's fitness is outweighed by benefits to relatives. This prediction has become known as Hamilton's rule.

Later in the decade, Griffing began to explore the consequences of what he called 'associate effects,' which occur when the genes of one individual affect the phenotype of another (Griffing, 1967). Griffing's contribution has received much less attention than Hamilton's, but as will be shown below, such associate effects (or indirect genetic effects (IGEs), as they are typically called today) may have major implications for the evolution of social phenotypes. Today, the work of Hamilton and Griffing has been integrated into the standard quantitative genetic model of evolution, allowing specific predictions for social phenotypes should evolve (Queller, 1992a,b; Moore *et al.*, 1997; Wolf *et al.*, 1999; Bijma *et al.*, 2007; Bijma and Wade, 2008; McGlothlin *et al.*, 2010, 2014). What follows is a brief overview of relevant theory and empirical approaches that explore how social interactions affect the evolutionary process.

Theoretical Background

Two necessary conditions must be met for a trait to evolve by natural selection. First, the trait must be predictably associated with fitness; that is, it must be under natural selection. Second, the trait must exhibit genetic variation such that offspring tend to resemble their parents. To show how these two conditions contribute to adaptive evolution, biologists tend to use the quantitative genetic model of phenotypic evolution, which shows that under many conditions, phenotypic evolution can be predicted by the breeder's equation:

$$\Delta\bar{z} = G\beta$$

(Lande, 1979; Lande and Arnold, 1983; Falconer and MacKay, 1996). This simple equation shows that evolutionary change in the population mean of a trait ($\Delta\bar{z}$) can be predicted by the product of additive genetic variance (G), a measure of the similarity between parents and offspring, and the selection gradient (β), which measures the slope of the relationship between a trait and fitness. Both G and β can be estimated empirically, making this model a powerful way to study evolution in natural populations (Endler, 1986; Mousseau and Roff, 1987, 1997; Kingsolver *et al.*, 2001).

Social interactions among individuals can lead to complexities that alter the predictions of the breeder's equation. Specifically, social interactions can lead to violation of two important assumptions: that traits can be neatly decomposed into genetic and environmental components and that an individual's fitness can be attributed primarily to an individual's own traits. Violation of these two assumptions lead to two pathways by which social interactions can affect evolutionary change: by altering the phenotypes of interacting individuals, giving rise to IGEs, and by directly influencing fitness, giving rise to social selection (**Figure 1**).

Indirect Genetic Effects

The simplest quantitative genetic model assumes that the expression of a trait, z, can be attributed to two sources: an additive genetic component, a, which contributes to the

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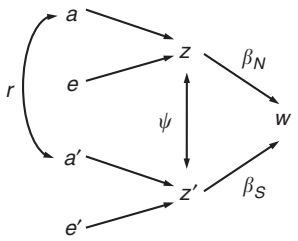

Figure 1 Pathways by which social interactions may influence evolutionary response to selection. In the absence of social interactions, an individual's fitness (w) is influenced only by its own traits (z), which are a function of its genes (a) and environment (e). The strength of this relationship is known as nonsocial selection (β_N). Social interactions may alter this pathway in two ways. First, an individual's phenotype may be influenced by the phenotypes of another individual (z') with strength ψ, leading to indirect genetic effects (IGEs). Because such interactions can involve feedback, this effect is shown as a double-headed arrow. Second, the social partner's phenotype may directly influence fitness, leading to social selection (β_S). Social selection can alter evolutionary response in the presence of IGEs, relatedness (r), or both.

similarity of parent and offspring traits, and an environmental component, e. To obtain an individual's phenotypic value, these components are simply added together:

$$z = a + e$$

Now consider a trait whose expression depends upon a trait in another individual. Animal behavior provides numerous examples of such traits. For example, a territorial holder's aggressive response might depend on the body size of the invader it encounters. These effects can also arise via feedback between the same phenotype expressed in different individuals. For example, a bird might sing more intensely when it hears song from another bird. At the same time, the second bird may be adjusting its song in response to the first. Although these effects on phenotypic expression may seem like just another part of the environment, they may alter evolutionary predictions because now the environment depends upon the traits of others and thus has a genetic component of its own.

The dependence of an individual's phenotype on genes found in another individual is known as an IGE. (Most of the treatment below follows the model of Moore *et al.* (1997), which introduced this term.) Such effects can be modeled by introducing another term into our phenotypic equation:

$$z_i = a_i + e_i + \psi_{ij} z'_j$$

The new term $\psi_{ij} z'_j$ represents the effect of a second individual's trait (z'_j) on the expression of the first individual's trait (z_i). The subscripts i and j allow the consideration of both cases sketched above, and the prime on the latter trait is used to indicate that the trait belongs to a second individual. When i and j are different, this equation represents cases like the adjustment of aggression based on invader body size; when i and j are the same, it represents cases of feedback, like the birdsong example. The coefficient ψ_{ij} represents the strength and direction of the interaction. If ψ_{ij} is positive, z_i increases in response to larger values of z'_j, whereas if ψ_{ij} is negative, the opposite occurs.

IGEs arise when the trait of the interacting individual is heritable. In this case, z'_j can be broken down into an additive genetic effect and an environmental effect of its own:

$$z_i = a_i + e_i + \psi_{ij}(a'_j + e'_j)$$

It is clear from this equation that the expression – and hence the evolution – of trait z_i will depend on both a direct genetic effect or DGE (a_i), which is attributable to an individual's own genes, and an IGE ($\psi_{ij} a'_j$), which is attributable to genes of its social partner. (Again, the primes denote that values belong to a second individual.) Even more complexity can arise when feedback is incorporated into the model. This may occur when one trait influences the same trait in another individual, as in the birdsong example, or when two traits influence each other in a loop. For example, animals might have both aggressive and submissive displays that they may use in an agonistic encounter. The aggressive display from one individual might elicit the submissive display from the other, and in turn, the submissive display might suppress the aggressive display. Such loops can be modeled by adding another term:

$$z_i = a_i + e_i + \psi_{ij}(a'_j + e'_j + \psi_{ji} z_i)$$

After some algebra, this can be written as

$$z_i = \frac{a_i + e_i + \psi_{ij}(a'_j + e'_j)}{1 - \psi_{ij}\psi_{ji}}$$

The denominator of this equation shows that feedback loops will influence the magnitude of both DGEs and IGEs. This effect will depend on the signs of the two ψ coefficients.

These social effects on the expression of phenotypes lead to alterations in the predictions of the breeder's equation. Consider the case of a phenotype that triggers a change in the same phenotype in an unrelated individual, like the birdsong example above. In this case, the predicted change in response to selection is

$$\Delta\bar{z} = \frac{G\beta}{(1-\psi)(1-\psi^2)}$$

The subscripts have been dropped here because this equation considers only a single trait. This equation shows that when a single trait is considered, IGEs affect evolutionary predictions in two ways. First, the term $(1-\psi)$ in the denominator shows that the simple presence of IGEs increases the response to selection when ψ is positive and decreases it when ψ is negative. This effect arises because the genes an individual passes on to its offspring will influence both their own phenotype and the phenotypes of others. When social interactions cause individuals to express more similar phenotypes, effective genetic variance (and thus response to selection) is increased. Social interactions that cause individuals to become more different from each other have the opposite effect.

Second, the term $(1-\psi^2)$ in the denominator arises from feedback in social interactions. Feedback is more important for very strong values of ψ, and cause a rapidly increasing response to selection as ψ becomes more positive. When ψ is negative,

feedback effects can overwhelm the depressive effect of IGEs on genetic variance, leading to a very strong response to selection for values of ψ around -0.9 and smaller. It is unknown whether such values are likely to be realistic, however. As will be discussed later, empirical estimates of ψ are limited, but there is currently no evidence for extreme negative values of ψ.

IGEs may also influence how responses to selection on multiple traits. In the absence of IGEs, a trait may evolve in response to selection on a second trait if the two traits are genetically correlated:

$$\Delta\bar{z}_1 = G_{11}\beta_1 + G_{12}\beta_2$$

In this equation, G_{11} refers to the additive genetic variance in trait z_1 and G_{12} is the additive genetic covariance between the two traits. Clearly, z_1 may evolve even when selection does not act directly on it (i.e., $\beta_1=0$) if z_2 is under selection ($\beta_2 \neq 0$) and the two traits covary genetically ($G_{12} \neq 0$). IGEs may alter this prediction by creating genetic relationships between traits that are otherwise uncorrelated. Consider the case when z_1 is influenced by z_2' in unrelated social partners ($\psi_{12} \neq 0$). If these two traits show no additive genetic correlation ($G_{12} \neq 0$), such IGEs can still cause the evolution of the two traits to be intertwined:

$$\Delta\bar{z}_1 = G_{11}\beta_1 + \psi_{12}G_{22}\beta_2$$

Note that the social effect causes z_1 to evolve in response to selection on z_2 even though the two traits are genetically uncorrelated. The quantity $\psi_{12}G_{22}$, which is the IGE coefficient multiplied by the genetic variance in trait 2, plays the same role as the additive genetic covariance G_{12} above. Interestingly, this equation also shows that z_1 may evolve in response to selection on z_2 even when it shows no additive genetic variance of its own ($G_{11}=0$). This would not be true in the absence of IGEs because genetic covariance is by definition absent when genetic variance is absent.

Social Selection

The second pathway by which social interactions may influence the evolutionary process is via direct effects on fitness. The effect of the phenotype of one individual on the fitness of another is known as social selection (Wolf *et al.*, 1999). Social selection may arise whenever social interactions have fitness consequences that depend on phenotype. For example, if agonistic encounters when larger individuals tend to inflict more harm, social selection would be acting through body size.

Like ordinary natural selection, or 'nonsocial' selection, social selection will only lead to evolutionary change under certain conditions. For nonsocial selection, the relevant variable is genetic variance, but what matters for social selection is the correlation between interacting individuals. Specifically, social selection will only lead to an evolutionary change when there is a nonrandom association between an individual's genes and the phenotype of its social partner (McGlothlin *et al.*, 2010). Using the example above, if large individuals inflicted harm and also sought out smaller individuals to bully, social selection would lead to an evolutionary increase in body size. It is easy to see why this is true: small individuals suffer the most from aggression and thus have the lowest fitness.

To add social selection to an evolutionary model, fitness must be divided into two components: one deriving from an individual's own traits and one deriving from those of social interactants. The simplest version of such a model considers the same trait in two socially interacting individuals. In this model, an equation for relative fitness can be written as

$$w = \alpha + \beta_N z + \beta_S z' + \varepsilon$$

where w is relative fitness, β_N is the nonsocial selection gradient, β_S is the social selection gradient, and α and ε are an intercept and an error term, respectively (Wolf *et al.*, 1999). Although social selection can involve any phenotype, it is easiest to envision acting through behavioral traits: β_S should be positive for behaviors that tend to help another individual (cooperation or altruism) and negative for behaviors that harm another individual (such as physical aggression).

As mentioned above, an evolutionary response to social selection depends on an association between one individual's genes and another's phenotype. This relationship can arise in two different ways: either individuals nonrandomly interact with one another, or IGEs may alter the expression of phenotypes during interactions. As noted above, IGEs are quantified with the parameter ψ. To model nonrandom association, the parameter r is used. This parameter is usually called relatedness, because one of the easiest ways to get a nonrandom phenotypic association is for relatives to interact. However, familial relatedness is not necessary; any nonrandom assortment (such as big individuals seeking out smaller individuals) will do.

Adding these effects to the breeder's equation yields:

$$\Delta\bar{z} = \frac{(1 + r\psi)G\beta_N + (r + \psi)G\beta_S}{(1 - \psi)(1 - \psi^2)}$$

This equation shows that evolutionary response to social selection depends on the quantity $(r+\psi)$, that is, on the presence of relatedness, IGEs, or both (McGlothlin *et al.*, 2010). In addition, the response to nonsocial selection is altered somewhat when both relatedness and IGEs are present $(1+r\psi)$. Social selection can either act in opposition to or in concert with nonsocial selection. The former case is the most interesting, because here the levels of selection are in conflict; in other words, different trait values are favored when we consider an individual's fitness versus the fitness of others. When levels of selection are in conflict, the evolutionary outcome will reflect a balance between nonsocial and social selection. This balance will be determined both by the strength of each selection gradient and the combined effect of relatedness and IGEs.

The most instructive case to examine here is the same one that concerned Hamilton: the evolution of altruism. Altruism occurs when others are helped at the expense of one's own fitness, and hence altruistic behaviors should have positive β_S (Hamilton's 'benefit') and negative β_N (Hamilton's 'cost'). Thus, an altruistic behavior should increase in response to selection ($\Delta\bar{z}>0$) when:

$$-\beta_N < \frac{r + \psi}{1 + r\psi}\beta_S$$

This inequality, which is a slight modification of Hamilton's rule, demonstrates that relatedness and reciprocity can have symmetrical and complementary effects on the evolution of altruism (McGlothlin *et al.*, 2010). In words, this inequality shows that altruism should increase in a population when fitness costs to oneself are outweighed by scaled benefits to others. As Hamilton identified, the effective benefit increases with relatedness; for example, altruistic behavior is more likely to evolve when full-sibs ($r=0.5$) benefit than when half-sibs ($r=0.25$) benefit. Similarly, IGEs enhance the evolution of altruism when ψ is positive, leading interacting individuals to be more similar to each other and slow its evolution when ψ is negative. Positive values of ψ can favor the evolution of reciprocal altruism, a form of cooperation where one individual's actions depend on the actions of its social partner. The modified form of Hamilton's rule indicates that this type of reciprocity can lead to the evolution of cooperation among unrelated individuals ($r=0$) when ψ is strong enough. When both factors are present, they can interact to influence the evolution of altruism.

Empirical Examples

The power of the framework outlined above is that relevant parameters can be estimated in natural populations, allowing evolutionary biologists to assess the importance of social interactions in the evolutionary process. The study of social interactions has traditionally been the domain of behavioral ecology, and its synthesis with evolutionary quantitative genetics is still in its infancy. Empirical work at the nexus of these two fields has begun to bear fruit in recent decades. This section will briefly explore empirical studies of IGEs and social selection and their relevance to understanding evolution interacting phenotypes.

Indirect Genetic Effects

IGEs can be studied empirically taking one of two approaches. Trait-based approaches follow directly from the theory outlined above and attempt to assess the importance of particular phenotypes in generating IGEs (McGlothlin and Brodie, 2009). In contrast, variance-partitioning approaches assess the total strength of IGEs on a particular phenotype without assigning these effects to a particular phenotype in an interacting individual (Wolf, 2003; Muir, 2005; Bijma *et al.*, 2007; Bijma, 2010). Each of these approaches has its advantages and disadvantages, and the choice depends upon the question being asked. Fortunately, the two frameworks are compatible in theory, and results from one framework may often be translated to the other (McGlothlin and Brodie, 2009; McGlothlin *et al.*, 2010).

Studies that measure IGEs use simple modifications of methods used to detect standard quantitative genetic parameters such as heritability. Two ingredients are of prime importance for such studies. First, as for any quantitative study, the investigator must have knowledge of the genetic relatedness among the individuals under study. This is often accomplished using a controlled breeding design, such as mating males to multiple females to generate half-sib families, but may also involve complex natural pedigrees or experimentally generated inbred lines (Falconer and MacKay, 1996). Second, associations among individuals must be known in order to quantify the effects of social interactions (Muir, 2005; Bijma *et al.*, 2007). This may derive from housing individuals together in a laboratory environment or observing natural social groups in the wild.

Most empirical studies of IGEs have used a variance-partitioning approach, and the bulk of this work has been conducted in domestic species such as laying hens or hogs (Bijma *et al.*, 2007; Ellen *et al.*, 2008; Wade *et al.*, 2010). One particularly nice example comes from a study of mortality in domestic fowl (Bijma *et al.*, 2007). Group-housed hens suffer socially induced mortality due to cannibalistic pecking. A large study of 4000 hens with a known pedigree showed that nearly two-thirds of the genetic variation in survival derived from IGEs rather than DGEs. There was no strong correlation between direct and indirect effects, suggesting that avoidance of pecking and pecking others are genetically independent.

Some work has been conducted in nondomestic species as well. In one study that quantified IGEs in aggressive interactions between male deer mice (*Peromyscus maniculatus*) in the laboratory, Wilson *et al.* (2009) detected evidence for IGEs in three of the five traits they measured. Notably, all three traits showed strong correlations between direct and indirect effects. Dominant individuals tend to mount subordinates, and the rate of mounting showed a strong negative correlation between direct and indirect effects. This relationship likely indicates a negative value of ψ: individuals that tend to be submissive induce dominant behavior in their partners and vice versa. This is predicted to lead to a slower response to selection than would occur in the absence of IGEs (cf. Wolf, 2003). Two other traits, rearing (a threat display) and time to initiate a fight, showed strong positive direct–indirect correlations, indicating the presence of positive values of ψ. This result suggests that IGEs should cause certain aggressive behaviors to respond very rapidly to selection.

Although the analyses presented by Wilson *et al.* (2009) suggest certain values of ψ, they cannot directly estimate it because they do not use a multivariate approach to tease apart relationships among correlated traits. Only a few studies have attempted to estimate ψ directly. In one pioneering study, Bleakley and Brodie (2009) used a clever experimental design to estimate ψ for predator inspection behaviors in guppies. This study took advantage of the many available designer guppy strains, which are similar to inbred lines, allowing the experimenters to control the genetic makeup of social groups. Several different strains were placed in groups with a focal individual, and the regression of focal on social group behaviors provided estimates of ψ. Significant estimates of ψ were overwhelmingly positive and tended to involve the same behavior in focal and social individuals. This suggests a pattern of reciprocity: guppies are more likely to approach a predator or school if others do as well. This positive feedback should allow these behaviors to respond rapidly to selection.

In certain systems, direct and IGEs may be assigned to particular regions of the chromosome, sometimes even to particular genes. For example, Mutic and Wolf (2007) used inbred lines of the plant *Arabidopsis thaliana* to map

quantitative-trait loci underlying direct and indirect effects on a number of traits related to plant size and development. Interestingly, direct and indirect effects of given loci tended to be of the same sign, suggesting a pattern of cooperation rather than competition among plants.

Social Selection

Studies that estimate natural selection in wild populations tend to use a regression-based method that teases apart the direct effects of correlated traits on fitness. The partial regression slopes generated by these analyses provide estimates of selection gradients, i.e., a measurement of direct natural selection on each trait (Lande and Arnold, 1983). Social selection can be measured in natural populations using a simple modification such analyses (Heisler and Damuth, 1987; Goodnight *et al.*, 1992; Wolf *et al.*, 1999). In addition to including an individual's own traits as predictors of fitness, a social selection analysis would include phenotypic values from one or more social interactants. The partial regression slopes for an indivdual's own traits would estimate nonsocial selection gradients (β_N), while the slopes for the traits of social interactants would estimate social selection (β_S).

Studies directly applying social selection analysis in wild populations are very rare. One example of social selection comes from a study of forked fungus beetles, which live in groups on bracket fungi (Formica *et al.*, 2011). Social interactions among males are often aggressive and involve competition over access to mates, and larger males typically have the advantage in such contests. Formica *et al.* (2011) found that mating success, which was estimated by observing copulations, was predicted not only by a male's own size but also that of the males with which he interacted. Specifically, males with the highest mating success were larger (positive β_N) and surrounded by smaller competitors (negative β_S). Such social selection is probably common in the context of sexual selection, where a male's trait relative to local competitors is likely to be more important than his absolute trait value. Interestingly, Formica *et al.* found that larger males tended to be surrounded by smaller males. Although they did not measure genetic relationships among individuals, this phenotypic correlation suggests the possibility of a nonrandom genetic association between interacting males, which would predict an evolutionary response to social selection.

Conclusion

Theory shows that when social interactions affect phenotypes, fitness, or both, evolutionary responses to selection may be drastically altered. Depending on the nature of the interaction, evolutionary change may be accelerated or slowed. In addition, traits that are not otherwise genetically correlated may coevolve via their roles in social interactions. The effects of social interactions on evolutionary change may be probed empirically using subtle tweaks of standard quantitative genetic methods. Although early work has provided foundational evidence that IGEs and social selection may be important in nature, the field is still in its infancy and much more work is necessary to establish the evolutionary roles played by these phenomena.

See also: Genotype-by-Environment Interaction. Natural Selection, Measuring

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Sociobiology, History of

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Glossary

Adaptation A product of evolution, such as a particular morphological feature or behavior that fulfills a particular function in a particular environment; also the process involved in the production of this feature or behavior.

Adaptationism According to critics of sociobiology, sociobiologists' belief that all traits of an organism are adaptations, or the attempt to study all traits of an organism as if they were adaptations. (The critics, in contrast, point to reasons why individual traits may not be adaptations, for instance, they may be by-products.)

Altruism A behavior that promotes the fitness of another organism at the expense of its own fitness. Note that 'altruism' is used in sociobiology to refer strictly to the outcome of an organism's behavior, it does not involve motives. Altruism was a puzzle for Darwinian theory until Hamilton developed the idea of inclusive fitness in 1964.

Eusociality The 'super sociality' typical of social insects, characterized especially by the existence of a sterile caste of workers who help raise the offspring of the queen.

Evolutionarily stable strategy (ESS) A pattern of behavior ('strategy') is 'evolutionarily stable' when it is the dominant one in the population and will prevail against any invading alternative behavior pattern. Natural selection tends to produce such evolutionarily stable patterns. ESS was coined by Maynard Smith and Price (1973) inspired by Hamilton's 'unbeatable strategy' (1967).

Fitness In biology, 'fitness' refers to the number of offspring produced by an organism, not other types of fitness. Hamilton invented 'inclusive fitness' to take into account also the effects of relatives on each other's reproduction (fitness). His inclusive fitness theory was extended even to formally unrelated individuals, which possessed 'superkinship' traits that enabled individuals with altruistic genes to find each other.

Game theory Game-theoretical reasoning involves considering taking into account what other individuals ('players') in a particular situation ('game') are doing when one considers what behavior ('strategy') to adopt in order to reach the most advantageous outcome for oneself.

Gene's eye view It is useful in Neo-Darwinian reasoning to adopt a 'gene's eye' perspective, since what ultimately counts is not the survival of the individual organism itself but rather the survival of copies of its genes. By adopting this kind of perspective one can easily understand the 'strategy' of a gene (see game theory).

Genetic relatedness The likelihood that two individuals possess copies of the same gene inherited by a common ancestor. The probability of sharing genes is 1/2 for parents and offspring or for full siblings, and 1/8 for first cousins. The principle is to go through the whole chain of related individuals and multiply all the probabilities of sharing genes for the involved individuals. So for two first cousins, starting with one cousin, it is 1/2 for his or her relationhip to his/her parent, times 1/2 for this parent's relationship to his/her sibling, times 1/2 for this siblings' relationship to his/her own offspring (i.e., the other first cousin), this makes the relatedness $1/2 \times 1/2 \times 1/2 = 1/8$.

Genome All the genes of an organism.

Group selection A process of natural selection among groups rather than individuals. An early assumption was that individuals sacrificed themselves 'for the good of the group.' Wynne-Edwards formulated a possible mechanism in 1962. Biologists however, declared group selection a possible but very unlikely phenomenon. The new paradigm of kin selection was accepted as an alternative. Following Maynard Smith and George Williams, many now saw kin selection as an alternative to group selection. However, Hamilton himself (1975) regarded these as alternative ways to describe the same phenomenon.

Haplodiploidy Almost all social and nonsocial Hymenoptera (bees, wasps, ants, etc.) are haplodiploid. The males are haploid, they have only one set of chromosomes from their mother, because they stem from unfertilized eggs. The females are diploid, they have two sets of chromosomes, one each from father and mother, since they stem from fertilized eggs. Haploid males pass on all their genes to their daughters, causing them to be more related to each other than to their own (hypothetical) daughters.

Hamilton's Rule Explains how natural selection can favor altruism between genetic relatives: this can happen if the reduction in fitness (number of offspring) of the donor (cost = c) is more than made up for by the increased fitness (number of offspring) of the recipient (benefit = b). An important consideration therefore is the coefficient of genetic relatedness between donor and recipient (r). One formulation of the rule is $br - c > 0$.

Inclusive fitness This concept, developed by Hamilton in 1964 and again in 1975 (based on the Price Equation), takes into account the fact that related animals affect one another's fitnesses. It explains among other things how it is possible for natural selection to favor altruism. This can happen if the benefits of altruism can be made to fall on individuals who are likely to be altruist rather than random members of the population. A typical case involves relatives but Hamilton intended 'inclusive fitness' to be a broader concept than both 'kin selection' and 'group selection.' It can even involve unrelated individuals. Technically, inclusive fitness of an organism is typically measured as its own fitness plus the effect of its behavior on its relatives' reproduction minus its relatives' effect on its own reproduction, multiplied by its genetic relatedness to each related organism. In practice, the shorthand Hamilton's Rule is used.

Kin selection A process of selection in which individuals are postulated to behave altruistically toward relatives with

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whom they (probabilistically) have genes in common. Kin selection (a term launched by Maynard Smith in 1964) explains how natural selection works under the assumption of 'inclusive fitness' (Hamilton, 1964) rather than the fitness of an individual organism. Kin selection has often been contrasted with group selection but Hamilton's (1975) reformulation of inclusive fitness presented kin selection and group selection as alternative ways of expressing the evolution of altruism by natural selection.

Pleiotropy The term for one gene influencing many traits at the same time.

Population genetics Population genetics expresses evolution mathematically as a change in gene frequencies in a population. Selection, for instance, is expressed population-genetically as the increase of one genotype at a greater rate than another in the population (Other processes for altering gene frequencies may also be mathematically formulated, such as mutation pressure, meiotic drive, genetic drift, and gene flow). Population genetics typically assumes free competition and recombination, that is, no linkages or interaction between genes. The opponents of sociobiology have typically criticized the lack of realism of these assumptions.

Reciprocal altruism/reciprocity Trivers in 1971 coined this term to refer to the repaying of altruistic acts among unrelated individuals, or at least the promise of repayment in the future. Hamilton wanted him to call this 'reciprocity' instead, since for him altruistic behavior necessarily had a self-sacrificial aspect, having suicide as the imagined outcome when taken to the extreme.

Sociobiology Wilson defined sociobiology in 1975 as "the scientific study of the biological basis of social behavior in all kinds of organisms including man." However, the actual research paradigm in sociobiology applies a few core theories and methods, such as reasoning based on inclusive fitness (or kin selection), reciprocity, game theory, and a gene's eye view. Many biologists prefer the name behavioral ecology or functional ethology. Human sociobiologists often call themselves Darwinian anthropologists or evolutionary psychologists.

The Modern Synthesis After the reconciliation between Darwinism and Mendelism in the early decades of the twentieth century many branches of evolutionary biology were reformulated in the language of population genetics, which expresses evolution mathematically as a change in gene frequencies in a population.

The Emergence of Sociobiology

The Rise of Neo-Darwinism

The development of sociobiology in the second part of the twentieth century was a natural outgrowth of the program of the Modern or Neo-Darwinian Synthesis, taking place between 1920 and 1950. The aim of that evolutionary synthesis was to integrate different areas of biology within a common framework using the language of mathematical population genetics, which formulated evolutionary processes as changes of gene frequencies in a population. The scientists responsible for this important basic translation were Ronald A. Fisher and J.B.S. Haldane in the United Kingdom and Sewall Wright in the United States. Later, in a continuing international effort, Ernst Mayr, Theodosius Dobzhansky, and others worked on integrating a number of biological fields. Julian Huxley (1942) brought the message to the general public with his popularly written *Evolution: The Modern Synthesis*.

The Theoretical Pioneers

The emergence of sociobiology as a research field was a continuation of these earlier efforts. Social behavior, too, was now seen as evolving, just like morphological traits, lending itself to quantitative treatment and hypothesis testing. The insight revolutionizing the field was W.D. (Bill) Hamilton's concept of inclusive fitness (also called 'kin selection') and his models for the four basic types of social behavior (altruism, selfishness, cooperation, and spite) (Hamilton, 1964). Other early core contributions were Robert Trivers's Hamilton-inspired theories of reciprocal altruism (1971), parental investment (1972), and parent–offspring conflict (1974); John Maynard Smith's and George Price's concept of Evolutionarily Stable Strategy (1973); and George Williams' (1966) influential *Adaptation and Natural Selection*.

A novel game-theoretical approach united these theoretical insights. Genes could be seen as strategists 'causing' their carriers to behave in 'selfish' ways, that is, ways that promoted their transmission to the next generation. 'Selfish' genes could 'cause' their carriers to behave altruistically, including sacrificing their lives. Hamilton used this imagery occasionally in early publications; Richard Dawkins took it to new pedagogical heights in *The Selfish Gene* (1976). In contrast, E.O. Wilson (1975) in *Sociobiology: The New Synthesis*, did not use game-theoretical imagery, but instead population genetic reasoning explained in popular form.

Spreading the New Ideas and Establishing the Field

The new ideas were brought to a larger public in the mid-1970s in textbooks (e.g., Alcock, 1975; Brown, 1975) and review articles (e.g., Alexander, 1974). The books that stole the show, however, were Wilson's *Sociobiology* and Dawkins' *The Selfish Gene*. They were written independently and were quite different, but together helped establish the field of sociobiology. Wilson demonstrated that a rich field of social behavior existed and gave it a name, while Dawkins explained how to think about it in Neo-Darwinian terms. And both were later attacked in the sociobiology controversy (see below).

The Name 'Sociobiology'

Before Wilson's book, the term 'sociobiology' itself had already been in use in a division of the Animal Behavior Society led by John Paul Scott and others. Sociobiology is

rooted in ethology, the study of animal behavior in natural environments. Unlike ethology, however, it only asks questions about the function or adaptive value of a behavior. Sociobiology often goes under the name behavioral ecology. Human sociobiologists typically call themselves Darwinian anthropologists or evolutionary psychologists.

Sociobiological Key Concepts

Inclusive Fitness

Inclusive fitness is the key concept in sociobiology, introduced by Hamilton in his path breaking 1964 paper 'The genetical evolution of social behaviour' when he was still a graduate student. The idea of inclusive fitness was able to explain the long-standing mystery how it is possible for altruistic behavior to evolve. Hamilton showed that altruistic behavior toward another individual can in fact be favored by natural selection as soon as the benefit of an altruistic act falls on someone that is likely to share the individual's genes, rather than on random members of the population.

Hamilton intended inclusive fitness to be an universal theoretical principle, applying to all forms of life (he often had plants in mind). Its definition is rather complicated. Assuming we are dealing with relatives (note: it may also apply to other individuals, see his 1964 paper), it is thought about as the organism's own individual fitness, plus the effect of its behavior has on the fitness (reproduction) of its relatives, minus the effect that its relatives have on its fitness, multiplied by the organism's genetic relatedness to each of these relatives (see e.g., Dawkins, 1982, Chapter 10; Grafen, 1984). Researchers, however, use the handy and powerful shorthand, 'Hamilton's Rule' (Hamilton, 1963).

Hamilton's Rule

Hamilton's rule is a simple cost-benefit calculation which can be formulated in many different ways. Basically it says that altruism can evolve if the reduction in fitness of the altruistic donor (the cost c) is more than made up for by the increased fitness for the beneficiary (the benefit b), taking into account their relatedness (r). So br needs to be bigger than c, or $br - c > 0$.

If we are dealing with relatives, the proportion of genes which two relatives are likely to share on average (their 'coefficients of relatedness') can be readily calculated for various relationships; it is 1/2 for parent–offspring and for two siblings, 1/8 for first cousins, and 1/32 for second cousins. Note that relatedness is only one consideration. In principle b and c can include any imaginable factors affecting the situation (typically ecological conditions). This makes Hamilton's Rule very generally applicable.

The Hymenoptera Case and the Haplodiploidy Hypothesis

Hamilton was able to dramatically apply the inclusive fitness concept to solve a long-standing problem in biology, that of sterility in Hymenoptera (social insects such as ants, bees, and wasps). How can it make sense for female workers to help raise their sister's offspring instead of their own? The answer has to do with the unusual genetic relationships (called haplodiploidy) in the Hymenoptera, which make the worker sisters more closely related to their sisters (including the queen) than to their own (hypothetical) daughters. Therefore, helping the queen is in the workers' genetic self-interest. The social insects became an important illustrative example in Hamilton's (1964) paper. He also briefly speculated about a possible relationship between a species' haplodiploid nature and its general ability to evolve eusociality, but later backed away from this idea in the face of empirical counterevidence. Although it was intended just as an example, many somehow got the false impression that inclusive fitness had to do particularly with the social insects. (For the evolution of Hamilton's own views, see Segerstrale, 2013, Chapter 11).

Kin Selection

Inclusive fitness is often understood in practice as the workings of 'kin selection,' natural selection acting on the family or kin group rather than the individual. This term was introduced by John Maynard Smith (1964). But, as Hamilton had noted already in 1964, kinship was only one way of getting the benefits of altruism to fall on individuals likely to be altruist. Moreover, Hamilton never intended kin selection to be an alternative to group selection (as Maynard Smith presented it). Later, however, these terms were used interchangeably. (For a discussion of the troubled relationship between Maynard Smith and Hamilton, see Segerstrale, 2000, 2013).

Kin Selection Explanation as Opposed to Group Selection Explanation

Group selection refers to a process of natural selection among groups rather than individuals. It was often implied in expressions such as 'the good of the species.'

In 1962, the Scottish zoologist Vero Wynne-Edwards (1962) proposed that the gatherings and displays of the Scottish grouse might be a way for the birds to self-regulate their breeding not to outrun their food resources. He postulated (with Darwin) that groups with cooperative individuals would fare better than groups with selfish ones, which would lead to the evolution of behavior that was 'good for the group.' Group extinction was seen as driving evolution. His book stimulated a strong critical response. Even if altruistic traits made a group more fit and this group would have an advantage over other groups, how would altruism initially be able to arise and establish itself in a group of largely selfish group members? Leading biologists declared group selection possible, but unlikely in nature (e.g., Maynard Smith and Williams). Kin selection was soon held up as the alternative. Still, some evolutionists maintained that group selection was a real phenomenon, among them E.O. Wilson.

Kin Selection and Group Selection Explanations as Equivalent

Hamilton, an adherent of individual selection, followed Fisher's population genetics tradition of treating individuals as assemblies of genes. This gave him a dual perspective that

enabled him to understand processes from both an individual selectionist and a 'gene's eye' point of view (see below). As for group selection, Hamilton declared himself 'allergic' to it, both for its seeming woolliness and its perceived association with totalitarianism (Hamilton, 1996). In the 1970s, however, he rederived his inclusive fitness formula as a hierarchical model of multilevel selection with group selection as one level (Hamilton, 1970, 1975). The helpful Price Equation, which makes it possible to assess the total distribution of genetic variance inside and outside groups in a population (Frank, 1995a), allowed him to again derive his original inclusive fitness formula, this time from group selectionist premises (see e.g., Segerstrale, 2013, Chapter 10).

This was also Hamilton's chance to finally clarify the group selection–kin selection relationship. The criterion for spreading of altruism was not relatedness (kinship) per se, but rather 'assortation' of individuals likely to carry the altruistic gene. That could happen in different ways:

> [I]n the assortative-settling model it obviously makes no difference if altruists settle with altruists because they are related (perhaps never having parted from them) or because they recognize fellow altruists as such, or settle together because of some pleiotropic effect on the gene on habitat preference. (Hamilton, 1975, p. 337)

Therefore, he reasoned,

> [i]f we insist that group selection is different than kin selection the term should be restricted to situations of assortation definitely not involving kin. But it seems on the whole preferable to retain a more flexible use of terms; to use group selection where groups are clearly in evidence and to qualify with mention of 'kin' (as in the kin-group selection referred to by Brown), 'relatedness' or 'low migration' (which is often the cause of relatedness in groups), or else 'assortation,' as appropriate. (Hamilton, 1975, p. 337)

This insight, however, hidden away in a book on biosocial anthropology, did not get much noticed. (Dawkins, 1976, however, discussed Hamilton's idea of altruists recognizing each other because of a shared superkinship trait as a 'greenbeard effect').

Reciprocal Altruism (or Reciprocity)

Reciprocal altruism (according to Trivers) is altruism that occurs between unrelated individuals when there will be repayment (or at least the promise of repayment) of the altruistic act in the future (Trivers, 1971). (Hamilton objected to this use of altruism, and suggested 'reciprocity' instead). An example is mutual grooming among many birds and mammals. Mutual assistance between members of different species is called mutualism or symbiosis (e.g., cleaner fish clean the mouths of larger fish, which in turn refrain from eating them).

For reciprocity to work, individuals would need to be able to recognize each other at a future time, and many animals seem capable of that. A problem is cheating, that is, taking advantage of a benefit offered but then not paying back. Trivers (1971) suggested that many of the human psychological characteristics have evolved for us to be able to cheat, to detect cheaters, and to avoid being detected if cheating. Cheater detection is one of the leading concepts of the field of evolutionary psychology.

Selfish Gene Theory

Taking a 'gene's eye' view means seeing the gene as a strategist promoting its own survival and propagation. The ostensibly altruistic behavior of an individual can be seen as actually serving the interest of its selfish genes. The gene's eye view was introduced by Hamilton's (1963, 1964) papers as a pedagogical device, and further developed by Dawkins in *The Selfish Gene* (1976). Dawkins later made an important distinction between 'replicators' and 'vehicles' (Dawkins, 1978). A gene was a replicator; organisms and groups, however, were vehicles, not replicators.

Behavioral Strategies and Game Theory

The gene's eye view can be used for thinking about behavioral 'strategies' adopted by interacting animals. Mating strategies is an example. Depending on the 'investment' its carrier has in its offspring, a gene will 'cause' its vehicle (male or female) to adopt a particular strategy. According to Trivers (1972), because a male typically invests little beyond the act of mating itself, while the female invests a lot, for a male, it is typically advantageous to mate with as many females as possible, while for a female it pays to be choosy. (For an alternative perspective see Hrdy, 1981.)

Reasoning in sociobiology is typically of a game-theoretical nature – an individual's best choice of behavioral strategy often has to do with what others do. An important concept is evolutionarily stable strategy, or ESS, a behavior pattern which, when dominant in a population, will prevail against any invading 'mutant' strategies. Natural selection tends to produce populations of organisms that are evolutionarily stable in this sense, for example, contain a stable ratio of 'hawk' and 'dove' genes (corresponding to two different patterns of behavior). The idea of ESS was developed by Maynard Smith and Price (1973) inspired by Hamilton's 'unbeatable strategy' (1967).

Game theory can be employed to explain a great variety of phenomena. Trivers (1974) used game theory to develop his theory of parent–offspring conflict (where both parties wish to increase their inclusive fitness). Game theory has been used to explain why sex ratios are typically 50:50 (Hamilton, 1967), and to model intragenomic conflicts of various kinds, for instance 'genomic imprinting,' whereby a gene is differently expressed depending on whether it stems from the father or the mother (Hamilton, 1967; Burt and Trivers, 2006). The existence of apparently stable genomes despite possible conflicts, suggests evolutionarily stable strategies (ESS) also at the level of the genome (Haig, 2002).

Also the evolution of cooperation between unrelated individuals (including bacteria) can be explained with the help of game theory (Axelrod and Hamilton, 1981). A famous early winning strategy was known as 'Tit for Tat'; many other strategies have been developed (see e.g., Axelrod, 1984; Nowak, 2006).

The Sociobiology Debate

The last quarter of the twentieth century featured the fierce sociobiology controversy around E.O. Wilson's book *Sociobiology: The New Synthesis* (Wilson, 1975), particularly the book's last chapter, where Wilson suggested that a number of human behaviors, too, including sex roles, aggression, altruism, and even moral and religious beliefs could well have a biological basis. Although he backed his tentative views with primate studies and behavioral genetics research, for a radical group of critics he represented a dangerous biological determinist view of humans. Wilson's own protests were of no avail. After the initial upheaval (including polemics, demonstrations, and Wilson famously getting ice-water poured into his neck at the 1978 AAAS sociobiology symposium in Washington, DC), the critical focus shifted to an attack on 'the adaptationist program' seen as underlying sociobiology. Sociobiologists in general were accused of believing that everything was perfectly adapted in the best of all possible worlds while ignoring other evolutionary forces (e.g., Gould and Lewontin, 1979).

The critics' scientific objection to what they called 'adaptationism' appeared to be based on a moral/political concern. If everything is optimally adapted, there is no point in changing society. But if instead of adaptation the emphasis is placed on such things as discontinuity, contingency, and chance, in a radically new environment new types of individuals will flourish. Everybody gets his chance. In other words, it is not a question of the survival of the fittest (see Gould and Lewontin, 1979). (For a history and analysis of the sociobiology controversy, see Segerstrale, 2000a).

As Wilson later moved on to address environmentalist concerns, the new protagonists became Dawkins and Gould. While Dawkins in *The Blind Watchmaker* (Dawkins, 1987) and other books showed how adaptation over time could give rise to complex design, Gould (especially in *Wonderful Life*; Gould, 1989) emphasized the role of chance and contingency instead. Dawkins' logical explanations of evolutionary mechanisms were treated as erroneous statements about the real world by Gould. This went on for years, producing numerous books.

The sociobiology debate can also be regarded as the next round in an ongoing conflict about the Modern Synthesis and the true meaning of Neo-Darwinism, which started already with the original architects. It was a continuation of the protest against 'beanbag genetics' (Ernst Mayr's term for the British approach) and an attempt to reopen the debate about the relationship between micro- and macro-evolution (Mayr, 1959; Segerstrale, 2000a).

Many have seen the sociobiology controversy as a conflict between the political left and right. But it was rather a conflict between a particular brand of academic activists who believed that dangerous-seeming ideas should be weeded out of science and traditional scientists who believed in the freedom of research and the democratic process (Segerstrale, 2000a).

Bringing in Culture and the Mind

Sociobiology and Anthropology

Some anthropologists had already earlier been thinking along lines similar to Wilson. In 1973, Robin Fox convened an early conference on 'biosocial anthropology' in Oxford, to which he invited Bill Hamilton (Fox, 1975). Together with Lionel Tiger, Fox had produced a number of popular books in a broad biosocial spirit, for example, *The Imperial Animal* (Tiger and Fox, 1971). Wilson acknowledged being influenced by their idea of an innate universal 'biogrammar' (in turn inspired by Chomsky). Later Chagnon and Irons (1979) edited the important *Human Social Behavior: An Anthropological Perspective*. At the University of Michigan and elsewhere Darwinian anthropologists' meanwhile applied kin selection and other theories directly to such things as marriage arrangements, patterns of inheritance, child abuse, and other phenomena (see e.g., Betzig, 1997; Daly and Wilson, 1988).

The Move Toward Coevolution

In the early 1980s Wilson, having been constantly criticized for ignoring human culture, decided to bring it in, his way. In *Genes, Mind, and Culture* (1981) produced with Charles Lumsden, a young theoretical physicist, Wilson demonstrated with the help of mathematical models and empirical evidence, how the human mind, influenced by genes, was in fact geared toward choosing survival-promoting cultural elements. Postulated innate biases, 'epigenetic rules,' guided the development of mind and connected genes with culture by affecting individuals' choices of 'culturgens': behaviors and artifacts seen as isolated units that individuals could either choose or reject (Lumsden and Wilson, 1981).

Wilson saw *Genes, Mind, and Culture* as a new version of sociobiology. Still, the book's particular type of coevolutionary process was clearly aimed at 'keeping culture on a leash.' Many social scientists disagreed with the depiction of culture as a 'sum of mental constructs and behaviors' (e.g., Leach, 1981). Others questioned the mathematics (e.g., Maynard Smith and Warren, 1982). Later, in *Consilience* Wilson (1998) presented a fuller picture of his reasoning, with special focus on the epigenetic rules.

Other coevolutionary modelers allowed culture more independence. It was not necessary to presume genetic predispositions for all adaptive behaviors, argued anthropologist William Durham (1978, 1991). Humans might be maximizing their inclusive fitness for both cultural and biological reasons. Sometimes a type of cultural selection could even replace natural selection. For example, people might maintain a behavior which was no longer biologically adaptive because of its continuing cultural association with high status. Boyd and Richerson (1985) worked with 'dual inheritance' models, which considered biological and cultural evolution at the same time.

The Emergence of Evolutionary Psychology

In the 1980s, Wilson moved away from sociobiology, focusing on environmental activism (Wilson, 1992). This opened an opportunity for the emerging field of evolutionary psychology, energetically promoted by Harvard-trained anthropologist John Tooby and psychologist Leda Cosmides. To many, evolutionary psychology may have seemed as an alternative paradigm to sociobiology. It was not dealing with genes but instead with universal features of the human mind, formed

once and for all in the ancestral Environment of Evolutionary Adaptation. The mind was compared to a Swiss army knife, composed of a set of modules for solving typical problems (shelter, food, mate finding, etc.). Culture was seen as based on a combination and extension of these modules. The 'bible' of evolutionary psychology is *The Adapted Mind* (Barkow *et al.*, 1992). A critical book is Buller (2006). Wilson persistently regarded evolutionary psychology as the same as human sociobiology.

A Change of Climate

In the 'pro-culturist' academic climate of the 1970s and 80s, the sociobiologists could not effectively rebut their critics' political interpretations of sociobiology. Already by the end of the 1980s, however, a social climate change was underway. By 2000 the post-war taboo on biological explanation of human behavior had been broken. With the development of biotechnology and especially the Human Genome Project, genetics was becoming practically a household word.

Instead of emphasizing cultural differences, new studies now documented the existence of human cultural universals (Brown, 1991). New research on language and culture presented animals as more similar to humans, and 'nicer' than earlier depicted (e.g., DeWaal, 1996). Nonverbal communication was reemphasized as a fundamental link between nature and nurture in both animals and humans (e.g., Segerstrale and Molnar, 1997). Finally, and ironically, a new 'cultural left' in academia showed little interest in traditional left-wing concerns such as science and ideology, or 'good' and 'bad' science, being instead preoccupied with postmodern concerns (Segerstrale, 2000b).

Later Developments

Group Selection, an Update

It is important to make a distinction between the 'old' and the 'new' group selection in sociobiology. The 'old' group selection refers to Wynne-Edwards 1960's model of evolution of group-beneficial traits. Wynne-Edwards was strongly criticized and for many kin selections was seen as 'the solution.' A 'new' type of group selection, also called trait group or intrademic selection, was championed especially by David Sloan Wilson in the mid-1970s. This form of selection recognizes a multilevel approach. What is central here are not group level traits but instead the traits of the individual members in the groups. However, in their later book espousing this perspective Sober and Wilson (1998) were criticized for confusing old and new group selection (e.g., Trivers, 1998; Maynard Smith, 1998).

Since group selection or multilevel selection models partition selection into within-group and between-group selection, the general opinion continues to be that group selection models can be readily translated to kin selection models (Hamilton, 1975; Frank, 1998; Lehmann and Keller, 2006). It is also argued that most (all?) examples that purportedly demonstrate the role of group selection – such as the cooperation between cells in a multicellular organism – can equally well be explained by kin selection (e.g., Queller, 2000). Some argue, however, that there are cases of 'pure' group selection. Researchers typically regard it as a matter of choice which approach to use. Working from kin selection is usually more convenient.

Cooperation – A Broader Umbrella for Prosocial Behavior

Hamilton (1964) identified cooperative behavior as behavior that is beneficial to both actor and recipient. However, cooperation can be defined in a number of ways. Some scientists use cooperation in a broader sense to refer to behaviors that are in the first place beneficial for the recipient and additionally either beneficial or costly for the actor. Also, both direct and indirect fitness benefits may be present, for example, in cases of cooperative breeding. (For a broad cross-disciplinary overview of cooperation research, see Hammerstein, 2003.)

When it comes to cooperation between unrelated individuals, reciprocal altruism, or better, 'reciprocity,' is only one of many ways in which this can take place (for other examples, see Clutton-Brock, 2009; Frank, 1995b; Lehmann and Keller, 2006; West *et al.*, 2007a). Interesting new experimental research on cooperation is being conducted with microbes (West *et al.*, 2006; Strassmann and Queller, 2011).

Later theorizing was concerned with the conditions under which the principle of reciprocity could be sustained and free riders and cheaters avoided. A distinction was made between *direct* and *indirect* reciprocity, where indirect reciprocity had to do with reputation (Alexander, 1987; Nowak and Sigmund, 2005). An individual with a reputation for helping is more likely to be helped by members of the same community. *Strong* reciprocity, again, involves not only reciprocation but also punishing 'defectors' or 'cheaters' who do not reciprocate (Bowles and Gintis, 2004). (This can even be taken to a higher level by punishing those who do not punish those who do not reciprocate).

The Role of Culture in Gene–Culture Coevolution

Some researchers give culture a larger role than before as a factor in human evolution. The 'group extinction' required by group selection theory can in fact happen in a number of ways – not necessarily by killing off the defeated group (as believed e.g., by Bowles, 2008; Wilson, 2012). According to Boyd and Richerson (2009), members of the defeated group may get absorbed by the winner and learn their culture by resocialization. They suggest that the human propensity for cooperation may well have arisen through gene-culture coevolution with *culture* as the driver. Culture can speed up evolution by quickly creating a new environment for adaptation and thus put pressure on the genes. The generation of cultural variation does not require inter-group competition or group extinction (Boyd and Richerson, 2005, 2009). Alternatively, a selection pressure for 'cooperative' genotypes may have been created by cultural rules alone (Bell *et al.*, 2009).

The Test of Sociobiology

The Flourishing of Sociobiology

In the first decade of the twenty-first century, the sociobiological paradigm was going strong.

It had been giving rise to burgeoning scientific industries around Hamilton's Rule and kin selection, ESS, and evolutionary game theory (and also different models of parasite-host coevolution, Hamilton's later interest). As new concepts proliferated, serious attempts were made to clarify and streamline the new terminology (for instance in regard to different types of reciprocity and other types of cooperative behaviors) and consistency in the semantics regarding altruism and cooperation as well as group selection (e.g., West *et al.*, 2007b, 2008). There seemed to be no empirical finding that the robust inclusive fitness paradigm could not handle: it could explain conflicts in the life of social insects, the workings of 'green-beard' genes, the social life of microbes, and even behaviors that were thought not to exist, such as spite (Ratnieks *et al.*, 2006; Strassmann and Queller, 2011; Foster *et al.*, 2001; West and Gardner, 2010).

Kin Selection – Attack and Defense

After the deaths of three founders of the sociobiological paradigm in the early 2000s (Hamilton in 2000, Maynard Smith in 2004, and Williams in 2010) an unusual challenge appeared. In an article in *Nature*, Edward O. Wilson, together with two younger mathematically trained Harvard colleagues, Martin Nowak and Corina Tarnita advocated the abandonment of kin selection, the ruling paradigm for 40 years. According to them inclusive fitness was difficult to calculate and had not generated any empirical results, and the theory of kin selection was not empirically supported. Therefore, the authors urged, the unworkable kin selection paradigm needed to be substituted for group – or rather multilevel selection. Contrary to the theory of kin selection, they argued, genetic relatedness was not needed for cooperation to emerge. Under particular ecological conditions (such as the need for common defense) the cooperation of unrelated insects could give rise to eusociality. What was needed was only the emergence of an allele (alternative form of a gene) for cooperation. They had developed a mathematical model to demonstrate this in Hymenoptera (Nowak *et al.*, 2010). (This was related to Wilson's special interest in the development of eusociality in Hymenoptera, just discussed in *The Superorganism* (Wilson and Hölldobler, 2009).

The surprisingly strongly worded attack in the *Nature* article met with a mass response from the sociobiological community, all in all some 150 signatories of five separate letters to the editor of *Nature* (see e.g., Abbot *et al.*, 2011). Together these (well referenced) letters formulated a clear rebuttal to what seemed as a number of persistent misconceptions (e.g., Wilson, 2005; Foster *et al.*, 2006). Kin and group selection were not opposites, they could be translated mathematically into one another (as Hamilton had already shown, based on Price). The kin selection paradigm had in fact generated an abundance of research. And ecological factors were already incorporated as part of the cost and benefit calculation in Hamilton's Rule. Finally, the letter writers noted, the critics had not demonstrated that relatedness was unimportant – they had just left relatedness out of their model. What was the critics' response? The Harvard trio did not budge (Nowak *et al.*, 2011).

The Mathematical Model – From Contention to Agreement?

In 2015, however, the mathematical model was challenged. After painstaking examination a team of researchers (Liao *et al.*, 2015) concluded that it represented a special case of a more general model; its results were obtained because of particular conditions. When – using the modelers' own assumptions – the result for the full range of conditions was calculated, however, it was shown that the outcome obtained by the Harvard critics was in fact the same as that for a model based on a kin selection approach. In other words, the model was not an alternative to kin selection, after all. Rather, it backed it up (Liao *et al.*, 2015; Nowak and Allen, 2015; Queller *et al.*, 2015).

Meanwhile, the persistent attack on kin selection had stimulated serious reviews by leading sociobiologists. How solid was in fact the kin selection (inclusive fitness) theory? The conclusion was that it was solid, both in regard to its general validity and applicability and its empirical support (for comprehensive reviews, see e.g., Bourke, 2011b, 2014). Seldom has a field gone through so much self-scrutiny, and seldom has it come out looking so good. Here, then, an external challenge not only had an integrative function on the field, it also suggested possible new ways of thinking, This would seem like a desirable situation around the 50th year anniversary of sociobiology.

See also: Cooperation and Public Goods, Bacterial. Inheritance: From Quantitative Genetics to Evolutionary Stable Strategies. Philosophy, Evolutionary Biology and. Synthetic Theory of Evolution, History of

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Speciation, Chromosomal Rearrangements and

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Glossary

Breakpoints Genomic regions delimiting chromosomal rearrangements.
Collinear regions Chromosomal regions with the same gene order (i.e., without rearrangements).
Dobzhansky–Muller incompatibilities (DMI) Epistatic interactions between alleles that manifest in hybrids by lowering their fitness.
Epistatic effect Effect of an allele on a trait (or on fitness) that depends on its interactions with alleles at other loci (genetic background).
Heterokaryotype Individual that is heterozygote for a chromosomal rearrangement.
Meiotic drive Distortion in the expected transmission ratio (50:50) of a particular allele or chromosome at meiosis.
Post-zygotic barrier Contribution to reproductive isolation that operates after zygote formation, for example, hybrid sterility, hybrid inviability or ecologically dependent selection against hybrids.
Pre-zygotic barrier Contribution to reproductive isolation that operates before zygote formation, for example, mate preference, habitat preference, temporal isolation, selection against immigrants, or gametic incompatibility.
Reinforcement The evolution or strengthening of a pre-zygotic barrier between two populations as a consequence of natural selection against hybrids.
Underdominance Case where the fitness of the heterozygote is lower than the fitness of either homozygote.

CRs Within and Between Natural Populations

The history of evolutionary genetics is inextricably linked with the classic work on chromosomal rearrangements (CRs) in *Drosophila* by Alfred Sturtevant, Theodosius Dobzhansky and other early-twentieth century pioneers. In the 1910s and 1920s, a series of genetic factors were discovered in *Drosophila* that prevented recombination as heterozygotes, but did not have the same effect as homozygotes (e.g., Sturtevant, 1917, reviewed in Graubard, 1932). Sturtevant (1926) explained this phenomenon by showing that these recombination suppressors were chromosomal inversions segregating within *Drosophila melanogaster* (Sturtevant, 1921; Sturtevant and Plunkett, 1926; Sturtevant, 1926).

Subsequently, population geneticists spent half a century investigating inversion polymorphisms within, and fixed inversion differences between, *Drosophila* species (Dobzhansky, 1970). Originally using the order of markers inferred from linkage maps, as in Sturtevant's pioneering work, the process was greatly facilitated by the ease with which rearrangements can be characterized in Diptera using giant polytene chromosomes from larval salivary glands (Dobzhansky and Sturtevant, 1938; Dobzhansky, 1970). For some beautiful examples of how abnormal pairing in heterokaryotypes allowed the elucidation of inversion polymorphisms in *Drosophila pseudoobscura* see Dobzhansky and Sturtevant (1938) and **Figure 1**. These studies provided early examples of adaptive polymorphism segregating within species, with evidence that the frequency of chromosomal inversions fluctuated cyclically and geographically in line with seasonal, altitudinal, and latitudinal climatic changes (Dobzhansky, 1943; Dobzhansky *et al.*, 1966; Krimbas and Powell, 1992). They also introduced the ideas of supergenes (Dobzhansky and Pavlovsky, 1958; Prakash and Lewontin, 1968) and of position effects, where inversions might be adaptive by changing the relative positions of genes (reviewed in Dobzhansky, 1970).

With the rise of modern genetics techniques, starting with allozymes in the 1960s, CRs were less frequently used as markers to study genetic variation, and this rich literature was largely forgotten (Kirkpatrick, 2010). In the genomic era, however, it seems that the importance of CRs has become apparent once more (Coghlan *et al.*, 2005). Modern sequencing techniques have afforded even greater insight into the prevalence of CRs, by empowering researchers to detect fine-scale structural changes that could not be resolved cytogenetically (Alkan *et al.*, 2011). It now seems clearer than ever that CRs are an important source of genetic variation and are widespread both within and between taxa, although this finding might not have surprised the classical *Drosophila* geneticists mentioned above. Genomic studies have shown that inversions are common and evolve rapidly in *Drosophila*, at a rate of around one new fixed inversion per million years (Ranz *et al.*, 2001). The rate of chromosomal change may be even faster in nematodes, up to four times the rate in *Drosophila* (Coghlan and Wolfe, 2002). The comparison of genome sequences has revealed around 1500 inversion differences

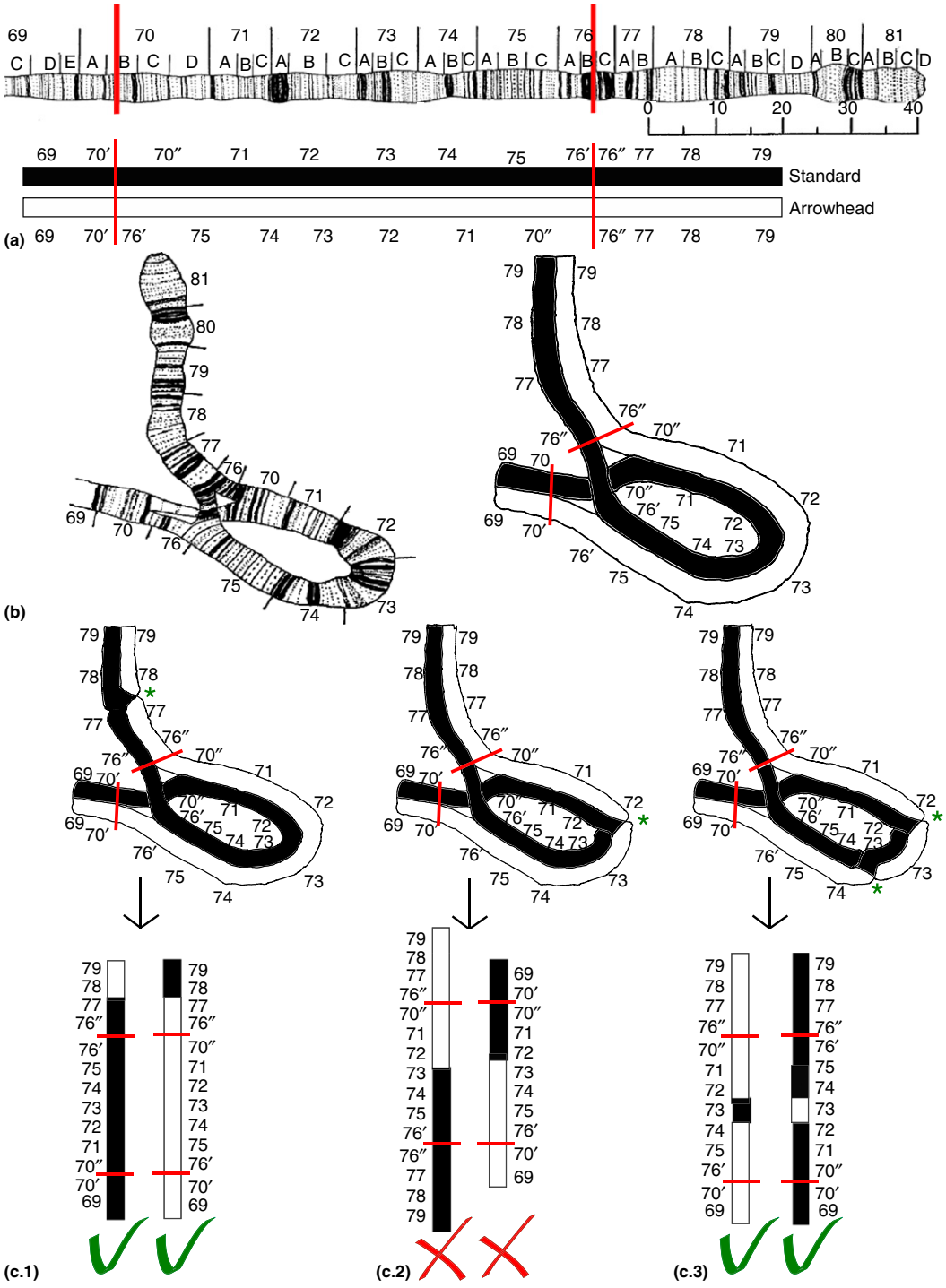

Figure 1 Representation of the pairing between homologous polytene chromosomes differing by a paracentric inversion in *Drosophila pseudoobscura* and possible consequences at meiosis. (a) Partial map of chromosome 3 (standard arrangement) showing several sections (scale in μm) and the location of the breakpoints (in red) of the 'Arrowhead' inversion, adapted from Dobzhansky, T., Sturtevant, A.H., 1938. Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics 23(1), 28–64 with permission from the Genetics Society of America; and schematic representation of part of the 'Standard' (black) and the 'Arrowhead' (white) chromosomes illustrating the reverse section order within the inversion. (b) Pairing of these homologous chromosomes in heterokaryotypes forming a loop structure (original representation reproduced from Dobzhansky, T., Sturtevant, A.H., 1938. Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics 23(1), 28–64) (left) and cartoon used in (c) (right). (c) Representation of three possible outcomes at meiosis after crossover(s) (green asterisk): C.1- one crossover in the collinear region resulting in full fertility/viability; C.2- one crossover within the inverted region generating unbalanced gametes (infertility/inviability); and C.3- double crossover (or gene conversion) within the inverted region resulting in viable gametes.

between chimpanzees and humans (Feuk *et al.*, 2005; Kirkpatrick, 2010). Despite many false positives (see also Chaisson *et al.*, 2006), the number of true inversions is much higher than the nine discovered by classical cytogenetics (Yunis and Prakash, 1982). What's more, when investigated further, many of these inversion differences were found to exist as polymorphisms within humans (Feuk *et al.*, 2005). There is also evidence for extensive chromosomal variation within and between plant taxa. Levin (2000) reviewed examples of the prevalence in CRs in the genera *Chaenactis*, *Clarkia*, *Coreopsis*, *Gaura*, *Gilia*, *Helianthus*, and *Lens*, with a focus on their role in reproductive isolation.

The recent access to whole genome sequences not only revealed a larger number of CRs but also a higher diversity of structural variants (including indels, copy-number variation, transposition, centromere repositioning) than previously considered (Rocchi *et al.*, 2012; Rogers and Gibbs, 2014). Here we focus on large-scale rearrangements, fusions/fissions, translocations, and inversions that affect many genes, rather than on small scale or insertion/deletion changes. We do not consider changes in ploidy, which are the focus of another chapter.

The Establishment of CRs in Natural Populations

If CRs are common in nature, how do they become established? Typically, newly arisen CRs show some level of underdominance and therefore should be eliminated from populations (details below, but see Dobzhansky, 1970). Because of this, rearrangements might be more easily established by drift in very small populations (e.g., Walsh, 1982; Lande, 1985; Spirito, 1998). The frequent demographic fluctuations experienced by annual plants together with self-fertilization, may explain why they tend to have a large number of CRs, since both traits reduce effective population size and selfing can generate homozygotes for rare variants, helping to overcome underdominance (Hoffmann and Rieseberg, 2008; Kirkpatrick, 2010).

In species with large effective population sizes, other mechanisms are needed to explain the establishment of underdominant CRs. One possibility is meiotic drive (White, 1978, chap. 6), which may explain the fixation of any type of rearrangement, but particularly those involving Robertsonian fusions/fissions that alter centromere properties and so may cause biased segregation in females (Pardo-Manuel de Villena and Sapienza, 2001a; Pardo-Manuel de Villena and Sapienza, 2001b). Association between CRs and segregation distortion is also expected because suppressed recombination within the region encompassed by the chromosomal rearrangement (**Figure 1**) can benefit a driving system by establishing linkage between drivers and responders (McDermott and Noor, 2010). However, evidence for segregation distortion of rearrangements is mixed (e.g., Gropp and Winking, 1981; Britton-Davidian *et al.*, 1990) and empirical studies of the association between segregation distortion and hybrid sterility have been limited largely to *Drosophila* (McDermott and Noor, 2010).

Other forms of selection may also favor the establishment of CRs. Mayr (1945) first articulated the idea that rearrangements could be adaptive to certain habitats (White, 1978). This might be through direct effects: a rearrangement's breakpoints may cause alterations in the open reading frames of genes or changes in gene expression due to promoter disruption or alteration of the relative positions of genes and regulatory regions (reviewed by Dobzhansky, 1970; Pérez-Ortín *et al.*, 2002; Avelar *et al.*, 2013). Alternatively, Dobzhansky suggested that inversions might hold together 'coadapted complexes of genes' (Dobzhansky, 1950; Wasserman, 1968), which can be interpreted as pairs or groups of loci with epistatic effects on fitness. Charlesworth and Charlesworth (1973) showed that the suppression of recombination by a newly arisen inversion could cause it to spread if it binds together an already-segregating combination of alleles presenting favorable epistatic interactions. A recent model (Kirkpatrick and Barton, 2006) shows how a CR can spread due to geographically divergent selection with gene flow, even in the absence of epistasis and when the CR itself has a small fitness cost. A new CR can become established if it traps advantageous alleles at two or more adaptive loci and if there is gene flow introducing locally maladaptive alleles at those loci. The inversion prevents the formation of suboptimal haplotypes (**Figure 1**), tying the locally adapted alleles together. This model can also explain why some inversions present a clinal distribution across environmental gradients, for example, in mosquitoes of the genus *Anopheles* (Costantini *et al.*, 2009; Ayala *et al.*, 2014) and in *Drosophila* (Krimbas and Powell, 1992; Hoffmann and Rieseberg, 2008).

The Kirkpatrick and Barton process may not be powerful enough to increase the frequency of rearrangements when they first appear as single copy in one heterokaryotypic individual (Feder *et al.*, 2011). Furthermore, the fixation of strongly underdominant CRs implies that several loci involved in adaptation must be captured in the same chromosomal region (Stathos and Fishman, 2014). This may be an unrealistic requirement for many systems. Feder *et al.* (2011) proposed a 'mixed geographic model' that relaxes these conditions: inversions can more easily become fixed during an alternating cycle of geographic isolation and gene flow. In an initial period of allopatry between two locally adapted populations, an inversion slightly increases in frequency due to drift; in a subsequent secondary contact the inversion spreads by the mechanism suggested by Kirkpatrick and Barton's model, because it carries alleles involved in local adaptation, to an equilibrium frequency maintained by selection and migration. In a new phase of allopatry, the inversion may end up being fixed.

Early Models for the Role of CRs in Speciation

The perceived importance of CRs in speciation peaked with the publication of M. J. D. White's book, *Modes of Speciation* (White, 1978) and was still being championed much later than this (King, 1993). Motivated by the frequent observation of chromosomal differences among closely related species and the observation that in some taxa speciation rates are significantly correlated with the rate of chromosomal evolution (Levin and Wilson, 1976; Bush *et al.*, 1977), White concluded that "it appears as if such [chromosomal] rearrangements, of many different types, have played the primary role in the majority of speciation events" (White, 1978, p. 336).

White was a major proponent of a class of 'chromosomal speciation' model whose general mechanism involves negative fitness effects in heterokaryotypes and can be summarized as follows: CRs that differ between populations generate intrinsic post-zygotic barriers to gene flow because heterokaryotes experience mechanical problems with chromosomes (misspairing and nondisjunction) at meiosis and/or their gametes suffer insertions or deletions (i.e., are unbalanced) (**Figure 1**; White, 1978; Davisson and Akeson, 1993). Thus rearrangements are strongly underdominant. White (1978, p. 55) cited the example of *Mus musculus* (house mouse; $2n=40$), which differs from *Mus poschiavinus* (tobacco mouse; $2n=26$) by multiple chromosome fusions: nondisjunction in meiosis occurs at a rate of 6–33%, strongly reducing hybrid fecundity.

Variants of these chromosomal speciation models (also called 'hybrid-dysfunction' models (Ayala and Coluzzi, 2005)) which date from between the 1960s and 1980s, are described by Rieseberg (2001). Most of them suffer from the same problem: in order to be strong barriers to gene flow, CRs must be strongly underdominant. But strongly underdominant rearrangements are unlikely to fix in natural populations because when they first arise they do so as heterokaryotypes, which are selected against (Spirito, 1998). An exception is the case where single rearrangements have very small effects on fitness but interactions between different rearrangements can have large effects: a chromosomal equivalent of the standard Dobzhansky–Muller model for intrinsic incompatibility (i.e., Dobzhansky–Muller incompatibilities – DMIs (Orr, 1995)). For example, the common shrew (*Sorex araneus*), exists as multiple chromosome races over its Palearctic range, each race having a different combination of fusions from an ancestral acrocentric karyotype. A single fusion has little effect on hybrid fertility but a pair of fusions can be highly incompatible (such as a *ko* fusion in one race and a *bk* fusion in another, described as 'monobrachial homology') (Searle, 1993). At the center of a hybrid zone between two races, shrews with acrocentric chromosomes 'reappear,' apparently favored by selection against the mismatched metacentric combinations (Searle, 1993).

The difficulty of fixing underdominant rearrangements means that these historic models for the role of CRs in speciation often rely on some sort of geographic isolation during the speciation process (Rieseberg, 2001). There are also problems with their generality. Because crossovers are suppressed within some CRs, no unbalanced gametes are produced at meiosis, which challenges the idea that CRs always reduce fertility (Coyne *et al.*, 1991, 1993). Hybrid inviability is at least as common as hybrid sterility as a barrier to gene flow, but heterozygosity for CRs is expected to cause the latter much more frequently. These and other patterns (Rieseberg, 2001; Coyne and Orr, 2004, pp. 259–260), suggest that genic factors are at least as important as chromosomal factors in generating incompatibilities. These problems ultimately led to hybrid-dysfunction models falling out of favor amongst most speciation biologists.

A New Role for CRs in Speciation

Interest in the role of CRs in speciation was rekindled by the simultaneous publication, in 2001, of works by Rieseberg (2001) and Noor *et al.* (2001). These authors hit upon the idea that the recombination reducing effect of CRs might facilitate speciation without reducing hybrid fitness (**Figure 2**). In fact, recombination-suppression models predate Rieseberg and Noor *et al.*'s work: see, for example, Dobzhansky (1950) who suggested that balanced inversion polymorphisms within populations maintained coadapted gene complexes, whilst heterokaryotypes for inversions derived from different populations were less fit; Coluzzi (1982) who suggested that inversions might maintain favorable (adapted) gene associations in novel ecological conditions; and Trickett and Butlin (1994) who showed that the recombination-suppression properties of CRs increased the likelihood of speciation in some older models (Felsenstein, 1981; Kirkpatrick, 1982). Rieseberg (2001) was inspired by his work in sunflowers (*Helianthus* spp.) from which he suggested that isolation genes within CRs might prevent gene flow across larger regions of the genome than isolation genes alone. Noor *et al.* (2001) were likewise inspired by their own results, in *Drosophila*, and suggested that CRs might delay the fusion of incipient species following secondary contact, by maintaining DMIs that would be lost from collinear regions due to recombination. Although their ideas are not mutually exclusive, Rieseberg (2001) put the emphasis on the role of CRs in accumulating the fitness effects of different isolation loci, due to suppressed recombination, and thus preventing gene flow across a larger fraction of the genome (Ortíz-Barrientos *et al.*, 2002). In contrast, Noor *et al.* (2001) focused on the asymmetric nature of incompatibilities, which would explain why they would be lost in collinear regions but maintained in CRs (Ortíz-Barrientos *et al.*, 2002). Noor *et al.* (2001) noted that, in their model, CRs increase the opportunity for the evolution of further pre-zygotic barriers between hybridizing taxa, i.e., reinforcement, but this is actually a general feature of chromosomal speciation models (**Figure 2**).

Rieseberg and Noor *et al.*'s models were followed by further theoretical developments. Navarro and Barton (2003a) showed that DMIs are more likely to accumulate if CRs reduce recombination. Under a scenario of divergence with gene flow, their simulations showed that CRs can act as barriers by delaying the fixation of universally advantageous alleles, allowing the establishment and accumulation of incompatibilities. Together, these three models reconciled previously distinct 'genic' and 'chromosomal' views of speciation, by showing that both processes can work together in establishing reproductive isolation. Although none of these models addressed the initial requirement for a CR to become established in a natural population in the presence of gene flow, this problem has been solved in a rather general way by the Kirkpatrick and Barton (2006) model described above (and see Kirkpatrick, 2010, for further generalization). Kirkpatrick and Barton's (2006) mechanism begins with local adaptation, resulting in some reproductive isolation. This is enhanced by the spread of the CR. The resulting chromosomal difference between demes may then facilitate speciation by the accumulation of further incompatibilities, further adaptive divergence, or reinforcement.

Taken together, these recombination-suppression models suggest that multiple classes of reproductive barriers, both pre- and post-zygotic, can be associated with CRs if they are involved in speciation and some of the empirical examples we discuss below support this prediction. The chronology of the

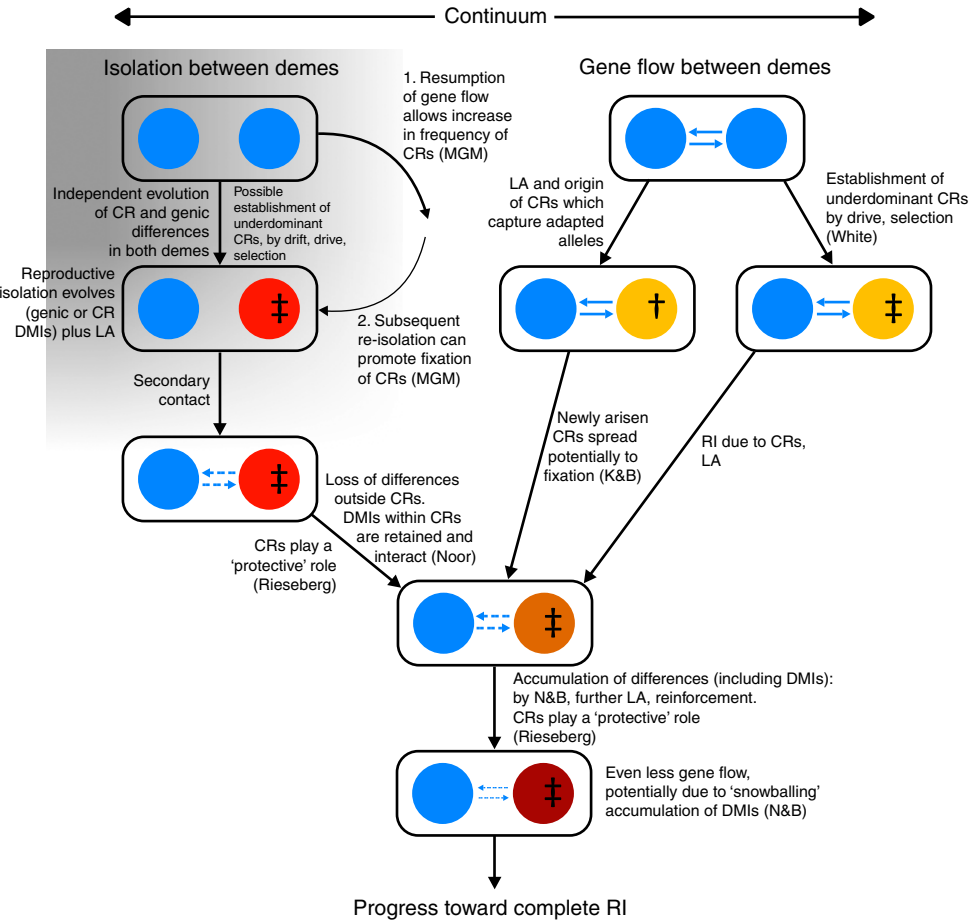

Figure 2 A schematic representation of some different paths toward reproductive isolation (RI) between two demes, invoking different processes that involve chromosomal rearrangements (CRs). We illustrate two starting points: the presence and absence of gene flow (blue arrows) between the demes, representing ends of a continuum. Different deme colors represent the accumulation of (genic or CR) differences from the ancestral (blue) condition: the accumulation of incompatibilities is represented by progress toward darker red in one of the two demes. A double dagger represents the situation where chromosomal rearrangements, including those that are (moderately) underdominant, may fix or have fixed. A single dagger represents the situation where only chromosomal rearrangements with lower direct fitness costs may fix or have fixed. These pathways are illustrative and are a simplification of the real world: other paths are possible in which the various processes occur in different combinations or sequences. Abbreviations and definitions: DMI: Dobzhansky–Muller incompatibility; LA: local adaptation; MGM: mixed geographic model (Feder *et al.*, 2011); K&B: Kirkpatrick and Barton (2006) model; N&B: Navarro and Barton (2003a) model; Noor: Noor *et al.*'s (2001) model; Rieseberg: Rieseberg's (2001) model; White: White's (1978) stasipatric or similar 'hybrid-dysfunction' model.

establishment of different categories of reproductive barrier that different models predict is, however, uncertain. To mention just two examples, one could propose that ecological divergence may contribute to the establishment of CRs (*sensu* Kirkpatrick and Barton, 2006), which in turn, may lead to the accumulation of genetic incompatibilities (*sensu* Navarro and Barton, 2003a) that subsequently promote reinforcement. Alternatively, underdominant rearrangements might themselves contribute to post-zygotic isolation, which paves the way for reinforcement to evolve (Stathos and Fishman, 2014). We present some possibilities in **Figure 2**.

Evidence for the Role of CRs in Speciation

Despite interest in the role of CRs in speciation for nearly a century, their role in reproductive isolation is still unclear. One common finding is an association between karyotypic differences and the opportunity for gene flow between taxa. Such a relationship has been observed at least in *Drosophila* (Noor *et al.*, 2001), butterflies (Kandul *et al.*, 2007), estrildid finches (Hooper and Price, 2015), and mice (Castiglia, 2014). However, this does not distinguish among models and many challenges remain.

The different mechanisms by which CR might contribute to speciation are associated with different geographic scenarios (**Figure 2**). For instance, most hybrid-dysfunction models assume strong underdominance or multiple weakly underdominant rearrangements that accumulate in the absence of gene flow until their combined effect is negative enough to act as a barrier on secondary contact (Rieseberg, 2001). However, there is now convincing evidence that gene flow during speciation is not as rare as previously thought (Pinho and Hey, 2010) and this is the situation in which

suppressed-recombination models are more likely to apply. In fact, the growing interest in understanding how speciation occurs in the face of gene flow may have contributed to a bias in the recent literature on CRs and speciation, which tend to be directed toward testing the predictions of suppressed-recombination models.

Although many closely related species show fixed CR differences (see above), empirical evidence for their role in reducing gene flow between natural populations and in the establishment of reproductive isolation in nature has been collected for only a handful of cases: in *Drosophila* (Noor *et al.*, 2001; Kulathinal *et al.*, 2009) sunflowers (Rieseberg *et al.*, 1999; but see Strasburg *et al.*, 2009), apple maggot flies (Feder *et al.*, 2003; Feder *et al.*, 2005), mosquitoes (Ayala and Coluzzi, 2005; Manoukis *et al.*, 2008; Ayala *et al.*, 2013), mice (Panithanarak *et al.*, 2004; Franchini *et al.*, 2010; Giménez *et al.*, 2013), shrews (Basset *et al.*, 2006; Yannic *et al.*, 2009), and plants of the genus *Mimulus* (Lowry and Willis, 2010; Stathos and Fishman, 2014). In many cases though, we still lack the information needed to form a complete picture of the processes by which CRs have contributed to speciation.

Among the model systems that have contributed the most to our understanding of the role of CRs in speciation, the *D. pseudoobscura* complex is perhaps the best characterized. *Drosophila persimilis* and *D. pseudoobscura* are sister species native to North America that started to diverge around 500 000 years ago and now have overlapping ranges (Noor *et al.*, 2001). They differ by three fixed (or nearly) inversions, two on the X chromosome and another on chromosome 2. Despite showing strong reproductive barriers (pre- and post-zygotic), they still hybridize in nature, although rarely (Noor *et al.*, 2001). Remarkably, all the reproductive barriers identified between these two species map exclusively to a few regions on chromosomes X and 2 in or near the inversions. In comparison, isolation loci identified between allopatric species pairs, such as between *D. persimilis* and *D. pseudoobscura bogotana*, are mostly located in collinear regions (Brown *et al.*, 2004; Chang and Noor, 2007). This suggests that reproductive barriers mapping to collinear regions between *D. persimilis* and *D. pseudoobscura* were eliminated in the face of gene flow, in contrast with those mapping to inversions, which have survived. Consistent with this pattern, divergence between these species is higher within inversions, whereas evidence for introgression is found in collinear regions (Machado *et al.*, 2002; Machado *et al.*, 2007), a trend that is not observed when comparing allopatric populations/species pairs and sympatric pairs where hybridization does not occur, such as between *D. persimilis–D. pseudoobscura* and *Drosophila miranda* (Kulathinal *et al.*, 2009; McGaugh and Noor, 2012).

Higher divergence within CRs compared with collinear regions was also observed between humans and chimpanzees when this prediction was first tested (Navarro and Barton, 2003b). However, this trend was not confirmed by subsequent studies, some based on full genome data (Zhang *et al.*, 2004; Mikkelsen *et al.*, 2005; Marques-Bonet *et al.*, 2007). Contradictory evidence has also been obtained in terms of higher divergence in gene expression patterns within CRs (Karaman *et al.*, 2003; Zhang *et al.*, 2004; Marquès-Bonet *et al.*, 2004). All this contributes to the overall impression of a minor role of chromosomal speciation in primates and, furthermore, it illustrates the complications of using solely divergence measures as evidence for the role of CRs in speciation. The difficulties in the study of speciation in the human lineage are indeed considerable. First, since our species and chimpanzees diverged about 4.5–6 million years ago (Locke *et al.*, 2011), the many other evolutionary processes that occurred since their split (including speciation processes) have shaped the divergence landscape (Ayala and Coluzzi, 2005). Second, the impossibility of mapping studies precludes obtaining the sources of evidence that are so helpful in, for instance, *Drosophila* studies. Finally, inversions could have been segregating before speciation started and thus higher divergence within CRs would not necessarily be associated with speciation (Noor and Bennett, 2009). Thus, despite enormous interest, we are still far from understanding the role that CRs have played in the speciation of humans and chimpanzees, or generally in speciation processes across the primate order. Still, structural variation is known to be high in this taxonomic group (Yunis and Prakash, 1982; Carbone *et al.*, 2014; Rogers and Gibbs, 2014) and is likely to have played a role in primate diversification. Perhaps the best way forward is to focus on recently diverged taxa or those with ongoing gene flow.

A more compelling example of the role of CRs in speciation can be found in the yellow monkeyflower, *Mimulus guttatus*. A perennial ecotype of this species is found in cool, wet coastal conditions, and an annual ecotype is found in inland habitats which experience a summer drought, both in Western North America (Hall and Willis, 2006; Lowry and Willis, 2010). These ecotypes differ in flowering time and morphology, and in other floral, vegetative, and life-history traits (Hall and Willis, 2006). An adaptive shift in flowering time has resulted in temporal pre-zygotic isolation between the two ecotypes. Immigrants are also selected against because of their maladaptive life-history traits (Hall and Willis, 2006). Fascinatingly, Lowry and Willis (2010) found that quantitative trait loci (QTL) for multiple relevant traits map to a chromosomal inversion which is polymorphic within *M. guttatus*, and which is in perfect association with the contrasting life histories of the perennial and annual ecotypes. Crossing experiments demonstrated that the effects of the inversion are independent of the wider genomic background, and reciprocal transplant experiments showed that the inversion contributes to local adaptation, which in turn causes reproductive isolation as described above (Lowry and Willis, 2010). Elsewhere in the genome variation is most strongly related to geography, which suggests that the inversion is maintaining adaptive polymorphism in the face of strong gene flow (Twyford and Friedman, 2015).

Concluding Remarks

Speciation is a process that extends over many generations. During this time, environments and species distributions change, altering population sizes, selection pressures, and opportunities for gene exchange (Abbott *et al.*, 2013). The accumulation of barriers to gene flow is, therefore, likely to occur at an uneven pace, even with existing barriers being lost in some cases, and to be influenced by multiple processes. Where do CRs fit into this picture?

CRs are simply one class of genetic variant. Any new variant with a negative effect on fitness as a heterozygote will be unlikely to spread and so its potential to contribute to isolation between populations fixed for different variants will only be realized under a restrictive set of conditions. These conditions are far more relaxed for variants with small effects in the genetic background on which they arise but with strong epistatic effects when they meet a different background through hybridization, as in the classic Dobzhansky–Muller model (Gavrilets, 2004). In these respects, CRs are no different unless they are particularly likely to fall into the latter class (as with monobrachial homology of chromosomal fusions, perhaps) or have special features, such as meiotic drive. These features are likely to be common only for some rearrangements and in some taxa (as would also be the case for most classes of genetic variants) suggesting that CRs in general do not play a special role in speciation via these routes.

However, CRs are special because of their effects on recombination. These effects vary across classes of rearrangements, with strong reduction in inversion heterokaryotes and new linkage patterns in translocations and fusions. Other classes of genetic variation can also modify recombination (Butlin, 2005; Baudat *et al.*, 2010) but, nevertheless, recombination reduction is a general property of CRs that may impact speciation. Because strong reproductive isolation depends on the build-up and maintenance of associations among traits that contribute barriers to gene flow, suppression of recombination between genes coding for these traits can facilitate speciation (Smadja and Butlin, 2011). The models discussed above show how recombination suppression can make the spread of new CRs more likely (Kirkpatrick and Barton, 2006), how this can result in the more rapid accumulation of both intrinsic and extrinsic reproductive isolation (Rieseberg, 2001; Navarro and Barton, 2003a) and how CRs can protect existing combinations of alleles that contribute to isolation from being disrupted by gene flow (Noor *et al.*, 2001).

Current empirical evidence strongly supports an association between CRs and barriers to gene flow. It would be good to extend the comparative evidence from *Drosophila* (Noor *et al.*, 2001) to more species and other taxa. Case studies show that genic differentiation in the face of gene flow is greater in or near CRs than elsewhere in the genome and that multiple traits associated with barriers to gene flow (intrinsic and extrinsic, pre- and post-zygotic) map to CRs, sometimes exclusively (Noor *et al.*, 2001; Lowry and Willis, 2010). However, it is rare for these studies to go from observing this association to dissecting its origin. We see potential using recently developed approaches to go further in two ways: understanding the relative timing of historical events and identifying loci within CRs that underlie the effect of recombination suppression on reproductive isolation.

Comparative analyses and modeling can address critical questions about the origin of CRs in relation to periods of allopatry and secondary contact, or in relation to the origin of adaptive alleles within CRs. Answers to these questions are critical to distinguishing the contributions of the various possible recombination suppression effects. The example of *D. pseudoobscura* and *D. persimilis* shows how comparisons among geographic regions and with outgroup species can help in unveiling the sequence of events (Kulathinal *et al.*, 2009; McGaugh and Noor, 2012). Recent work on *Drosophila mojavensis* and *Drosophila arizonae* using genome sequence data places the origin of inversions at the time of species divergence as well as demonstrating their impact on subsequent introgression (Lohse *et al.*, 2015). Approaches of this type have great potential for application to other systems.

Finding the genes that matter within CRs has always been a problem. The suppression of recombination usually precludes classical mapping approaches and compromises genome-scan methods. Low levels of double crossovers and gene conversion may actually hold background differentiation within inversions at low enough levels to detect excess divergence at selected loci (Feder and Nosil, 2009; Stevison *et al.*, 2011; Guerrero *et al.*, 2012). More formal models are needed to understand these processes and make predictions for empirical tests. There is also potential to use gene expression, now readily available through deep-sequencing techniques, to identify candidate loci within inversions. This approach has started to produce interesting insights into candidate loci for adaptation in large-scale inversion clines (e.g., Zhao *et al.*, 2015) but, to our knowledge, it has yet to be applied to the role of CRs in reproductive isolation. If candidates can be identified then, at least in principle, their effects on phenotypes and on reproductive isolation can be tested using RNA interference (Mohr *et al.*, 2014) or genome editing (Harrison *et al.*, 2014) methods while coalescent approaches can be used to test the ages of selective sweeps. In these ways, it may finally be possible to say which loci diverged before and which after the origin of the rearrangements, through which traits they contribute to fitness and to barriers to gene flow and whether their effects on fitness are independent or epistatic. We will then have a full picture of the role of CRs in speciation.

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See also: Reproductive Isolation, Postzygotic. Species Concepts and Speciation

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Speciation Continuum

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Glossary

Allopatry It refers to geographically isolated populations.
Allopolyploid A new species resulting from hybridization between two parental species, where the hybrid species has twice the chromosome number of the parental species.
Assortative mating When individuals with similar genotypes mate preferentially with each other at greater frequency than if mating was random.
Autopolyploid A new species resulting from the doubling of the genome of one parental species is called autopolypoid.
Chromosome doubling test A test conducted to determine if chromosome rearrangements are involved in hybrid sterility. Diploid hybrid fertility is compared to artificial allopolyploid hybrid fertility. If the allopolyploid hybrids have increased fertility, then it can be concluded that chromosomal rearrangements are involved in the sterility of hybrids.
Dobzhansky–Muller incompatibility Hybrid sterility, inviability, or lethality that typically results from a negative epistatic interaction between two or more genetic loci. These incompatibilities are thought to evolve in allopatry.
Ecogeographic isolation Reproductive isolation that occurs when two ecotypes or species are geographically isolated as a result of adaptation to local environmental conditions.
Gametic isolation A post-mating prezygotic reproductive isolating barrier caused by the failure of the gametes of two species to generate a fertilized embryo.
Homoploid hybrid speciation A new species resulting from hybridization between two parental species where the hybrid species has the same chromosome number as the parental species.
Parallel speciation Repeated evolution of reproductively isolated populations by the same mechanisms as a result of similar selection pressures in different geographic regions.
Reciprocal monophyly When phylogenetic analysis reveals that two groups form distinct monophyletic clades.
Reinforcement The process by which prezygotic isolation evolves by natural selection to avoid costly mating between species that results from postzygotic isolation.
Sympatry It refers to populations with overlapping geographic ranges.

Introduction

The biological diversity that we observe on earth is the product of a chain of speciation events stretching back to the origin of life. But what do we mean by a speciation event? The formation of a new species is not instantaneous. Rather, each speciation 'event' is a complex continuum that occurs over time as interbreeding populations bifurcate into different species and eventually, distinct phylogenetic lineages. This process generally takes considerable time and thus we can only observe brief snapshots of the continuum in a human lifespan. Over the past century and a half, many evolutionary biologists have attempted to understand the speciation continuum, but many questions still remain (Sobel *et al.*, 2010; Baack *et al.*, 2015).

One of the approaches that evolutionary biologists have adopted is to classify parts of the continuum as stages in the process of speciation. Historically, there were three major points when evolutionary biologists divided the speciation continuum into distinct stages (reviewed in Lowry, 2012). Alfred R. Wallace was the first to clearly articulate the idea of stages in the process of speciation (Wallace, 1865). During the middle part of the twentieth century, multiple plant evolutionary biologists, most notably Gote Turesson and Jens Clausen, developed their own scheme for describing stages in the process of speciation (Turesson, 1922; Clausen, 1951). Recently, a number of zoologists have resurrected the idea of stages in the process of speciation (Wu, 2001; Nosil *et al.*, 2009; Seehausen *et al.*, 2014). By focusing on different stages of the continuum of speciation, it is possible to gain insights into the different evolutionary mechanisms involved along the entire continuum. Recent studies now regularly split the speciation continuum into stages to understand the entire process better (Nosil *et al.*, 2009; Hendry *et al.*, 2009; Powell *et al.*, 2013; Conflitti *et al.*, 2014; Seehausen *et al.*, 2014).

Mathematical modeling can be very useful for clarifying the details of individual stages of the speciation continuum, especially when tailored to particular biological systems (Gavrilets *et al.*, 2014). However, the entire process of speciation is not easily captured in a single model. Instead, the process of speciation is more often constructed as a verbal narrative, whose story arc is revised and edited in response to the positive feedback between modeling and new empirical data. This article explores the reproductive isolating mechanisms that accumulate along the speciation continuum, how the process of speciation is completed, and why disagreements between species concepts could be the result of the continuous nature of speciation.

Reproductive Isolating Barriers along the Continuum

Mechanisms that Initiate the Speciation Process

The process of speciation always begins with the evolution of one or more reproductive isolating barriers. Isolating barriers

are typically categorized as prezygotic or postzygotic, acting before or after the formation of a hybrid zygote. Barriers are also divided between extrinsic (dependent on the external environment) or intrinsic (independent of environment) mechanisms. Prezygotic extrinsic barriers cause *assortative mating* between populations, ecotypes, or species. These barriers typically result from adaptive evolutionary changes in behavior, habitat, and/or pollinators. In contrast, intrinsic postzygotic barriers cause hybrid inviability, sterility, and lethality. Among intrinsic and extrinsic isolating mechanisms, postzygotic barriers tend to be weaker than prezygotic barriers early in speciation (Nosil *et al.*, 2005; Lowry *et al.*, 2008a; Sobel *et al.*, 2010). The initiation of the speciation process occurs most often in groups that are spatially or temporally isolated due to geography and/or ecology. Following this initial isolation, incipient species progress through the speciation continuum by accumulating stronger reproductive isolating mechanisms over time. Much of contemporary speciation research aims to determine the order in which isolating mechanisms evolve throughout the process (Seehausen *et al.*, 2014).

The Genetic Basis of Isolating Barriers

Early theory on the population genetic basis of reproductive isolation recognized that epistatic interactions between two or more loci, which evolved in allopatry, could cause intrinsic postzygotic isolation (Bateson, 1909; Dobzhansky, 1934; Muller, 1942). *Dobzhansky–Muller incompatibilities* constitute mutations that reduce hybrid fitness when populations come into secondary contact with each other following a period of allopatry. Chromosomal rearrangements can cause hybrid sterility through the production of unbalanced gametes in meiosis. While intrinsic barriers can evolve by selection or drift, extrinsic barriers to gene flow result from the evolution of traits in response to selection. Extrinsic barriers can thus result from any gene that has evolved in response to natural or sexual selection.

The genetic basis of several reproductive isolating barriers has been determined in recent years (Presgraves, 2010; Rieseberg and Blackman, 2010; Seehausen *et al.*, 2014). This research has confirmed the Dobzhansky–Muller model of intrinsic postzygotic isolation by identifying the genes that interact epistatically to cause reductions in hybrid fitness. The evolution of incompatibility genes is thought to be driven by an array of mechanisms including genomic conflict, selfish genetic elements, and rapidly evolving immunity genes. Further, ecological adaptations can drive the spread of incompatibilities when the loci under natural selection are genetically linked to incompatibility loci (Wright *et al.*, 2013). Very few studies have identified genes involved in extrinsic barriers to reproductive isolation. Successful gene identification has only occurred in cases where the genetic architecture underlying the barrier was simple (e.g., Hopkins and Rausher, 2011). The dearth of known ecological reproductive isolation genes may reflect the complex genetics underlying the evolution of morphological and physiological traits involved in most extrinsic barriers.

Different Narratives of Speciation

The Selection-Based Narrative of Speciation

Most of the evolutionary biologists who have divided speciation into stages have focused on scenarios in which the initial divergence leading to speciation is driven by selection. We describe the narrative here for speciation driven by selection (**Figure 1**):

The first stage of speciation in this narrative occurs when different populations diverge due to selection. We now have strong evidence that natural and sexual selection can lead to assortative mating, and thus drive reproductive isolation between populations. This is especially true for studies of *parallel speciation*, where reproductive isolation occurs repeatedly between independent pairs of populations due to strong ecological selection. An excellent example of the early stages of parallel speciation is found in the stick insect species *Timema cristinae* (**Figures 2(a)** and **2(b)**). These insects repeatedly form

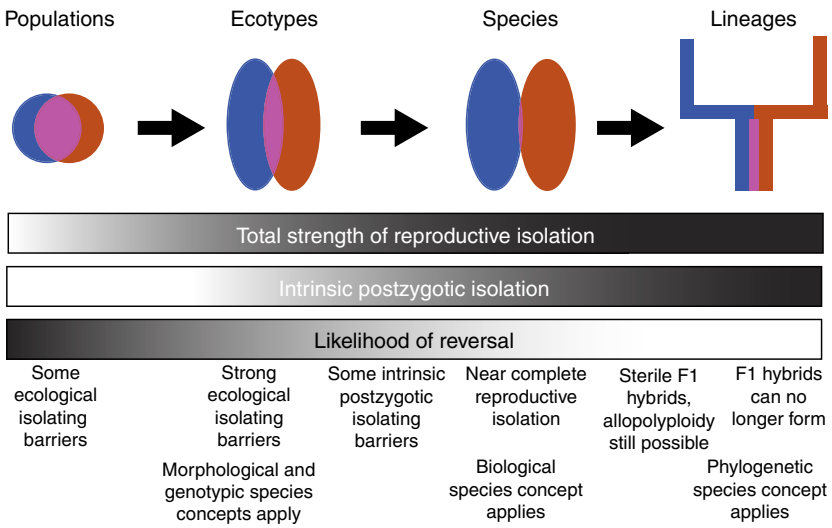

Figure 1 A selection-based narrative of the continuum of speciation. Orange and blue shapes represent diverging groups, with pink indicating potential gene flow between those groups.

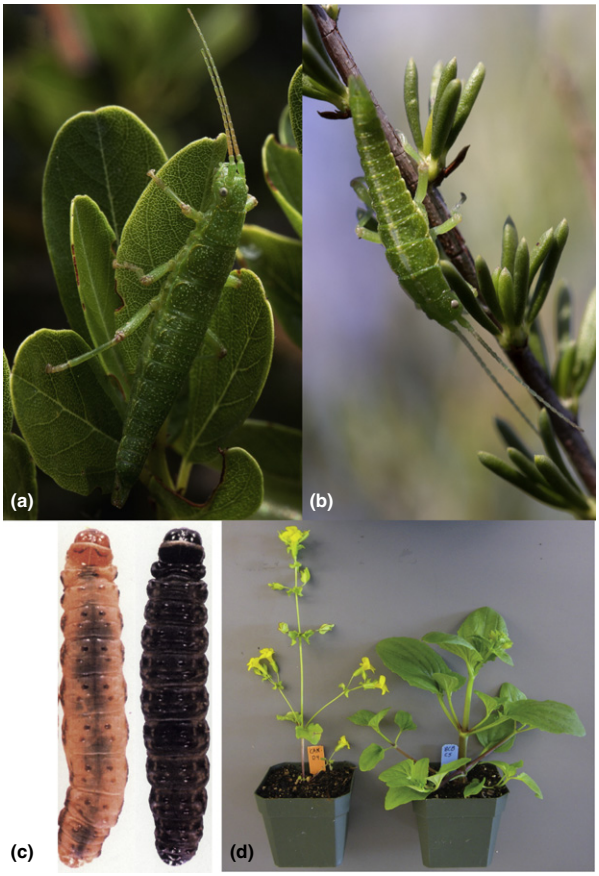

Figure 2 Divergent populations and ecotypes. Parallel patterns of reproductive isolation have occurred between nearby populations of *Timema cristinae* stick insects that are adapted to (a) *Ceanothus* and (b) *Adenostoma* host plants (see Soria-Carrasco *et al.*, 2014; photo credit: A. Comeault). (c) Ecotypes of the larch budmoth *Zeiraphera diniana* adapted to pine (left) and larch (right) trees (see Emelianov *et al.*, 2004; photo credit: W. Baltensweiler and J. Mallet). (d) Inland annual (left) and coastal perennial (right) ecotypes of the yellow monkeyflower *Mimulus guttatus* (see Lowry, 2012; photo credit: D. Lowry).

reproductively isolated pairs of adjacent populations that are adapted to living on different plant host species (Soria-Carrasco *et al.*, 2014).

The next stage of speciation is the formation of ecotypes, which encompass multiple populations that share a set of traits in common (**Figure 2**). Ecotypes have gone by different names, including ecological races, host races, varieties, and subspecies (Clausen, 1951; Mayr, 1982; Dres and Mallet, 2002; Nosil, 2012). The discrepancy in the names of these groups reflects differences in the interests of various biologists and what evolutionary forces are thought to be responsible for the evolution of these groups. Since ecotypes involve multi-trait divergence, they are best characterized by the principal components of the set of traits that define them (Lowry, 2012). Ecotypes are not distinct species. While they have some reproductive isolation that prevents gene flow between them, it is not strong enough to prevent collapse through mating if environmental conditions change or sexual selection shifts. An example of ecotypes can be found in the yellow monkeyflower, *Mimulus guttatus* (Lowry *et al.*, 2008b). Within *M. guttatus*, a geographically widespread perennial ecotype occurs in cool-wet coastal habitats which differs strongly from a closely related annual ecotype adapted to hot and dry inland habitats (**Figure 2(d)**).

Once reproductive isolation reaches a very high level, divergent populations have reached the species stage. Gene flow is rare at this stage, but is still possible. Reversal of speciation is also much less likely at this point. Unlike ecotypes, the species stage is far less controversial and most biologists agree that species exist (Coyne and Orr, 2004). A good example of the species stage is represented by *Helianthus annuus* and *Helianthus petiolaris*, which are two widespread species of sunflowers that have partially overlapping ranges in North America. Reproductive isolation is extremely high between these two species. However, reproductive isolation is not fully complete and recent gene flow has been detected between these sunflower species through molecular methods (Sambatti *et al.*, 2012).

Some biologists assume species is the final stage in the process of speciation, but this view ignores the fact that gene flow often does still occur between many species. Gene flow can also reverse the process of speciation and lead to species collapse if environmental conditions change. Therefore, the final stage of the speciation continuum is two distinct phylogenetic lineages, which will no longer exchange genes in the future through sexual reproduction. To achieve those distinct irreversible lineages requires complete intrinsic postzygotic isolation. While not all evolutionary biologists currently agree, a renewed focus on the continuum of speciation has revived the view that complete intrinsic postzygotic isolation is necessary to complete the process (Seehausen *et al.*, 2014).

Alternatives to the Selection-Based Narrative of Speciation

Speciation that results from natural selection and sexual selection has come to dominate the field of evolutionary biology over the past 20 years. There is now sufficient empirical evidence to suggest that the general selection narrative (**Figure 1**) described above applies to many biological systems (Sobel *et al.*, 2010; Lowry, 2012; Soria-Carrasco *et al.*, 2014; Baack *et al.*, 2015). However, there are many alternative narratives by which speciation can occur.

One major alternative narrative for speciation to occur is through the initial accumulation of strong intrinsic postzygotic reproductive isolation (**Figure 3**). In this scenario, two populations become geographically isolated from each other. Over time, those two populations accumulate genetic changes through drift or selection that contribute to intrinsic postzygotic isolation. When those species come back into secondary contact, the two species can no longer produce fertile offspring. This is the classic allopatric model of speciation and is still a common narrative for the speciation continuum (Grant, 1981; Coyne and Orr, 2004).

While biologists have constructed different verbal narratives of the speciation process, most speciation events likely defy simple classification. It has long been recognized that the entire process of speciation often involves the simultaneous divergences of morphology and physiology with the accumulation of reproductive isolating barriers (Clausen, 1951, p. 90).

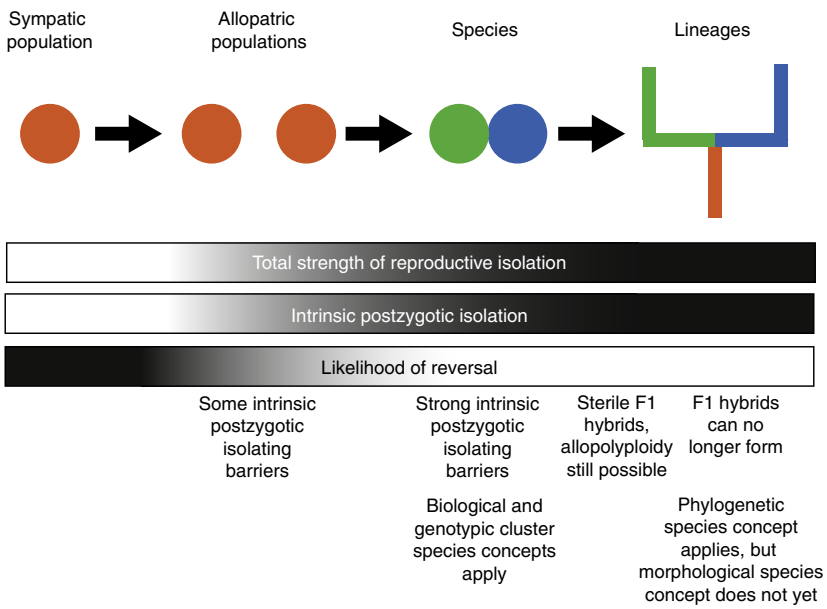

Figure 3 A geographic isolation narrative of the speciation continuum, where most reproductive isolation is the result of intrinsic postzygotic isolation accumulated in allopatry by selection or genetic drift.

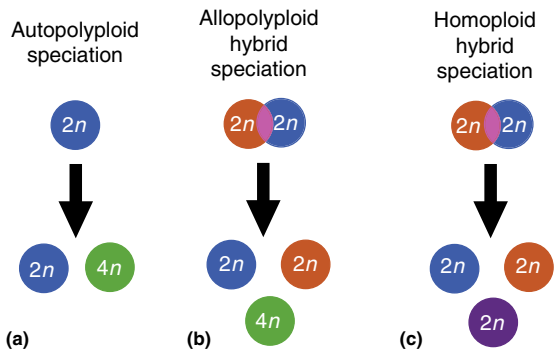

Figure 4 Diagram of three modes of rapid speciation: (a) autopolyploidy, (b) allopolyploid hybrid speciation, and (c) homoploid hybrid speciation. Diploids are represented by 2*n* and tetraploids by 4*n*.

Overall, while there are general patterns of speciation, such as the progressive accumulation of reproductive isolation over time, the process is most commonly the result of a complex combination of multiple selective forces and chance events. The specific details of individual speciation narratives are likely as unique as snowflakes.

The length of time for the speciation process can also be considerably shorter in cases of polyploidy and hybrid speciation (**Figure 4**). Autopolyploid speciation occurs through spontaneous genome doubling, with a tetraploid daughter species instantaneously reproductively isolated from a parental diploid species (Soltis *et al.*, 2007). Hybrid speciation occurs when related species successfully reproduce with the result being the formation of a new species. Allopolyploid hybrid speciation is very frequent in plants and occurs when the hybridization between two diploid species results in a tetraploid (or higher ploidy) daughter species. Reproductive isolation is often immediately strong between diploid parent and polyploid daughter species because hybrids are triploid and can be sterile. Further, both autopolyploids and allopolyploids can have different ecological niches than parents simply as a by-product of changes in genome complement and those differences can lead to immediate habitat isolation (Ramsey, 2011). Homoploid hybrid speciation occurs, for example, when the hybridization between two diploid species results in a new species that is also diploid. There are few good examples of homoploid hybrid speciation and so it is thought to be far less common than allopolyploid hybrid speciation (Schumer *et al.*, 2014).

Geography and the Continuum

The geographic context of speciation is a key component of the speciation. Allopatric separation is thought to occur at least at one point during the process in most of cases of speciation. Allopatry can occur early in speciation and be followed by a secondary contact phase in which speciation is completed. Alternatively, initial ecological divergences among sympatric

or parapatric populations could facilitate a subsequent phase of allopatry in which the accumulation of intrinsic postzygotic isolating barriers completes the process. There are many documented cases where local adaptation to different habitats maintains the allopatric distribution of populations and thus could lead to the further accumulation of reproductive isolating barriers. Allopatry that is maintained by ecological divergence is called *ecogeographic isolation*, and is thought to be very important in speciation (Schemske, 2000; Sobel, 2014).

Alternatively, speciation could occur without an allopatric phase if natural selection was strong enough to restrict gene flow between diverging taxa. Parapatric speciation along environmental gradients likely occurs often and may be the major mode of speciation in the ocean (Bowen *et al.*, 2013). Theoretical models overwhelmingly support the likelihood of parapatric speciation (Coyne and Orr, 2004; Gavrilets, 2014). However, proving that any given speciation event was entirely parapatric, without an allopatric phase, is difficult. Empirical evidence for speciation in *sympatry* is also sparse. There are only a handful of compelling cases of completely sympatric speciation (Barluenga *et al.*, 2006; Savolainen *et al.*, 2006) and those have been brought into question by modeling tailored to those systems (Gavrilets, 2014). Further, classic cases of sympatric speciation, mostly notably *Rhagoletis* fruit flies, now appear to involve genetic variation that first arose in allopatry (Feder *et al.*, 2005).

Mechanisms that Complete the Speciation Process

The Reversibility of the Speciation Continuum

The process of speciation is unstable until new species reach a point at which the process can no longer be reversed (**Figures 1** and **3**). While extrinsic ecological barriers to gene flow can be quite strong, they are contingent on current environmental conditions. If those environmental conditions change, ecological barriers can disappear leading to high levels of gene flow and subsequent species collapse. Reversals along the speciation continuum, linked to environmental changes, have now been documented in multiple fish species. For example, eutrophication has been linked with recurrent declines in differentiation between divergent sympatric lake whitefish subspecies (Vonlanthen *et al.*, 2012) and cichlid fish in Lake Victoria, Africa (Seehausen *et al.*, 2008). Similarly, stickleback fish researchers have documented the collapse of two species into a hybrid swarm following the introduction of an exotic crayfish (Taylor *et al.*, 2006). It should be mentioned that reversals in the speciation process could also occur for intrinsic postzygotic barriers, but those reversals are yet to be documented.

Reinforcement

While intrinsic postzygotic isolating barriers are ultimately responsible for the completion of the speciation process, the accumulation of those postzygotic barriers can actually drive the evolution of strong prezygotic barriers near the end of speciation. Reinforcement is the process by which new prezygotic barriers are selected for in order to avoid costly hybridization between species. Avoidance of hybridization is advantageous because hybrids are less fit than progeny produced by crosses within parental ecotypes or species. While reinforcement was controversial for much of the twentieth century, there are now many examples of reinforcement in both plants and animals (Yukilevich, 2012; Hopkins, 2013). Reinforcement is thought to typically occur when previously allopatric species come into secondary contact. Once in secondary contact, reinforcement is predicted to occur in regions of sympatry but not allopatry. One can assess the occurrence of reinforcement by comparing the strength of prezygotic isolating barriers in allopatric versus sympatric populations.

Hopkins and Rausher's (2012) recent research on *Phlox* identified the first genes to be involved in the process of reinforcement. *Phlox cuspidata* and *Phlox drummondii* come in contact with each other in central Texas, USA. These species produce highly sterile hybrids when they are crossed with each other. In the sympatric zone of these species' range, *P. drummondii* takes on a bright red coloration, which reduces deleterious hybridization with *P. cuspidata*. Two genes in the anthocyanin pigmentation pathway are ultimately responsible for the transition between pink and red flowers (Hopkins and Rausher, 2012). The *Drosophilia* of the island of São Tomé, Africa provide an excellent example of how reinforcement can evolve without changes in mating preference. Both *Drosophilia yakuba* and *Drosophilia santomea* form a hybrid zone on the island and male hybrids between the two species are sterile. Matute (2010) found that these *Drosophilia* species evolved reinforcement through the evolution of *gametic isolation* in sympatric populations. Thus, reproductive isolation that occurs after mating, but before fertilization, can evolve through reinforcement.

Mechanisms Involved in the Completion of Speciation

While recent studies have established that natural selection can initiate the process of speciation through the formation of divergent populations and ecotypes, we have a poor understanding of how diverging groups subsequently acquire intrinsic postzygotic isolation to complete the speciation process. It is largely unknown how often the same environmental factors driving the formation of initial ecological barriers also directly drive the evolution of intrinsic postzygotic barriers. Intrinsic isolating barriers can result from both intrinsic and extrinsic selective forces. Further, intrinsic barriers can spread by genetic drift within populations that are already reproductively isolated by strong ecological barriers. It would be particularly interesting to determine whether ecogeographic isolation could be a major factor facilitating the spread of intrinsic barriers, since this barrier functions by promoting allopatry.

Dobzhansky–Muller incompatibilities have been localized in different types of organisms and are thus thought to be frequently involved in speciation (Presgraves, 2010; Rieseberg and Blackman, 2010). There is also strong evidence that chromosomal rearrangements are commonly involved in plant speciation. This conclusion comes from studies of species that have a high level of sterility in F1 hybrids. When the genomes of those species are artificially doubled to make allopolyploid hybrids, hybrid fertility is often restored. The proposed mechanism for restored fertility is that, rearranged chromosomes no longer pair

at meiosis and unbalanced gametes are in turn no longer produced. Ramsey and Schemske (2002) surveyed multiple highly reproductively isolated plant species and found that diploid hybrids only had on average 17% fertility. When synthetic tetraploid hybrids were made between these same species, fertility was elevated to 71%. A major conclusion of these *chromosome doubling tests* is that rearrangements are largely responsible for hybrid sterility in the later stages of plant speciation. While chromosomal reproductive isolation is common in plants, it does not appear to be as common in animals. There are multiple hypotheses for why chromosomal reproductive isolation is more common in plants than animals (Coyne and Orr, 2004, pp. 266–267), but this question has yet to be resolved.

Another profound implication of the chromosome doubling test is that as long as two species can make F1 hybrids there still may be potential for those species to combine to form a new allopolyploid hybrid species. Therefore, the gradual accumulation of postzygotic isolation barriers will not only lead to the formation of two separate phylogenetic lineages, but will also create the potential to instantaneously form a third hybrid lineage through allopolyploidization. As a result of this observation, Clausen (1951) argued that the continuum of speciation is only finally complete when two species can no longer produce any viable hybrids. For some organisms, it may take a very long time until F1 hybrids can no longer be produced. A recent study identified a natural F1 hybrid fern that had resulted from the hybridization between two species that had been separated for ~60 million years (Rothfels *et al.*, 2015).

Species Concepts and the Continuum of Speciation

Most of the twentieth century was filled with fierce debate over the definition of species. However, much of the differences between species definitions can be attributed to biologists focusing on particular points of the speciation continuum. This controversy is analogous to the Indian parable of the blind men and the elephant. Each of the blind men had very different descriptions of the elephant because they had only touched one particular part of the elephant's body: ears, trunk, tail, tusks, etc. This resulted in a major disagreement between the men about the true nature of the elephant. The disagreement is only settled when a wise man informs the men that they are all correct because the elephant contained all the elements that the men had described.

Viewed as a continuum, it is quite easy to see how the major species concepts have arisen out of a focus on particular points along the continuum. This is clearly illustrated by revisiting the selection narrative of speciation (**Figure 1**). The morphological species concept is often used by taxonomists and primarily relies on a combination of traits quantified from museum specimens to define species. Many taxonomists prefer the morphological species concept because it does not require extensive additional research that other species concepts require. The genotypic cluster species concept defines species as a 'distinguishable groups of individuals which have few or no intermediates when in contact' (Mallet, 1995). A similar definition has recently been proposed for the term ecotype (Lowry, 2012). The reliance of the morphological and genotypic cluster species concepts on distinct traits is likely to bias them toward the earlier points along the speciation continuum. The biological species concept utilizes a strict criterion of reproductive isolation to define species and therefore corresponds well with the species stage of the continuum (**Figure 1**). The phylogenetic species concept defines species by *reciprocal monophyly* between taxa, at least at a large portion of loci across the genome (Baum and Donoghue, 1995; Queiroz, 2007). In other words, species are only recognizable by the phylogenetic species concept when gene flow has ceased long enough that allelic differences become fixed between species. Such a situation will only occur after complete reproductive isolation has occurred between taxa for numerous generations, as shared ancestral polymorphism must be eliminated before gene tree topologies will reflect actual species trees (Cruickshank and Hahn, 2014; Fontaine *et al.*, 2014). Thus, the phylogenetic species concept will, by definition, be biased toward the very end of the speciation continuum.

If speciation only occurred by a single type of narrative, it would be clear that different species concepts simply refer to different points along the continuum of speciation. However, because there are many different narratives of speciation, confusion still persists over different species concepts. For example, imagine a scenario where speciation primarily occurs through the accumulation of intrinsic postzygotic isolation in allopatry without much morphological divergence (**Figure 3**). In this case, different species would be readily identifiable by the biological species concept, and perhaps even the phylogenetic species concept, before those species would be identifiable by the morphological species concept. Essentially, they would be considered as cryptic species. The key take away from this thought exercise is that viewing speciation as a continuum, without assuming any particular narrative, can free us from the need for rigid species concepts.

Going forward, researchers should try to build a more comprehensive understanding of the different stages of speciation. Studies over the past two decades have produced many new insights regarding the role of ecological and sexual selection in the early stages of speciation. A major challenge in the future will be to understand the mechanisms that complete the speciation process and forever maintain distinct non-recombining phylogenetic lineages. A key component of this future research will include the interrogation of the causes of hybrid sterility through combining the chromosome doubling test with modern techniques (e.g., Stathos and Fishman, 2014) as well as the examination of what eventually prevents the formation of F1 hybrids. Overall, we must always keep in mind that there are many routes to producing new species and that no one model will capture the diversity of forms of the speciation continuum.

See also: Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Species Concepts and Speciation

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Speciation Genes

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Glossary

Bateson–Dobzhansky–Muller incompatibility Combination of alleles at two or more loci that interact epistatically to reduce the fitness of hybrid progeny.

***Cis*-regulatory variation** Polymorphisms affecting the transcription of a gene that are located within noncoding DNA sequences that are proximate to the gene itself.

Coalescence The merging of all descendant gene copies within a lineage at a single ancestral gene copy occurring within that lineage going back in time.

Divergent selection Selection that favors different optima for one or multiple traits in alternate environments or niches.

Ecological speciation The process by which barriers to gene flow evolve between populations as a result of ecologically based divergent selection.

Epistasis Dependency of the effects of an allele at one locus on the genotype at one or more additional loci.

Genome editing Targeted alteration of DNA through expression of factors that bind to specific nucleotide sequences and make double strand breaks proximate to them.

Genomic conflict A phenomenon where the proliferation or non-Mendelian inheritance of selfish genetic elements occurs at the expense of the host genome, which may in turn evolve to suppress the deleterious effects of those elements.

High-throughput genotyping Determination of the allelic composition at thousands of loci for hundreds to thousands of individuals using next-generation sequencing or oligonucleotide array-based technologies.

Hybrid zone Geographic region where two species ranges intersect and interbreeding naturally yields hybrid offspring at some frequency.

Incipient species Populations that share a recent common ancestor and that are partially reproductively isolated.

Knockout mutants Organisms carrying mutations that eliminate the function of a given gene.

Linkage disequilibrium Nonrandom association of alleles.

Meiotic drive Distortion of Mendelian inheritance during meiosis by alleles that are preferentially transmitted to the mature gamete pool (e.g., by inactivating sperm or pollen carrying alternate alleles).

Near isogenic lines Accessions generated by repeated backcrossing that have identical genomes except for differences at one or a few narrow genomic blocks.

Reproductive isolating barriers Biological features of organisms that impede the exchange of genes with members of other populations.

RNAi RNA interference; includes several methods by which dsRNA can be introduced to trigger the degradation or inhibit the translation of specific endogenous transcripts.

Sexual conflict A phenomenon where alleles that confer higher fitness to one sex also confer lower fitness to another sex, which may lead to antagonistic coevolution.

SNP Single nucleotide polymorphism.

***Trans*-acting variants** Polymorphisms affecting the transcription of a gene that are not located within or nearby that gene but are instead located elsewhere in the genome.

As speciation proceeds, genetic changes accumulate that restrict and eventually lead to the cessation of gene flow between populations sharing common ancestry, allowing these lineages to become increasingly evolutionarily independent. Because many questions about the nature of the molecular mechanisms and evolutionary forces that drive speciation can only be addressed with knowledge of the specific genes involved, researchers have long sought to identify the genes contributing to this process. However, these loci, dubbed 'speciation genes,' have been understandably quite difficult to identify. Genetic analysis is a formidable challenge when the organisms of interest are naturally recalcitrant to mating or their offspring cannot produce progeny of their own.

Fortunately, through advances in genetic and genomic resources, creative experimental design, and considerable brute force effort, the field of speciation genetics has made rapid progress over the past two decades (Bomblies, 2010; Moyle *et al.*, 2014; Noor and Feder, 2006; Nosil and Schluter, 2011; Orr, 2005; Presgraves, 2010; Rieseberg and Blackman, 2010; Wu and Ting, 2004). Allelic differences between lineages that cause reproductive isolation (RI) by compromising successful fertilization (pre- or postmating prezygotic RI) or the fitness of hybrid progeny (postzygotic RI) have been identified in diverse systems. Moreover, although their number is still modest, these case studies – whether considered individually or as set – have validated, deepened, and at times challenged longstanding ideas as well as promoted new directions for investigation. The aims of this entry are twofold. First, the conceptual history of the term 'speciation gene' and, following from this, empirical approaches for meeting the criteria implied by that definition will be reviewed. Second, insights into the speciation process that have emerged from the study of known speciation genes will be highlighted. Though the present focus is on genic changes that increase RI, how chromosomal rearrangements and polyploidy may also contribute is reviewed elsewhere in this volume.

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What are Speciation Genes and How are They Identified?

An Inclusive Definition

As emphasis on particular forms of RI has shifted over time, how authors have defined the term 'speciation gene' has evolved as well. Most early discussions were restricted in scope, focusing solely on genes contributing to intrinsic postzygotic isolation in sexually reproducing organisms (Orr *et al.*, 2004; Orr, 2005; Orr and Presgraves, 2000). This narrow definition was partly motivated by the argument that although prezygotic barriers or extrinsic postzygotic barriers may be important early in speciation, they may also erode due to shifts in the environment or may be insufficient to maintain divergence when allopatric populations come into secondary contact. In contrast, intrinsic hybrid inviability and sterility are generally predicted to be environment-independent and were considered essential to complete speciation (Muller, 1942). Moreover, the genes underlying intrinsic postzygotic barriers have historically been subject to more fervent attention by geneticists and in the literature due to the greater inherent mystery surrounding their identity (Coyne and Orr, 2004). Traits contributing to prezygotic isolation can be studied within species, and it was expected that the proteins that ordinarily function in the genetic networks regulating these phenotypes within species would also contribute to divergence between species. In contrast, intrinsic hybrid inviability and sterility were predominantly studied only in interspecific crosses. Consequently, beyond the prediction that alleles from each parent genome would interact epistatically (Bateson, 1909; Dobzhansky, 1936; Muller, 1942), few *a priori* expectations existed regarding the functions of genes harboring variants affecting intrinsic postzygotic barriers.

The scope of the term 'speciation gene' has since broadened to also include loci contributing to prezygotic barriers and extrinsic postzygotic barriers. As defined by Rieseberg and Blackman (2010), a "speciation gene can be strictly defined as a gene that contributes to the splitting of two lineages by reducing the amount of gene flow between them." Additional authors have advanced similar definitions (Noor and Feder, 2006; Nosil and Schluter, 2011; Wu and Ting, 2004). This more inclusive definition has emerged in part because many have recognized that the speciation process is best understood as continuum proceeding from absent to complete RI. In addition, as the genetics of both prezygotic and postzygotic barriers have received more intense study, it has become clear that postzygotic incompatibilities can diverge contemporaneously with prezygotic barriers, often segregate within species (Cutter, 2012), and may even be genetically related to prezygotic barriers through pleiotropy (Lee *et al.*, 2008).

Genetic Characterization of Speciation Genes

To satisfy the above definition, the contribution of allelic differences to a contemporary barrier phenotype must be characterized with sufficient empirical rigor to make a compelling case for causality. In addition, it has been argued that speciation genes should fulfill several evolutionary criteria that demonstrate these changes contributed to speciation historically, though assessing these requirements can be less than straightforward.

Candidate gene identification

Quests for speciation genes most commonly start with forward genetics approaches in controlled crosses (**Figure 1**). Because winnowing the genomic regions containing causal loci down to segments of tens to several hundred possible candidates requires performing quantitative trait locus (QTL) mapping on panels of related individuals segregating for variation in focal barrier phenotypes, this strategy has often meant working in systems where speciation is incipient and incomplete (**Figures 1(a)** and **1(b)**). That is, existing barriers can be somehow overcome, and F_2 or BC_1 generation progeny descended from partially or fully fertile F_1s are recoverable for at least one of the cross directions between two parental populations. Only classic model organisms, most notably *Drosophila*, where more advanced genetic toolboxes allow for deficiency and introgression mapping in F_1s, have been exceptions to this rule (**Figure 1(d)**; Orr, 2005). When feasible, QTL intervals are further narrowed by fine mapping in advanced-generation crosses (**Figure 1(c)**), by linkage disequilibrium (LD) mapping approaches that exploit historical recombination events in natural populations, or both. Investigators working in systems without sequenced genomes often supplement their mapping efforts with polymorphic markers specifically developed in candidate genes chosen *a priori* for their known functions in the gene networks governing barrier traits (e.g., Kronforst *et al.*, 2006). Even when promising associations between these targeted markers and phenotypic variation are found, fine mapping or LD mapping remain worthwhile pursuits, as they exclude any potential contributions of neighboring genes.

Fueled by advances in array- and sequencing-based methods for high-throughput genotyping, population genomic screens for speciation genes based on patterns of sequence diversity within and among natural populations have become increasingly common alternative strategies to forward genetics approaches (Noor and Feder, 2006; Seehausen *et al.*, 2014). Differentiation studies scour the genomes of incipient species pairs for regions of elevated divergence relative to polymorphism using metrics like Fst (e.g., Chapman *et al.*, 2013). Hybrid zone studies scan for SNPs that do not introgress and thus maintain much steeper allele frequency clines across space relative to genome-wide patterns of introgression (e.g., Teeter *et al.*, 2010). Although powerful, a drawback of these methods is that do not provide any immediate, concrete connection from genotypes back to specific barrier phenotypes, and establishing this link is often challenging. Moreover, positional information alone may be insufficient for assigning highly differentiated SNPs residing in noncoding regions to the genes they functionally affect. In addition, evolutionary causes other than the presence of a speciation gene may produce outliers of high differentiation, leading to false positives (Cruickshank and Hahn, 2014). Consequently, the need to follow up these studies with functional and evolutionary characterization of specific loci is compelling.

Functional characterization of speciation genes

The gold standard for validating candidate speciation genes is experimental manipulation by genetic transformation. Before

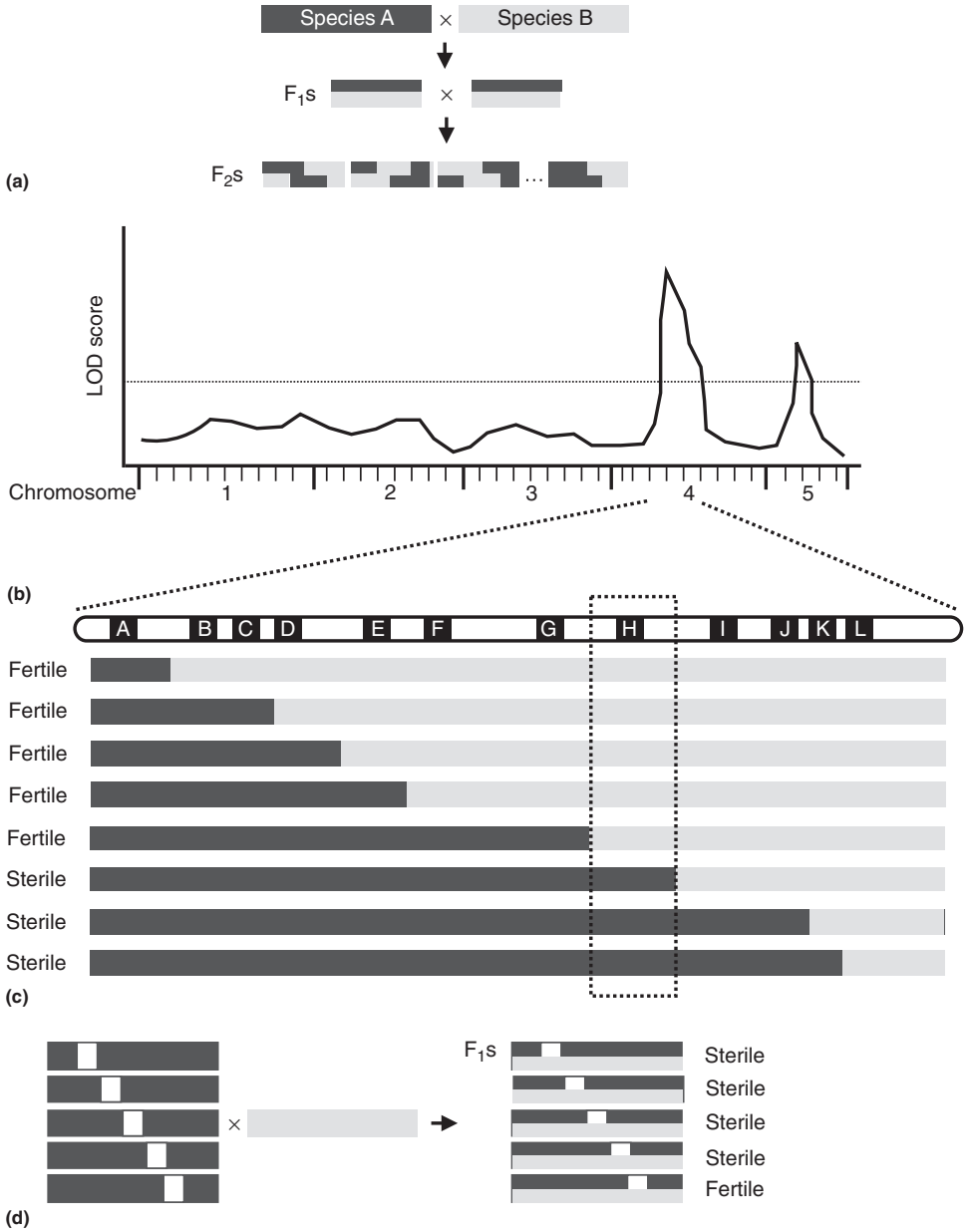

Figure 1 Identifying speciation genes by forward genetics. (a) Genetic mapping requires generating recombinant progeny, a prospect that may be impossible because reproductive isolating barriers in interspecific crosses prevent production of F_1s or leave them infertile. If RI barriers can be overcome, then the phenotypes of recombinants segregating for parental genomic segments can be obtained in the F_2 (shown) or BC_1 generation (not shown). Note that only effects detectable when comparing the heterozygous genotype to the backcross parent homozygote can be studied in a BC_1 generation while effects differentiating the heterozygote and either parental homozygote genotype can be studied in an F_2 generation. (b) Recombinant progeny are genotyped for polymorphic markers throughout the genome, and the effect of the genotype across a coarse genomic interval on segregating variation in a barrier trait is then detected by QTL mapping. The LOD score is the $\log_{10}$ likelihood ratio comparing a model with a QTL present in the interval to a model with no QTL present. (c) Finally, fine mapping may be conducted in the current panel and/or subsequent generations. The QTL interval is narrowed to a region containing one or a handful of genes by genotyping of the subset of progeny recombinant in that interval for additional markers. In the schematic, the region of chromosome 4 contained in the dashed line box is the minimal interval defined by recombination breakpoints affecting hybrid sterility, strongly implicating gene H as the causal locus. (d) In species with libraries of deletions or knockout lines, deficiency mapping can be performed for genes causing F_1 hybrid dysfunction. In the schematic, the genomic interval of Species A spanned by the rightmost deficiency segment contains a hybrid sterility allele.

proceeding with these studies, comparisons of parental coding sequences and gene expression levels in relevant tissues may provide circumstantial evidence useful for reducing the candidate gene set and bolstering support for particular genes in systems where transformation is prohibitively difficult. Allele-specific expression patterns in F_1 hybrids may be evaluated to corroborate that observed expression differences derive wholly or in part from *cis*-regulatory variation affecting the gene itself

rather than *trans*-acting variants located elsewhere in the genome (e.g., Hopkins and Rausher, 2011; Klahre *et al.*, 2011). Network-centered approaches that integrate mapping of barrier traits and gene expression (i.e., QTL and eQTL mapping, respectively) may facilitate description of complex gene interactions often characteristic of postzygotic isolation phenotypes as well (Turner *et al.*, 2014).

Allelic replacement of one species' sequence with that of another, and vice versa, by genetic transformation is the most rigorous experiment for validating a speciation gene. Such tests have historically been possible in only a few model systems, but new genome editing approaches promise to extend the capability for allelic replacement to any transformable system (Turner, 2014). Even when conversion of one species' sequence to that of another is not possible, alternative manipulative experiments may yield solid support. For instance, if crosses involving knockout mutants or individuals carrying RNAi constructs that knockdown expression of candidate gene exhibit reduced RI relative to control crosses, then the candidate gene is necessary for the isolating barrier (e.g., Presgraves *et al.*, 2003). Likewise, if introducing another species' sequence for a candidate gene causes increased RI in an intraspecific cross, then the candidate gene is sufficient for enhancing the isolating barrier (e.g., Phadnis and Orr, 2009). For genes whose protein function can be assessed biochemically, the effects of interspecific substitutions can be determined by *in vitro* assays (e.g., Wessinger and Rausher, 2015). Although not sufficiently rigorous to show causality of a specific gene since contributions of linked variation cannot be excluded, near isogenic lines carrying alternate species alleles in either background can be particularly advantageous for demonstrating a genomic region's effect on RI in field settings (e.g., Bradshaw and Schemske, 2003).

Evolutionary Characterization of Speciation Genes

Recognizing that the evolution of RI is a temporally dynamic process that begins prior to and continues after speciation, the definition advanced above also requires that the gene 'contribute to the splitting of two lineages.' Genetic changes that contribute to contemporary barriers to reproduction between fully isolated species may have accumulated long after speciation was already complete. Likewise, the relative importance of different barriers to gene flow, and consequently the impact of particular alleles on total RI, may change over time. Therefore, it has been argued that speciation genes must meet two additional evolutionary criteria (Nosil and Schluter, 2011). First, divergence at the locus must have occurred before speciation was complete. Second, the gene should have had a measurable effect on RI at the time it diverged. Obtaining empirical data addressing the first criterion can be straightforward, but demonstrating a gene meets the second criterion is potentially far more challenging.

The divergence time criterion is automatically met for genes that contribute to barriers between incipient species. For species pairs that are already completely isolated, divergence time can be assessed with gene trees or genealogies (Nosil and Schluter, 2011). If divergence at a putative speciation gene occurred prior to full cessation of gene flow, then its genealogy should be discordant with genealogies of unlinked neutral loci, which are more likely to show shallower coalescence and greater evidence of gene flow. Such tests can be problematic, however, in that they may yield false negatives depending on the evolutionary process driving divergence (Lessios, 2011). For instance, RI between species often arises as a by-product of accelerated sequence evolution driven by arms races mediated by sexual or genomic conflict within species that continue well after speciation is complete. Thus, a pattern of coalescence within species, rather than a pattern of coalescence prior to the timing of species divergence, may be expected and has been observed for some speciation genes evolving in this manner (Palumbi, 2009).

As for the second criterion, it is unclear whether a speciation gene's effect on RI relative to all other current barriers at the time of its divergence can be rigorously estimated for any species. The absolute effect of allelic divergence at a speciation gene on the strength of a contemporary RI barrier may be readily estimated by the methods discussed above. Moreover, a speciation gene's relative contribution to contemporary RI as a whole may also be estimated to the extent that the full genetic architecture for the barrier trait and the relative strength of that barrier relative to other barrier traits on RI are known. All these parameters are important because isolating barriers, to a large extent, act sequentially to prevent successful fertilization or to impair hybrid fitness, and hence it is possible that loci with major effects on later acting barriers (e.g., hybrid dysfunction) may only have minor contributions to overall RI.

By extension then, estimating a speciation gene's contribution to historical levels of RI would require not only knowledge of the temporal dynamics of evolution at a given locus (and interacting loci if RI is caused by epistatic incompatibilities) in one or both lineages, but also the past history of all other loci contributing to the cessation of gene flow. Thus, meeting this criteria may only be possible in rare systems where speciation in action can be followed over observable time scales. Alternatively, in species pairs isolated by few barriers with tractable genetics, it is conceivable that the historical series of genotypes at speciation loci could be reconstructed if the order of substitutions causing RI were inferable from genes trees or observable in ancient DNA time series. Estimates of absolute and relative contributions to contemporary RI may help determine the bounds to paths historically possible in other systems. However, given the pragmatic hurdles to determining historical dynamics (irrespective of any complexity introduced by gene × environment effects), it seems sufficient to demonstrate a speciation gene affects contemporary isolation and acknowledge the caveat that the specific contribution to total RI at the time of divergence is unknown.

What Have We Learned from Known Speciation Genes?

Beyond important basic knowledge of whether the types of genes or mutations involved follow predictable functional patterns, identifying speciation genes allows many models about the molecular and evolutionary mechanisms that drive speciation to be tested. In addition, while some questions are

addressable without knowing the underlying loci, having causal allelic sequences in hand is often a boon. For instance, patterns of intraspecific polymorphism for RI can be more efficiently assessed by sequencing-based surveys of allelic diversity than through expansive crossing schemes in most systems. Although only a modest set of speciation genes and other strong candidates is known (**Table 1**), the emerging picture supports a plurality of mechanisms while also revealing some broad patterns.

The Identities of Speciation Genes are Predictable for Some Isolating Barriers

As noted above, speciation genes for prezygotic barriers were broadly predicted to be members of networks that govern these phenotypes within species. Although the number of genes known remains insufficient to test this prediction for some barriers (e.g., temporal and mechanical isolation), identification of speciation genes affecting certain forms of RI in multiple systems has proven informative and largely confirms the expectation (**Table 1**). Pollinator isolation stands out in this regard (Yuan *et al.*, 2013a). For floral color and scent, enzymes in pigment and volatile biosynthetic pathways and components of the transcriptional complexes that regulate their expression (often R2R3-MYB transcription factors) are responsible for species differences (Byers *et al.*, 2014; Hoballah *et al.*, 2007; Hopkins and Rausher, 2011; Klahre *et al.*, 2011; Streisfeld *et al.*, 2013; Yuan *et al.*, 2013b). One particularly impressive example of convergence at the genetic level is in the genus *Penstemon*, where function-compromising variants in F3′5′H contribute to flower color shifts in 13 independent transitions from bee to hummingbird pollination (Wessinger and Rausher, 2015). The repeated observation of rapid protein evolution affecting gamete recognition systems in free-spawning marine invertebrates represents a similar case of predictable speciation gene identity (Lessios, 2011).

More surprisingly, the molecular basis of some postzygotic barrier traits in plants also appears predictable. Intra- and interspecific crosses in various groups often yield progeny exhibiting a form of hybrid inviability – hybrid necrosis – in which F_1 or F_2 hybrids show lesioning and compromised growth (Bomblies and Weigel, 2007). Across several systems, the vast majority of loci involved are immune receptors whose faulty epistatic interactions trigger errant autoimmune responses (Bomblies *et al.*, 2007; Chae *et al.*, 2014; Chen *et al.*, 2014; Krüger *et al.*, 2002; Rooney *et al.*, 2005; Todesco *et al.*, 2010). The genetic basis of cytoplasmic male sterility (CMS) barriers appears similarly predictable (Rieseberg and Blackman, 2010). Chimeric mitochondrial transcripts that cause sterility evolve as selfish elements, and the majority of nuclear fertility restorer genes identified to date are members of the pentatricopeptide repeat (PPR) gene family. Although most genetic studies of CMS have been conducted in crosses of cultivated plants to wild species, exciting work in fully wild crosses signals these results will bear out for natural populations as well (Barr and Fishman, 2010; Case and Willis, 2008).

Similar patterns of repeated involvement of common genes or gene families have not emerged for postzygotic isolation barriers in animals or fungi (Presgraves, 2010). An absence of trends here may signal that disruptions to viability or fertility in hybrids are more phenotypically idiosyncratic and taxon-specific in these groups relative to plants, where hybrid necrosis and CMS are widespread isolating barriers. Alternatively, the number of genes identified to date may be too small to reveal trends, but existing examples that implicate particular complexes of proteins regulating recombination, transposable element suppression, and chromosome segregation may prove general (**Table 1**). For instance, allelic differences in the histone methyltransferase PRDM9, a major determinant of recombination hotspots in mammals, have been shown to cause hybrid male sterility in some crosses between subspecies of *Mus musculus* (Flachs *et al.*, 2012; Mihola *et al.*, 2009). Signatures of positive selection altering the number and sequence of zinc finger domains across diverse rodent, primate, and other metazoan species raise the possibility that PRDM9 gene contributes to hybrid sterility in diverse systems (Oliver *et al.*, 2009).

Hybrid Incompatibilities Evolve by Diverse Paths and are Often Polymorphic

The Bateson–Dobzhansky–Muller (BDM) model of reproductive incompatibilities provides a simple and powerful explanatory framework for how alleles that cause hybrid inviability or sterility evolve among lineages that recently shared freely interbreeding ancestors (Bateson, 1909; Dobzhansky, 1936; Muller, 1942). In its most commonly expressed form, Lineage 1 fixes a derived allele at Locus A and Lineage 2 fixes a derived allele at Locus B, but when newly brought onto the same genomic background through hybridization, negative epistatic interactions leading to dysfunction emerge (**Figure 2(a)**). Empirical examples of such BDM incompatibilities have been described (e.g., *Lhr*/*Hmr*; Brideau *et al.*, 2006), as have additional mechanisms. For instance, recent work has described how substitutions of multiple derived alleles in a single lineage can lead to ancestral-derived incompatibilities between lineages involving two unlinked loci (e.g., Krüger *et al.*, 2002; Phadnis and Orr, 2009; Rooney *et al.*, 2005; **Figure 2(b)**) or multiple alleles of a single locus (e.g., Chae *et al.*, 2014; Todesco *et al.*, 2010; **Figure 2(c)**).

In the classic BDM model, the time required for fixation of derived alleles is assumed to be instantaneous, relative to the time spent in allopatry, for mathematical convenience (Orr, 1995; Cutter, 2012). However, numerous empirical studies involving many well-known speciation genes have found that alleles involved in BDM incompatibilities are polymorphic (e.g., Brideau *et al.*, 2006; Mihola *et al.*, 2009; Phadnis and Orr, 2009). In other words, only some strains will produce dysfunctional hybrids in interspecific crosses because the contributing alleles segregate within species. Moreover, intraspecific standing variation in BDM incompatibilities is often found (e.g., Bomblies *et al.*, 2007; Bikard *et al.*, 2009). Describing the evolutionary causes of these polymorphisms and determining whether variable reproductive isolation maintained within an ancestral lineage eventually contributes to postzygotic RI among lineages are major theoretical and empirical challenges in the field of speciation genetics (Cutter, 2012).

Table 1 Representative speciation genes and candidates

Stage	*Isolation type*	*Gene(s)*	*Species*	*Phenotype(s)*	*References*
Prezygotic, Premating	Habitat	Odorant-binding proteins *OBP57d/OBP57e*	*Drosophila sechellia/ Drosophila simulans*	Oviposition preference	Matsuo *et al.*, 2007
		Ectodysplasin (Eda)	Marine and freshwater *Gasterosteus aculeatus*	Lateral plate number and growth rate*	Colosimo *et al.*, 2005; Barrett *et al.*, 2009
	Temporal	*FLOWERING LOCUS C* (*FLC*)	*Arabidopsis suecica/ Arabidopsis arenosa,* and *Arabidopsis thaliana*	Delayed flowering of allopolyploid	Wang *et al.*, 2006
	Pollinator/ behavioral	*R2R3-* and *R3-MYB* transcription factors (e.g., *anthocyanin2* and *ROSE INTENSITY 1*)	Various *Petunia, Antirrhinum, Phlox,* and *Mimulus* species pairs	Flower color differences leading to pollinator shift	Quattrocchio *et al.*, 1999; Hoballah *et al.*, 2007; Schwinn *et al.*, 2006; Hopkins and Rausher 2011, 2012; Streisfeld *et al.*, 2013; Yuan *et al.*, 2013a, 2013b
		Flavonoid 3′,5′-hydroxylase (F3′5′H)	Various *Penstemon* species; *Phlox drummondii/Phlox cuspidata*	Flower color differences leading to pollinator shift	Hopkins and Rausher 2011, 2012; Wessinger and Rausher 2014, 2015
		Methyl-branched CHC-specific fatty acid synthase (*mFAS*)	*Drosophila serrata/ Drosophila birchii*	Cuticular hydrocarbon profile differences affect mate choice	Chung *et al.*, 2014
		ODORANT1	*Petunia axillaris/ Petunia exserta*	Floral scent differences leading to pollinator shift	Klahre *et al.*, 2011
		Stearoyl-acyl carrier protein desaturases 1 and 2 (*SAD1/SAD2)*	Various *Ophyrus* species	Floral scent differences leading to pollinator shift	Xu *et al.*, 2012
		OCIMENE SYNTHASE	*Mimulus lewisii Mimulus cardinalis*	Floral scent differences leading to pollinator shift	Byers *et al.*, 2014
		optix	Various *Heliconius* species	Wing color pattern leading to assortative mating*	Heliconius Genome Consortium, 2012; Reed *et al.*, 2011
Prezygotic, Postmating	Gametic	*ui1.1* (Cullin 1)*; ui6.1* (S-Locus F-box)	Various *Solanum* species	Unilateral incompatibility accompanying mating system shift	Li and Chetelat, 2010, 2015
		Bindin	Various sea urchin genera	Species-specific fertilization	Palumbi 2009; Lessios 2011
		Lysin	*Haliotis rufescens/ Haliotis corrugata*	Species-specific fertilization	Palumbi 2009; Lessios 2011
		Cysteine-rice peptide *LURE1*	*Torenia concolor/ Torenia fournieri; A. thaliana/ Arabidopsis lyrata*	Species-specific chemoattraction of pollen tubes	Kanaoka *et al.*, 2011; Takeuchi and Higashiyama, 2012
Postzygotic, Extrinsic	Ecological	*optix*	Various *Heliconius* species	Wing color pattern intermediates suffer greater predation*	Heliconius Genome Consortium 2012; Reed *et al.*, 2011
	Pollinator/ behavioral	*R2R3-MYB* transcription factor	*Phlox cuspidate/ Phlox drummondii*	Flower color intermediates less attractive to pollinators	Hopkins and Rausher, 2011, 2012

(*Continued*)

Table 1 Continued

Stage	*Isolation type*	*Gene(s)*	*Species*	*Phenotype(s)*	*References*
Postzygotic, Intrinsic	Hybrid Inviability	Various NLR immune receptors (e.g., *DANGEROUS MIX 1*)	*A. thaliana* (intraspecific); *Oryza rufipogon/ Oryza sativa*; *Solanum lycopersicum/ Solanum pimpinellifolium*	Hybrid necrosis	Krüger *et al.*, 2002; Bomblies *et al.*, 2007; Todesco *et al.*, 2010; Chae *et al.*, 2014; Chen *et al.*, 2014
		HISTIDINOL-PHOSPHATE AMINO-TRANSFERASES 1 and 2 (*HPA1/HAP2*)	*A. thaliana* (intraspecific)	Arrest of seed development	Bikard *et al.*, 2009
		Nucleoporins Nup96 and Nup160	*D. melanogaster/ D. simulans*	Hybrid lethality	Presgraves *et al.*, 2003; Tang and Presgraves, 2009
		Lethal hybrid rescue (Lhr)/ Hybrid male rescue (Hmr)	*D. melanogaster/ D. simulans*	Hybrid lethality	Barbash *et al.*, 2003; Brideau *et al.*, 2006; Thomae *et al.*, 2013; Satyaki *et al.*, 2014
	Hybrid Sterility	*Odysseus (Ods)*	*D. simulans/ D. mauritiana*	Hybrid male sterility	Ting *et al.*, 1998; Sun *et al.*, 2004; Bayes and Malik, 2009
		JYAlpha	*D. melanogaster/ D. simulans*	Hybrid male sterility	Masly *et al.*, 2006
		PR domain-containing 9 (PRDM9)	*Mus musculus musculus/Mus musculus domesticus*	Hybrid male sterility	Mihola *et al.*, 2009
		Overdrive	*Drosophila pseudoobscura pseudoobscura/ Drosophila pseudoobscura bogotana*	Hybrid male sterility	Phadnis and Orr, 2009

Genes were selected to illustrate the breadth of isolation barriers for which speciation genes or strong candidates have been characterized, and the table is not intended to be comprehensive. Although compelling evidence linking genotype to phenotype has been provided in all cases, the direct impact of allelic variation on RI may not be fully demonstrated for some candidates (asterisks (*)). That is, an impact on the prevention of fertilization or hybrid dysfunction is assumed based on the phenotypes changed by these alleles but reproductive isolation has not been explicitly examined following allelic replacement by transformation or introgression for these candidates.

Diverse Evolutionary Models Supported

Because many evolutionary models in the field of speciation genetics are grounded in specific molecular mechanisms, identifying speciation genes is essential to test their biological merit. In addition, knowing key sequences facilitates the application of population genetic tests to determine whether drift, selection, and/or migration have been the predominant forces driving the evolution of RI. The speciation genes identified to date lend support to diverse processes.

Ecological speciation

A classic model of speciation – ecological speciation – postulates that RI evolves as a by-product of ecologically based divergent natural selection (Schluter and Conte, 2009). In other words, the mechanism by which individual populations differentially adapt to local selection pressures is genetically linked to the evolution of RI between populations. Pleiotropy, tight physical linkage, capture by a chromosomal inversion, or one-allele assortative mating all represent alternative mechanisms by which recombination between the loci contributing to locally adaptive phenotypes and the loci causing RI may be sufficiently frustrated to allow the joint evolution of both traits (Nosil, 2012).

Speciation genes provide ample empirical evidence that supports this model for prezygotic or extrinsic postzygotic barriers. For instance, *cis*-regulatory changes that eliminate expression of mFAS, a fatty acid synthase responsible for methyl-branched cuticular hydrocarbon production, in the humid habitat specialist *Drosophila birchii* relative to the habitat generalist *Drosophila serrata* are pleiotropic (Chung *et al.*, 2014; Chung and Carroll, 2015). They alter both desiccation sensitivity and mate choice. Likewise, allelic differences that specialize plants to different pollinators or to alternative mating systems necessarily also reduce gene flow between plant species (e.g., Hoballah *et al.*, 2007; Byers *et al.*, 2014; Li and Chetelat, 2015). In *Heliconius* butterflies, wing color patterns are essential for both predation avoidance through mimicry and mate choice (Jiggins *et al.*, 2001). Thus, genetic differences that have evolved in response to divergent selection favoring

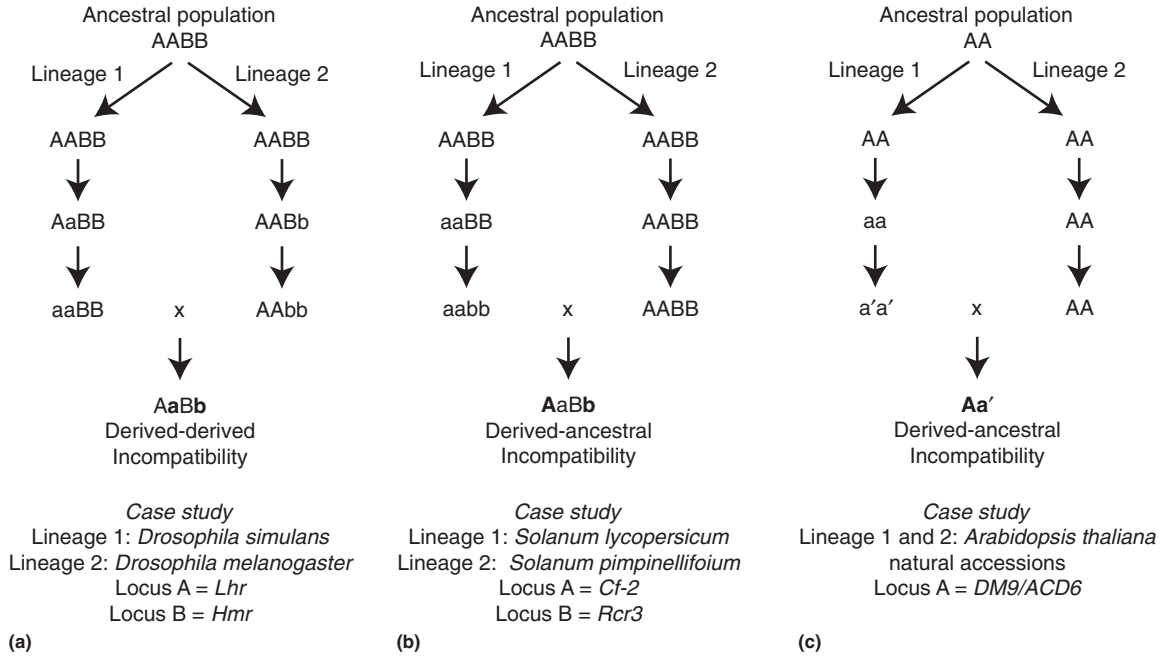

Figure 2 BDM incompatibilities evolve by multiple evolutionary mechanisms. (a) Classic BDM model where incompatible derived substitutions fix at alternative loci in each lineage, as observed for F_1 hybrid male lethality in crosses of two *Drosophila* species (Brideau *et al.*, 2006). (b) Substitution of a derived allele at Locus A permits the evolution of a second derived allele at Locus B in the same lineage, leading to incompatibility with the ancestral allele at Locus A. In the case study, hybrid necrosis arises in 3/16 of F_2 progeny of crosses between wild tomato species, because bb homozygotes are incompatible with AA or Aa genotypes (Rooney *et al.*, 2005). (c) A succession of substitutions at a single locus leads to the evolution of an incompatibility between a derived and ancestral allele. Some loci involved in incompatibilities that cause hybrid necrosis in intraspecific crosses among *Arabidopsis thaliana* accessions likely evolved by this mechanism (Chae *et al.*, 2014; Todesco *et al.*, 2010). Incompatible alleles are highlighted in bold.

alternative mimicry patterns (Kronforst *et al.*, 2006; Kronforst and Papa, 2015) may lead to behavioral isolation between color pattern races as well as extrinsic postzygotic isolation since hybrids with intermediate, non-mimicking color patterns are more susceptible to predation.

Theoretical models and experimental evolution studies both provide strong evidence that intrinsic postzygotic RI can evolve through ecological speciation as well (Dettman *et al.*, 2007; Gavrilets, 2004; Schluter and Conte, 2009). Although several speciation genes exhibit evolutionary patterns consistent with this process, their histories may also be consistent with other models or their contribution to interspecific barriers remains to be fully affirmed (Chae *et al.*, 2014; Lee *et al.*, 2008).

Evolutionary arms races, mutation pressure, and hybrid incompatibilities

Although multiple processes may drive the fixation of alleles within lineages that cause postzygotic incompatibilities between lineages, incompatibilities are predicted to arise more rapidly if selection is involved. Consistent with this expectation, many speciation genes known to be involved in BDM incompatibilities appear to rapidly evolve within lineages due to ongoing evolutionary arms races. These patterns of substitution and counter-substitution seem to be largely driven by mutation pressure and selection to resolve genomic conflicts rather than ecological pressures (Presgraves, 2010). Within lineages, selfish genetic elements may parasitize host genomes through elevated transposition rates or biasing transmission in their favor through meiotic drive and gamete-killing segregation distortion. Host genomes respond to these strong selection pressures by evolving mechanisms that suppress these activities or compensate for their deleterious effects. If parasitic elements are separated from their corresponding suppressors or if different host genome compensatory mechanisms are incompatible in hybrid genetic backgrounds, dysfunction may manifest.

For instance, the speciation genes *Lhr* and *Hmr* interact as part of a protein complex that represses satellite DNA and transposable elements (TEs), and epistatic interactions between *Lhr* and *Hmr* in *Drosophila melanogaster* × *Drosophila simulans* hybrids lead to mis-expression of TEs and consequently hybrid lethality (Brideau *et al.*, 2006; Satyaki *et al.*, 2014; Thomae *et al.*, 2013). Rapid coevolution of repetitive DNA regions and the proteins that regulate their segregation during meiosis and mitosis, and other systems that compensate for mechanisms that distort chromosomal segregation patterns, have also been implicated in intrinsic postzygotic barriers (Bayes and Malik, 2009; Ferree and Barbash, 2009; Fishman and Saunders, 2008; Phadnis and Orr, 2009), providing support for a prescient mechanistic model (Henikoff *et al.*, 2001). The evolution of CMS and restorer genes reflects a similar case of postzygotic RI that evolves due to genomic conflict within lineages (Rieseberg and Blackman, 2010). The speciation genes involved in these types of arms races – as well as host–pathogen systems related to hybrid necrosis – exhibit high levels of coding sequence divergence, often in tandem

with copy number evolution. Whether the cumulative effects of all substitutions are necessary for BDM incompatibilities, or whether substitutions that cause incompatibilities are rare and only these types of genes evolve rapidly enough to hit upon them, remains an open question.

Relaxation of purifying selection, particularly following gene or genome duplication, may also accelerate rates of substitution through genetic drift. In the case of gene or genome duplication, if resolution of functional redundancy among recent duplicates occurs by gene silencing or subfunctionalization within populations such that different descendant lineages maintain function in separate map locations, postzygotic RI may also result (Lynch and Force, 2000; Werth and Windham, 1991). In this scenario, F_1 gametophytes or F_2 generation hybrids may inherit a chromosome set that lacks either a full complement of ancestral functions or any functional copy period. Examples of speciation genes consistent with this form of RI arising from gene movement driven by passive mutational silencing of gene duplicates have been observed in intraspecific crosses in *Arabidopsis* and interspecific crosses among rice and *Drosophila* species (Bikard *et al.*, 2009; Masly *et al.*, 2006; Yamagata *et al.*, 2010).

Hybridization as a driver of speciation

Reinforcement, allopolyploid speciation, and homoploid hybrid speciation are documented ways that hybridization may foster speciation. Speciation gene sequences have revealed an additional, perhaps surprising way that hybridization may actually promote the cessation of gene flow. In several cases, gene flow has facilitated the spread of alleles contributing to the evolution of RI between lineages. For instance, *optix* sequences involved in wing pattern divergence have been passed among *Heliconius* species and repeatedly reused in the independent evolution of mimetic races (Heliconius Genome Consortium, 2012; Reed *et al.*, 2011). In the threespine stickleback, the *Eda* allele that confers adaptation to freshwater through loss of lateral plates, and that may contribute to behavioral isolation between marine and freshwater populations due to effects on growth rate in a species that assortatively mates based on size, was likely transported to many freshwater populations worldwide through the marine gene pool (Barrett *et al.*, 2009; Colosimo *et al.*, 2005; McKinnon *et al.*, 2004; Schluter and Conte, 2009). It remains to be determined whether alleles that initiate or resolve genomic conflicts similarly introgress among species and, if so, whether gene flow of such alleles would facilitate or impede the speciation process.

See also: Ecological Speciation and Its Consequences. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Speciation Continuum. Speciation Genomics

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Speciation Genomics

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Glossary

Adaptive radiation Process in which organisms rapidly diversify into a plethora of new forms, particularly when a change in the environment makes new resources available, creates new challenges, and opens yet-environmental niches.

Allopatry Geographical separation enabling genetic divergence of populations and taxa in isolation.

Assembly, genome assembly Computational reconstruction of the genome sequence; typically involves collapsing reads into contigs, joining contigs into scaffolds, and linking scaffolds into chromosomes.

Bioinformatics Interdisciplinary field that develops methods and software tools for understanding biological sequence data.

de Bruijn graph assemblers Software programs for genome assembly based on a set of a number of symbols and dimensions. For genomics applications, the set of symbols consists of four characters ('A,' 'C,' 'T,' 'G') and dimension is equivalent to the chosen k-mer size.

Comparative genomics Branch of genomics in which genome features of different organisms are compared.

Contigs, contiguous sequence Genomic fragment generated by a genome assembler, based on overlapping reads.

Dobzhansky–Muller incompatibilities Genetic incompatibilities that evolve between populations or closely related species without going through an adaptive valley. It requires changes in two or more loci, where a new allele arises at one locus in one population, but is incompatible with another new allele that evolved independently in the second population. When combined in hybrids, these incompatibilities cause reduced fitness.

Greedy assemblers Software programs for genome assembly based on greedy algorithms, i.e., those finding the shortest common supersequence for a set of sequence fragments.

Linkage disequilibrium (LD) The nonrandom association of alleles at two or more loci. Such associations are facilitated by the physical proximity of genes on a chromosome (linkage) and/or by strong interactions of alleles with one another that affect the fitness of an organism more strongly than either allele alone (epistasis). Strong epistasis can cause LD between genes without them being closely physically linked on a chromosome.

Next-generation sequencing (NGS) High-throughput (massively parallel) methods of DNA sequencing, provided by such technologies as Illumina, 454, and Pacific Biosciences, enabling whole genome analyses.

Reproductive isolation Barriers to gene exchange between populations or species.

Secondary contact Recent geographic overlap of two or more divergent evolutionary lineages (i.e., populations or species) after a period of geographic isolation, which allowed for some degree of differentiation to occur from a common ancestor.

Smith–Waterman algorithm Local sequence alignment, in which similar regions between two strings or nucleotide or protein sequences are computationally determined.

Overview: Speciation Genomics – Toward the Periodic Table of Speciation

In the process of writing his famous book, *The Principles of Chemistry*, Dmitri I. Mendeleev (Mendeleyev and Lawson, 1891) created the Periodic Table of Elements that listed the majority of then-known elements according to increasing order of atomic weights. When he organized the table into horizontal rows, a system of elements became apparent – but only if he left blanks in the table. With the empty classes included, later to be filled with newly discovered elements, a distinct pattern of elements with similar physical and chemical properties emerged at regular intervals – periodically – in vertical columns of the table. It turned out that the properties of elements were accounted for, or at least correlated with, a single organizing variable, namely the positive charge of the nucleus, which in Mendeleev's times could be only approximated by atomic weight.

Compared with the Periodic Table, biological classification systems, including taxonomy, fall short. The human genome project and the origin of genomics, leading to the plethora of complete or near-complete genome sequences for multiple organisms across the tree of life were believed by many to hold promise of a new organizational understanding. To uncover new guiding principles, a systems approach known as systems biology, lying at the interface of genetics, genomics, and bioinformatics, was created. Systems biology has been less appealing to evolutionary biologists who were skeptical that categorizing and organizing principles other than evolutionary process itself can be informative or even feasible. Indeed, the most common and natural reason for hierarchical organization in biological systems is evolution by descent with divergence from the common ancestor.

As exemplified by the Periodic Table invention, when we examine a large amount of data, what we see depends a lot not only on what we want to see, but also on how we organize the data. Similarly, when we analyze multiple genomes, which are in the realm of comparative genomics, our perspective and novel ways of visualization determine what we will notice. One of the uncharted areas of comparative genomics is

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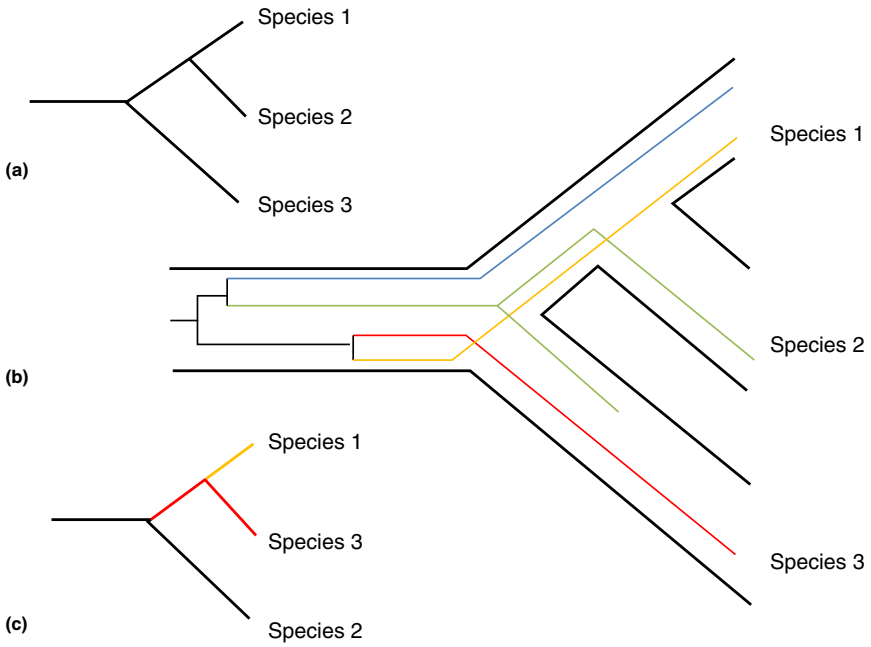


Figure 1 Speciation and incomplete lineage sorting that leads to gene genealogies incongruent with the species phylogeny (a). As the common ancestor diverges into Species 1, 2, and 3, not all ancestral alleles will end up in founding populations of the new species (b). For example, the most recent common ancestor of Species 1 and 2 has by chance lost the red allele, while the founding population of Species 3 did not have blue and orange alleles and then lost the green allele as well. Phylogenies constructed on the basis of the present day allele similarities between the three modern species will thus produce different species histories, some of which will be incongruent with the species phylogeny (a), dependent on which gene lineage we look at. For example, the orange allele in Species 1 is most related to the red allele in Species 3, so according to this allele Species 1 and 3 will be clustered together as most closely related (c).

inference of speciation, or the origin of new species, "the mystery of mysteries" as Darwin called it (Darwin, 1859). Speciation occurs as genetically-based barriers to gene flow evolve and cause previously interbreeding populations to become reproductively isolated from one another (Coyne and Orr, 2004). Reproductive isolation, either pre- or postzygotic, and its genetic determinants are thus central to describing and understanding speciation, at least in its formulation consistent with the prevalent Biological Species Concept (de Queiroz, 2005; Seehausen *et al.*, 2014). Most, if not all, discoveries in the field of speciation genetics, such as the identification of genes responsible for hybrid incompatibilities (Orr *et al.*, 2004; Presgraves, 2010; Rieseberg and Blackman, 2010), involved traditional experimentation, genetic mapping, and single candidate gene approaches, which necessarily limited the research to a handful of experimentally tractable organisms. On the other hand, comparative genetics for a long time was focused on model organisms and phylogenetically distant, 'flashy' representatives of major taxonomic groups, thus failing to provide a finer evolutionary timescale necessary for addressing questions related to speciation.

As next-generation sequencing (NGS) technologies are becoming increasingly available at a lower cost, comparative genomics has recently expanded to multigenome analysis of divergence between closely related species, as well as polymorphism within populations, laying the foundations for, respectively, phylogenomics and population genomics. 'Speciation genomics' is budding at the interface between these two areas of research (as well as functional genomics), but novel ways of thinking, data analysis, and visualization will be required to surface before this new field flourishes (Seehausen *et al.*, 2014). Reconstructing the sequence of speciation events and identifying speciation-driving genes in closely related and potentially hybridizing species is extremely challenging due to such processes as incomplete lineage sorting and interspecific gene flow. These processes result in local gene genealogies that differ in their topology from the species tree, and analyses of different loci with a single sequence per species are likely to produce conflicting or even misleading results (**Figure 1**). Moreover, if hybridization is involved in speciation and adaptive radiation, and we do keep finding out more and more that hybridization is a widespread phenomenon producing evolutionary novelties (e.g., Heliconius Genome, 2012; Martin *et al.*, 2013; Lamichhaney *et al.*, 2015), a simple tree is no longer an ideal representation of evolution, and more complex graphs or multidimensional tables will need to be explored instead. Most sequence divergence between species that we observe today has accumulated either before or after a speciation event, and even if it happened precisely at the tree bifurcation moment (node), it can still be coincidental rather than causative with respect to isolating mechanisms or local adaptations. Conversely, especially in species that diverged in separation (allopatry), some genes that differentiated after a speciation event are predicted to be a part of negative epistatic interactions known as Dobzhansky–Muller (hybrid) incompatibilities, essential for postzygotic reproductive isolation upon secondary contact and reinforcement of sexual isolation (Coyne and Orr, 2004). Also in the case of sympatric

speciation due to host shifting and ecological divergence, genetic variation responsible for new local adaptations that accumulated in populations after parting of their way may be considered a causative speciation factor. Therefore, it is critical for speciation genomics to employ gene-function annotation tools, genetic interactions information (pathways and networks), as well as population genetics tools, such as McDonald–Kreitman tests (Mcdonald and Kreitman, 1991; Messer and Petrov, 2013) and Pool-hmm (Boitard *et al.*, 2013) for dissecting adaptive sequence changes from neutral changes. The following sections will give a brief introduction to available methods and present two case studies that exemplify how advances of comparative genomics and phylogenomics provide new insights into speciation.

How are Whole Genomes Analyzed?

The ultimate goal of genome projects is to provide an organism's complete genomic sequence information but 100% completeness is still barely achievable in organisms other than microbes. The first genomes were sequenced with the classical Sanger (chain termination) method, employing its capillary-based, semi-automated implementations, while new genome projects rely more and more on the next generation, massively parallel sequencing technologies, primarily Illumina which has largely dominated today's market. Since most sequencing methods produce relatively short strands of 100–1000 base pairs, longer chromosomal sequences are partitioned into smaller fragments that can be sequenced separately, and subsequently reassembled to provide the original genomic sequence. The assembly process is one of the most challenging steps in *de novo* genome sequencing projects and involves either a laborious process of 'chromosome walking' that progresses through the entire strand piece by piece or faster but computationally demanding 'shotgun sequencing' that randomly breaks up DNA into numerous short segments, or a combination of both. Resequencing genome projects are easier, once a genome scaffold exists and can be used as a reference for mapping new genomes from the same species. Shotgun sequencing is largely dependent on redundant coverage, in which on average every nucleotide is sequenced many times over, as required to produce a high-quality assembly with a minimum number of gaps. Another benefit of redundancy is greatly increased accuracy in terms of the sequencing error control: Where a single read has an error rate of 1%, eightfold coverage produces an error rate as low as 10^{-16} when eight high-quality reads agree with one another (Schatz *et al.*, 2010). High coverage is also required for sequencing polymorphic sites within diploid or polyploid genomes.

A major challenge in the assembly process, even under high sequence coverage, is to reconstruct repetitive sequences, such as mononucleotide stretches, microsatellites, minisatellites, transposable elements, and clusters of tRNAs and rRNAs, since these sequences are very abundant in eukaryotic genomes, and tend to be longer than the read length. The best solution to resolving repetitive sequences is use of sequencing technologies (e.g., Pacific Biosciences) that generate long reads, the longer the better. Since NGS long-read solutions are not cost-effective, many genome projects have employed hybrid approaches combining long reads, mate-pair information, and high volumes of shorter reads (Illumina). Early genome assemblers were based on a simple 'greedy' algorithm, in which all pairs of reads are compared with each other, and the ones that overlap most are merged first. To allow for sequencing errors, assemblers generate these overlaps with a variant of the Smith–Waterman algorithm (Smith and Waterman, 1981) that allows for a small number of differences (1–10%) in the overlapping sequence. Once all overlaps are recovered, the reads with the longest overlap are concatenated to form a contiguous sequence (contig). The process then cycles, each time merging the sequences with the longest overlap until all overlaps are used (Schatz *et al.*, 2010). While greedy assemblers perform well with long reads and compact genomes, they fail to process shorter reads, especially from genomes enriched in repetitive sequences. To address these and other challenges of NGS, new assemblers replace the overlap algorithm with a de Bruijn graph, in which the reads are decomposed into k-mers becoming the graph nodes, edges between nodes are drawn, and nonbranching paths are translated into unambiguous contigs.

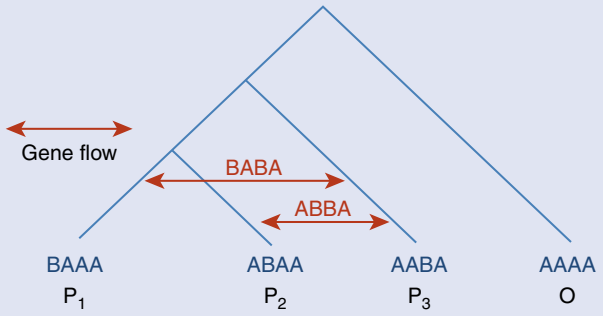
Although generating draft assemblies from NGS is relatively easy, finishing a genome to the chromosome level is still difficult and costly. Post-assembly genome processing involves quality assessment, gene prediction and annotation, as well as finishing steps, such as closing gaps through targeted Sanger sequencing, and integrating sequence information with genetic mapping, restriction maps or *in situ* hybridization results. Comparative genomics then establishes the level of homology between two or more genomes and expands formerly gene-oriented methods to a new genome-wide perspective. However, comparative genomics in the era of complete genomes is not only 'more of the same.' The entire genome level provides unprecedented opportunity to develop new methods, models, and statistical tests, such as the four-taxon ABBA/BABA test for introgression across genomes (**Box 1**), originally developed for estimating gene admixture between modern human and Neanderthal populations from single nucleotide polymorphism patterns (Green *et al.*, 2010; Sousa and Hey, 2013). The discovery of Neanderthal and Denisovan admixture with modern humans through ancient interbreeding (Reich *et al.*, 2011; Skoglund and Jakobsson, 2011) has been one of the most unexpected findings of comparative genomics and human genetics lately ($\sim$2.7% of the author's genome is estimated to derive from Neanderthals).

Genome-Wide Evidence for Speciation with Gene Flow in *Heliconius* Butterflies

The closely related neotropical butterfly species *Heliconius melpomene*, *Heliconius cydno*, and *Heliconius timareta* are distasteful to predators and often exhibit Müllerian mimicry to more distantly related species (Martin *et al.*, 2013). All three species include various wing pattern forms, or races, which can be viewed as incipient stages of speciation, as it seems that selection for Müllerian mimicry can lead to wing pattern divergence and color-based assortative mating with no geographic separation (Chamberlain *et al.*, 2009). *Heliconius cydno* and *H. timareta* together form a clade that is sister to

Box 1 The ABBA/BABA introgression test

Whole genome sequence information from closely related taxa may be used for identification and estimation of recent gene admixtures between populations or species. One of the methods, known as the four-taxon ABBA/BABA test, relies on comparing the taxa tree (assumed to be known) with the gene trees inferred at a specific site. Incongruent patterns between the population tree and the gene tree can be due to unequal mutation rates (rather unlikely), incomplete lineage sorting (shared ancestral polymorphism that by chance does not make it to all considered taxa) or to gene flow (introgressive hybridization). A certain statistic, called '*D*,' was specifically designed to detect introgressions from one population to another (Durand *et al.*, 2011; Sousa and Hey, 2013). Obtaining *D* estimates requires genome information from each of two sister populations (P_1 and P_2), a genome from a third population (P_3, a potential source of introgressed genes) and a fourth outgroup genome (O) to identify the ancestral state (identified as the A allele) and the derived state (B allele). If alleles at a specific site are scored in the order of P_1, P_2, P_3, and O, there are two phylogeny-incongruent allele patterns to be found: ABBA and BABA, with ABBA potentially originating from hybridization between P_2 and P_3, and BABA from hybridization between P_1 and P_3. Under the hypothesis of shared ancestral polymorphism, the number of tree topologies of ABBA and BABA are expected to be equal, and the expected *D* will be zero, which can be rigorously tested.



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H. melpomene, but unlike *H. cydno*, several *H. timareta* races have *H. melpomene*-like patterns (**Figure 2**). Martin *et al.* (2013) provided evidence that 20–40% of the genome in *H. melpomene* shows a discordant phylogenetic pattern, consistent with admixture with *H. cydno* or *H. timareta* in sympatry. Estimates of admixture by means of the ABBA/BABA method increased with time period examined, implying continued gene flow during speciation as opposed to a recent burst. Additionally, linkage disequilibrium (LD) was strongest between derived alleles that were shared during the recent time period, indicating the existence of introgressed haplotype blocks that are yet to be broken down fully by recombination. Remarkably, blocks of shared sequence variation containing the *B*/*D* region, known to be responsible for red mimicry patterns between races of *H. melpomene*, were exchanged between postman *H. timareta* and postman *H. melpomene* (Heliconius Genome, 2012). Interspecific F_{ST} between sympatric species tended to be lower and more variable than between the corresponding allopatric populations, as expected under a model of admixture with variable selection against introgressing alleles. There was a significantly reduced signature of admixture on the Z chromosome compared with autosomes, which can be associated with Z-autosome incompatibilities known to cause female hybrid sterility in the system (Jiggins *et al.*, 2001; Naisbit *et al.*, 2003). Genomics thus provides empirical data for addressing the ongoing debate between recent proponents of sympatric speciation and the classical view of ubiquitous allopatric speciation.

The Genomic Landscape of Phenotypic Integrity in the Face of Gene Flow in European Crows

Carrion crows (*Corvus* [*corone*] *corone*) and hooded crows (*Corvus* [*corone*] *cornix*) are two European corvid [sub-]species with very distinct coloration (**Figure 3**) which fuels assortative mating (Poelstra *et al.*, 2014). The geographical distribution pattern of these species suggests a population history shaped by glaciation cycles during the Pleistocene, and is characterized by well-defined, narrow hybrid zones in the areas where the ranges meet, mostly across central Europe and Siberia. Extensive gene flow between hooded crows and the German carrion crow population was detected using whole-genome analyses, consistent with a scenario of admixture upon secondary contact (Poelstra *et al.*, 2014). All populations but Spanish showed signatures of expansion and had higher nucleotide diversity than that of the assumed refugial Spanish population.

A 1.95-Mb genomic region containing extreme genetic differentiation between carrion and hooded crows was mapped to a single chromosome. This region, potentially

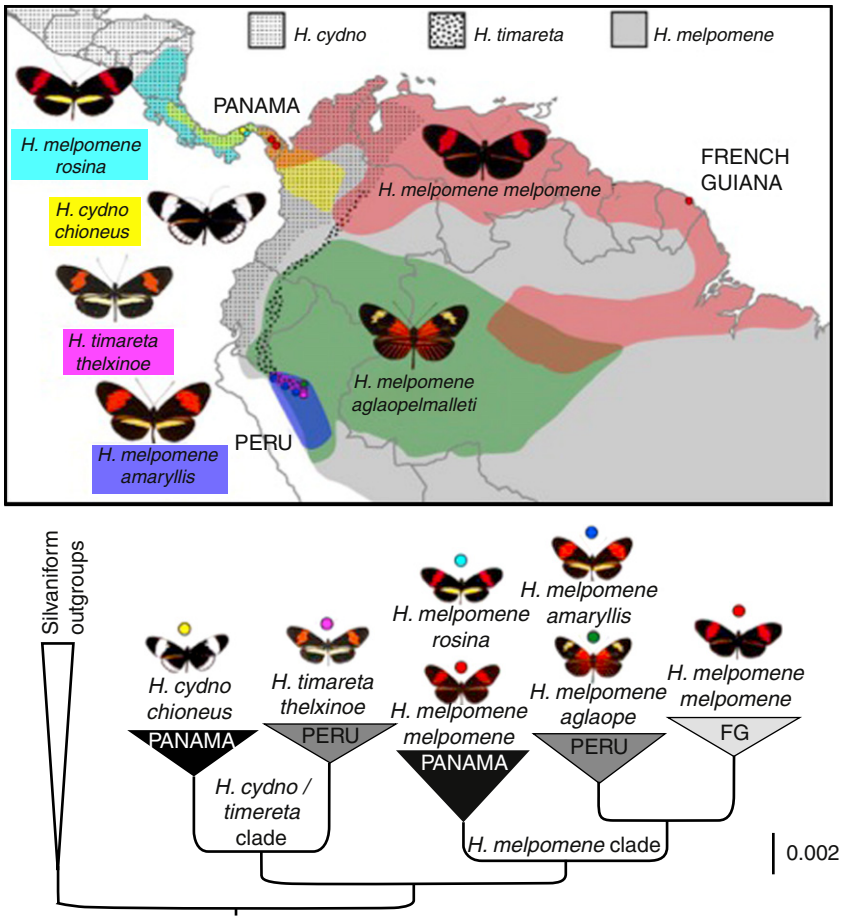

Figure 2 Distributions and phylogeny of *Heliconius* species and races. The phylogeny is a compressed version of the whole-genome maximum likelihood tree. Reproduced from Martin, S.H., Dasmahapatra, K.K., Nadeau, N.J., *et al.*, 2013. Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. Genome Research 23 (11), 1817–1828, with permission from Cold Spring Harbor Laboratory Press.

associated with an inversion and harboring 81 of all 82 fixed sites between carrion and hooded crows, including 40 annotated protein-coding genes, was characterized by significantly reduced levels of nucleotide diversity and increased LD. A ~4.1 kb section of the region, associated with a local F_{ST} peak, had longer haplotype blocks and provided evidence for recent, hooded crow-specific, positive selection. This region turns out to contain a tandem array of voltage-dependent calcium channel subunits, including those encoded by members of the CACNG gene family responsible for transmembrane regulators of AMPA receptors, linked to the microphthalmia-associated transcription factor gene MITF, a central regulatory element of the melanogenesis pathway. The group (Poelstra *et al.*, 2014) also conducted an extensive gene expression profiling and found that at least 11 of the 21 melanogenesis genes underexpressed in gray hooded crow feather follicles were directly regulated by MITF (e.g., TYR, TYRP1, SLC45A2, RAB38, EDNRB2) or interacted with MITF in feedback loops (MC1R, HPGDS). This tentatively links gene expression, color phenotype, and a signature of divergent selection within a single divergence peak.

Another highly divergent 'island' within the region contained a hooded-derived fixed variant in the regulatory region of RGS9 (*regulator of G protein signaling 9*), a highly expressed gene in eyes (with lower expression in hooded crows), known to affect visual perception in vertebrates, as well as influence dopamine and opioid signaling in the brain (Martemyanov and Arshavsky, 2009). This finding is of special interest because it may point to a pleiotropy underlying color-based assortative mate choice promoting speciation in the system.

Conclusion

Thanks to the new methodological developments in genomics and bioinformatics the whole-genome perspective is a potential 'game-changer' to speciation studies. The field of speciation genetics may finally progress from approximations with model systems, such as hybrid inviability in crosses between relatively distant *Drosophila melanogaster* and *Drosophila simulans* (e.g., Barbash and Ashburner, 2003; Brideau *et al.*, 2006), to natural systems undergoing incipient speciation and adaptive radiation. The pattern that emerges from these and similar case studies is that gene flow between taxa is a pervasive feature of speciation and post-speciation processes, even including our own species, as well as island systems that so

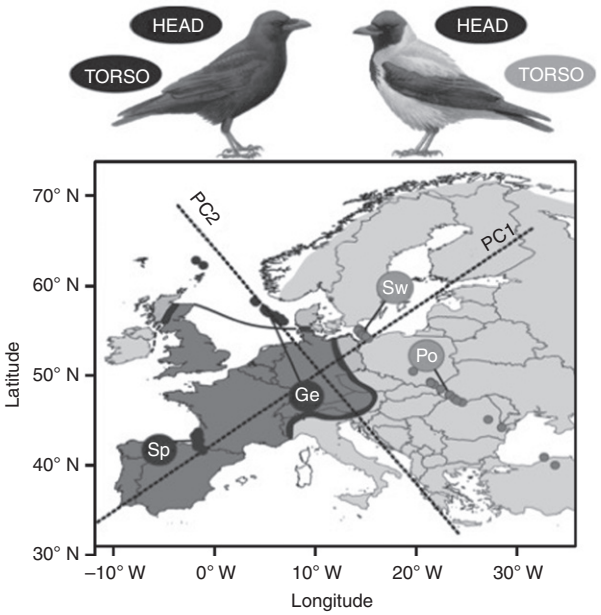

Figure 3 The European distribution of the carrion crow (dark gray area) and hooded crow (light gray area); the hybrid zone is shown as a black solid line. Reproduced from Poelstra, J.W., Vijay, N., Bossu, C.M., *et al.*, 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science 344 (6190), 1410–1414, with permission from AAAS.

far have been used as textbook examples of allopatric speciation, such as Darwin finches (Grant and Grant, 2014; Lamichhaney *et al.*, 2015). Genomic signatures of speciation are being detected as local peaks of divergence (often called 'speciation islands' (Turner *et al.*, 2005; Feder *et al.*, 2012)) that exhibit resistance to gene flow, in addition to other sequence, structural, and functional features, including eroded LD, footprints of selective sweeps, enrichment in inversions, and mate choice- or reproduction-related genes.

See also: Ecological Speciation and Its Consequences. Genetic Variation in Populations. Reproductive Isolation, Prezygotic. Speciation Continuum. Speciation-with-Gene-Flow. Species Concepts and Speciation

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http://en.wikipedia.org/wiki/Speciation

Speciation, Geography of

JM Sobel, Binghamton University (State University of New York), Binghamton, NY, USA

Introduction

> Is not the biological laboratory which leaves out the ocean and the mountains and meadows a monstrous absurdity? Was not the greatest scientific generalization of your times reached independently by two men who were eminent in their familiarity with living beings in their homes? Brooks (1899, p. 41).

In all of evolutionary biology, few subjects have instigated such contention as the geography of speciation. It is well established that geographic isolation can facilitate the speciation process by removing the constraints on divergence imposed by gene flow (Mayr, 1942, 1963; Coyne and Orr, 2004). However, a long history of debate surrounds whether geographic separation is indeed required for species formation. It is now clear that divergence can be achieved despite substantial gene flow, resulting in highly discontinuous taxa in nature (e.g., Michel *et al.*, 2010). Arguments have thus shifted to the nature of the frequency distribution of speciation events across a spectrum of potential rates of gene flow. Some authors maintain that the vast majority of speciation events in nature occur without gene flow (Coyne and Orr, 2004), while others are convinced that speciation with gene flow may be quite common (Via, 2001; Nosi, 2008). Unfortunately, aspects of this debate are bound to continue for several reasons. First, for any given pair of closely related sympatric species, it is extremely difficult to determine whether a period of allopatry preceded current contact (Pinho and Hey, 2010). Second, in cases where highly differentiated, yet incompletely isolated taxa occur in sympatry, we do not know which will continue along the path toward becoming full species. These difficulties have spurred some to suggest that geographic context is not a useful categorization of speciation events (Kirkpatrick and Ravigne, 2002). However, one only needs to consider how vastly different the biodiversity of our planet could be if the 'oceans and the mountains and meadows' were arranged differently to appreciate the importance of geography to speciation. Therefore, regardless of how we classify each event, the topic of geography will undoubtedly continue to be vital in our understanding of the origin of species.

History

From Darwin to Jordan

Given the famous impact that island faunas had on both Darwin's (1859) and Wallace's (1880) thinking as they developed their ideas on evolution by natural selection, it is not surprising that early work favors geographic isolation as a potent force in the emergence of species. However, the beginning of a later controversy surrounding the geographic context of speciation can be detected at the very emergence of the field. Darwin notes that geographic isolation is important to divergence, stating "In a confined or isolated area … natural selection will tend to modify all the individuals of a varying species throughout the area in the same manner … Intercrosses, also, with the individuals of the same species, which otherwise would have inhabited the surrounding and differently circumstanced districts, will be prevented." (Darwin, 1859, p. 104). However, in considering the connection between patterns on islands and the diversity on continents, he goes on to say, "Although I do not doubt that isolation is of considerable importance in the production of new species, on the whole I am inclined to believe that largeness of area is of more importance, more especially in the production of new species…" As thoroughly explored by Sulloway (1979), Darwin's further discussions of 'partial' and 'ecological' isolation also suggest that he viewed speciation in sympatry as common.

At least partially in response to Darwin's perceived endorsement of sympatric speciation, several authors in the late nineteenth and early twentieth centuries, voiced dissenting views in strong support of allopatric speciation (see Sulloway, 1979). These arguments focused strongly on the biogeography of closely related species pairs, as determined by morphological analysis. The ichthyologist David Starr Jordan was particularly influential in advocating the role of geography in speciation, stating what would later be named Jordan's rule: "Given any species in any region, the nearest related species is not likely to be found in the same region nor in a remote region, but in a neighboring district separated from the first by a barrier of some sort…" (Jordan and Kellogg, 1907, p. 120). Jordan viewed the conditions for geographic isolation as ubiquitous in nature, perhaps influenced by his extensive work in freshwater fishes.

The Great Debate: Allopatric versus Sympatric Speciation

The modern synthesis made tremendous strides toward understanding the speciation process, largely through incorporation of Mendelian genetics and the articulation of the Biological Species Concept (BSC). Darwin's species concept was famously poorly developed; however, later workers such as Poulton initiated debate intended to more carefully define species (Poulton, 1908). With influences from many others, Mayr formulated the BSC as, "… groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr, 1942). Mayr also held firmly that geographic isolation was essential to the process, writing several scathing critiques of sympatric speciation (Mayr, 1947, 1963). In his view, physical barriers that confer geographic isolation act as indiscriminant external agents that separate emerging taxa while internal barriers to reproduction accumulate through time (**Figure 1**). Intrinsic reproductive isolation therefore stems from biological differences between organisms, and it is assumed to typically arise as a by-product of divergence during this allopatric phase (Coyne and Orr, 2004; Schluter, 2001). Dobzhansky (1937) and others additionally favored the idea that speciation is

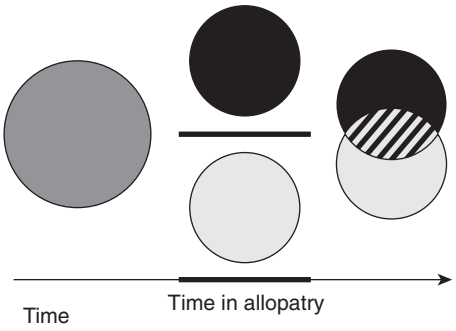

Figure 1 The traditional view of allopatric speciation. A formerly contiguous group of populations is separated by a physical barrier. During this time in allopatry there is no gene flow, and reproductive isolation arises as a by-product of divergence. Upon secondary contact, stable coexistence verifies that speciation is complete.

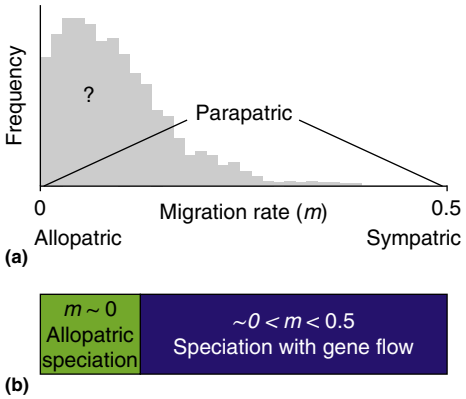

Figure 2 Redefining the geography of speciation. (a) A population genetic definition of the geography of speciation places allopatric and sympatric speciation at either extreme ends of a continuum in migration rate, while parapatric speciation describes everything in between. The geography of speciation may be approached by characterizing the unknown frequency distribution (?) of events across a range of potential migration rates (gray bars). (b) Because it will often be impossible to determine historic rates of gene flow or to rule out past episodes of allopatry, a rough dichotomy that separates events into fully allopatric speciation (green, $m \approx 0$) and speciation with gene flow (blue, $\approx 0 < m < 0.5$) may be more useful.

often completed through the process of reinforcement, which states that upon secondary contact natural selection may directly favor the evolution of early acting reproductive barriers due to the maladaptive nature of hybrids (Levin, 1970; Hopkins, 2013).

While this view of speciation dominated the literature throughout the mid-twentieth century, the vestige of sympatric speciation left by Darwin and other early workers (e.g., Walsh, 1864) never completely disappeared. In response to Mayr's criticisms, theoreticians explored potential conditions under which speciation could occur with gene flow (e.g., Maynard Smith, 1966), while empiricists sought to characterize potential examples. Guy Bush's experimental work on apple and hawthorn races of *Rhagoletis* fruit flies provided the most compelling case of sympatric speciation emerging from this period (1969). One of the key insights to the *Rhagoletis* system involves their mating behavior. Rather than forming a mating flight, *Rhagoletis* mate on their food source, providing a means for stable assortative mating to facilitate divergence. This work influenced the development of a number of similar empirical studies (e.g., Via, 2009), and there is continued speculation about whether host shifts in phytophagous insects are common examples of speciation with gene flow (Berlocher and Feder, 2002).

Redefining the Geography of Speciation

Mayr and others defined the geography of speciation spatially. Allopatric speciation occurs when geographic isolation is required, while sympatric speciation occurs when divergence happens in the same place, i.e., while each emerging species is within the 'cruising range' of each other (Mayr, 1963). Mayr also recognized parapatric speciation as an intermediate category that included examples where two taxa are mostly allopatric, but have abutting ranges with a narrow band of contact. More recently, these same terms have been defined from a population genetic point of view (Endler, 1977). The rate of migration (m) serves to indicate the magnitude of geographic isolation during speciation. Allopatric speciation is thus the subset of events where $m = 0$ throughout the process. The upper limit of migration $m = 0.5$ would occur if two taxa mated completely randomly at the onset of divergence, so this has been used as the condition of sympatric speciation. In contrast to the original spatial definition, parapatric speciation includes events where the value of m lies anywhere between the two extremes (**Figure 2(a)**).

Several criticisms of this approach provide motivation to redefine the geography of speciation. Allopatric speciation has been described by some as common and uncontroversial (Coyne and Orr, 2004). Colonization of oceanic islands following long distance dispersal to these isolated environments is regarded as an example. However, in order to truly achieve $m = 0$, no additional dispersal can occur to that 'isolated' environment during the entire time span of speciation. On the other end of the scale, criticisms have been levied against using $m = 0.5$ as the defining feature of sympatric speciation. For example, Mallet *et al.* (2009) argue that true panmixis of $m = 0.5$ is rarely, if ever, achieved in nature; therefore, it is a definition predetermined to fail. Instead, while acknowledging the potential importance of spatial separation in many cases, they favor defining sympatry less strictly, for example: Two populations are *sympatric* if individuals of each are physically capable of encountering one another with moderately high frequency. However, given the contentious history of this term, this redefinition seems unlikely to persist.

Given the perceived failures of categorization systems for the geography of speciation, a useful redefinition may be to examine events as either: (1) allopatric speciation (or nearly so), $m \approx 0$; or 2) speciation with gene flow ($0 < m < 0.5$). The former category would include events where geographic isolation is strong but potentially incomplete, such as long distance dispersal and subsequent colonization of isolated habitats. However, the defining feature would be consistently low or absent migration throughout divergence. The latter category would include those events for which gene flow has imposed a significant constraint on divergence. As Butlin *et al.* (2008) argue, migration rate can be viewed as a continuum,

and the value may fluctuate during different stages of the speciation process. In addition, past and future values of m are extremely difficult to estimate or predict. Therefore, rather than attempting to characterize a potentially unknowable frequency distribution (**Figure 2(a)**), a more useful approach may be to examine the dynamics of migration and selection in systems with ongoing gene flow (Butlin *et al.*, 2008), or to ask how often extant taxa exhibit the conditions favorable to the process (Bolnick and Fitzpatrick, 2007).

Speciation with Minimal Gene Flow ($m \approx 0$): Allopatric or Nearly So

Allopatric events include those for which migration is minimal throughout divergence. This would include both 'vicariant' and 'peripatric' subcategories of traditional allopatric speciation. Vicariant speciation refers to events in which a contiguous population is physically separated by some climatological or geologic event (Cracraft, 1982). Peripatric speciation is essentially the same with regard to gene flow, but occurs when small numbers of individuals in peripheral populations become separated from a progenitor through similar forces or long distance dispersal to isolated habitats (Coyne and Orr, 2004).

Theoretical support for allopatric speciation

Much of the theory associated with allopatric speciation focuses on the evolution of intrinsic postzygotic isolation (Dobzhansky, 1937, Muller and Pontecorvo, 1942). When emerging taxa are geographically isolated, two or more loci may fix for different alleles in each population. If these alleles produce negative epistatic interactions, hybrids between the taxa may be sterile or inviable (Orr, 2001). This form of isolation is often considered a hallmark of allopatric speciation because selection is expected to remove such negative interactions if they arose in the face of gene flow. While many other forms of isolation can also arise in allopatry, their evolution is often modeled indirectly. Indeed, theoretical treatment of allopatric speciation are simply models of sympatric speciation with the constraints imposed by gene flow removed. Felsenstein (1981) famously described the antagonism between selection and recombination when divergence occurs in the face of gene flow. His initial model considers a simplified case of two loci involved in divergent selection in alternate habitats. If mating is random, selection alone produces linkage disequilibrium between coadapted alleles; therefore, speciation cannot occur under these conditions unless selection is strong enough to remove every recombinant. If a locus conferring a pre-zygotic barrier is added in the form of assortative mating, speciation can occur; however, recombination severely limits favorable conditions by breaking up associations between assortative mating and adaptive loci. However, reductions in migration between alternative habitats facilitates the speciation process, with the lower extreme of allopatric speciation ($m = 0$) completely free of the constraints imposed by recombination.

Empirical support for allopatric speciation

Jordan's rule suggests that evidence for allopatric speciation should be ubiquitous, and it specifically proposes the most closely related species pairs as targets for assessing the geographic mode of speciation. Therefore, we might expect that the youngest species pairs are the most likely to exhibit allopatry, and this may often involve an obvious geographic barrier. Thus, phylogenetic studies of the time since speciation and the degree of range overlap might yield important insights into the geography of speciation. However, these studies, known as age-range correlations (ARCs), are very sensitive to mismatches between the timescales of speciation and range shifts, providing a limited glimpse into the process (see **Box 1**). Therefore, some of the best unambiguous examples of

Box 1 Age-range correlations

Age-range correlations (ARCs) aim to examine the geography of speciation by measuring the degree of geographic overlap in extant taxa across a range of different genetic distances (Barraclough and Vogler, 2000). Genetic distance serves as a proxy for time since speciation; therefore, the assumption is that the youngest taxa will reveal the nature of geographic distributions at the time of speciation. For example, if allopatric speciation is the norm, geographic overlap should be lowest for young taxa, and increase stochastically through time as range shifts and expand (**Figure B1(a)**). Alternatively, if speciation with gene flow is common, then many young species pairs will have overlapping geographic distributions (**Figure B1(b)**). These approaches have yielded mixed results, with signatures of allopatric speciation in some groups such as birds and mammals, and mixed results in groups such as phytophagous insects (Barraclough and Vogler, 2000). Despite the intuitive appeal of this approach, a number of significant criticisms have emerged (see Bolnick and Fitzpatrick, 2007; Warren *et al.*, 2014). For example, Losos and Glor (2003) argue that range shifts can be far too dynamic to be captured by this method. Indeed, range shifts associated with Pleistocene climatic fluctuations have likely produced frequent shifts between allopatry and sympatry across a timescale that is far too short for most speciation events (Fitzpatrick and Turelli, 2006). This method is also highly sensitive to the species concepts employed in taxonomic treatment of different groups. For example, sessile organisms with morphologically defined species may lead to the overprediction of allopatric speciation events. At best, this may therefore be a one-directional test, which should be interpreted with caution.

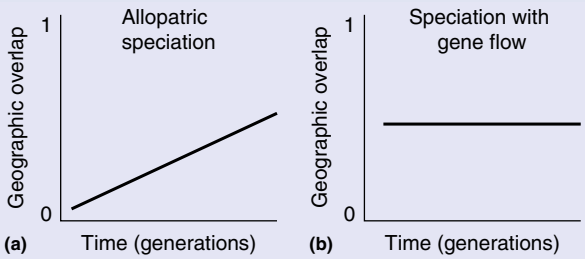

Figure B1 Age-range correlations examine the degree of geographic overlap between taxa over evolutionary time scales. (a) If allopatric speciation is common, geographic overlap should be low for the most recently diverged species pairs, and increase through time as ranges change. (b) If speciation with gene flow is common, geographic overlap may be high at the earliest stages, and post-speciation range shifts may stochastically impact geographic overlap.

allopatric speciation come from known geologic events that likely resulted in simultaneous allopatry for multiple species pairs. For example, as the Isthmus of Panama arose approximately 3 million years ago, many formerly contiguous marine species were completely isolated by this major geologic event. Several well-described examples of emerging species pairs exhibit additional forms of reproductive isolation, suggesting that speciation is either occurring or complete in many of these taxa (Knowlton *et al.*, 1997; Lessios, 1998).

Similarly, the highly isolated nature of oceanic islands provides ample opportunity for long distance colonists to diverge, either mostly or completely unimpeded by gene flow. These examples also illustrate the power and importance of geographic isolation to promote evolutionary divergence, with spectacular examples of island floras and faunas with extremely high rates of endemism (e.g., Amadon, 1950; Templeton, 1979). Further emphasizing the importance of spatial separation, *in situ* speciation on islands appears to be relatively rare (Coyne and Price, 2000). For example, Igea *et al.* (2015) find that among the 33 endemic plant species on the highly isolated Cocos Island, none are the result of *in situ* speciation, but rather result from mainland colonization and subsequent divergence.

Speciation with Gene Flow ($\sim 0 < m < 0.5$)

Speciation that occurs in the presence of recurrent gene flow faces significant constraints. Specific conditions may impact the probability of speciation with gene flow, such as polyploidy or homoploid hybrid speciation; however, these topics are covered elsewhere (Ramsey and Schemske, 1998; Abbott *et al.*, 2013). In recent years, genomic studies have helped illustrate the heterogeneous nature of genetic variation when divergence and gene flow occur simultaneously (Nosil *et al.*, 2009). Indeed, these studies have been instrumental in illustrating the maintenance of substantial phenotypic differentiation despite apparent introgression at neutral loci. However, others have demonstrated the need for caution in inferring patterns of gene flow and isolation from indirect evidence (e.g., Noor and Bennett, 2010; Cruickshank and Hahn, 2014), providing fodder for continued debate over whether the the current enthusiasm for divergence with gene flow is warranted (Coyne and Orr, 2004). Therefore, it is important to examine the theoretical and empirical support for speciation with gene flow critically.

Theoretical support for speciation with gene flow

As mentioned above, Felsenstein (1981) elegantly described the difficulties associated with divergence with gene flow due to the antagonism between selection and recombination. As a result, the subsequent theoretical work focuses on describing conditions that would overcome these difficulties (reviewed in Bolnick and Fitzpatrick, 2007; Gavrilets, 2003). For example, many theoretical models have been proposed that invoke pleiotropy or tight genetic linkage to prevent recombination (Ortiz-Barrientos *et al.*, 2002; Butlin, 2005; Bolnick and Fitzpatrick, 2007). For example, so-called 'one-allele' models describe situations such as Bush's (1969) *Rhagoletis*, whereby a single adaptive allele simultaneously impacts fitness on a new resource and assortative mating (Ortiz-Barrientos and Noor, 2005). Critics have labeled these 'magic' traits; however, others suggest this may be more common than expected (Servedio *et al.*, 2011). Models of speciation with gene flow that incorporate two or more loci are perhaps more realistic, and they demonstrate increased difficulty for the process. When recombination can breakup emerging associations, speciation with gene flow requires the strength of selection to be very strong or genetic loci involved to be very closely linked (**Figure 3**). However, in considering migration as a continuum, varying amounts of migration will alter the probability of speciation in emerging taxa. Low, but nonzero values of migration may often be surmountable, and the strength of selection or the degree of genetic association will have a positive impact on the potential for speciation (**Figure 3(b)**).

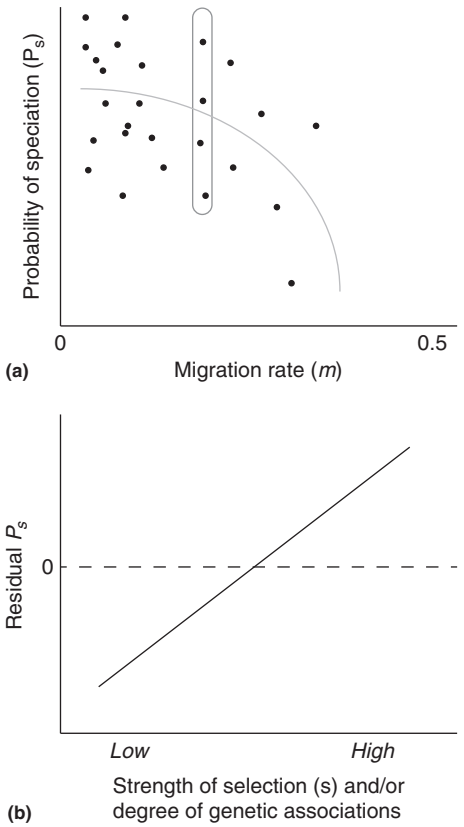

Figure 3 Factors impacting speciation in the face of gene flow. (a) Increasing migration will generally impede speciation, with higher rates of migration impeding divergence with gene flow. Residual variation in the probability of speciation is shown by the gray oval. (b) Two of the key factors impacting speciation in the face of gene flow are the strength of selection and the degree of genetic associations between loci involved in local adaptation and assortative mating. Residual variation in speciation probability is expected to have a positive relationship with these factors. For example, strong selection will facilitate speciation for a given degree of migration.

Empirical support for speciation with gene flow

The most common forms of evidence proposed for cases of speciation with gene flow comes from isolated environments

that are unlikely to provide the conditions of allopatry (Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007). While it may be impossible to determine the previous rate of migration between emerging taxa in these conditions, the geographic structure of isolated environments suggests that migration between groups was initially high before reproductive isolation emerged. Undeniably, it is often very difficult to unambiguously rule out periods of allopatry from any putative case of speciation with gene flow (see **Box 2**), but these cases provide some of the most compelling candidates. Examples have been proposed in fish species flocks inhabiting isolated lakes (Schliewen *et al.*, 1994) or plants on isolated islands (Savolainen *et al.*, 2006). For example, on Lord Howe Island, a species pair of palm trees occurs in sympatry on this remote oceanic outcrop (Savolainen *et al.*, 2006). These trees appear to be capable of hybridizing, but habitat and temporal isolation are strong enough to maintain these distinct taxa in nature. Interestingly, in contrast to the Cocos Island example above, Lorde Howe Island appears to have a relatively high rate of *in situ* speciation, suggesting that this island may provide unique conditions favoring divergence with gene flow (Papadopulos *et al.*, 2011).

While these examples appear compelling, a number of issues prevent ruling out an impact of geographic isolation completely. First, multiple invasion events from a source taxon may facilitate divergence (Gray and Cavers, 2014). For example, the first colonists to reach an island may establish a population and begin to diverge from the progenitor. If a second group of colonists arrive later, their success may depend on establishing in a different habitat from the original colonists. Even low levels of hybridization between the two groups could make them appear to be each other's closest relative, and the ecological character displacement that occurs due to competition may produce two stably differentiated taxa (Schluter and McPhail, 1992). Another issue with putative cases of divergence with gene flow is the potential for standing genetic variation to impact divergence (Seehausen, 2002). For example, in Bush's *Rhagoletis* fruit flies, a chromosomal inversion that arose in allopatry may explain how recombination is prevented between coadapted allele combinations (Feder *et al.*, 2005). Sympatric divergence may not be possible without this inversion, so this appears to be a case where the lines between sympatric and allopatric speciation are blurred.

Indeed, the most interesting speciation events may involve a mixture of processes in both geographic isolation and in sympatry (e.g., Heliconius Genome Consortium, 2012; Martin *et al.*, 2015; Stankowski and Streisfeld, 2015). Mayr's notion of parapatric speciation, with taxa exhibiting abutting ranges, represents just one simple case where gene flow may be high in some regions and low in others. For example, Mallet *et al.* (2009) describe 'mosaic sympatry' in which patchy alternative habitats for diverging taxa produce a mix of physical isolation and contact. Host shifts, as in phytophagous insects may represent one relatively common example of this form of divergence (Nosil, 2007). In plants, edaphic specialization is similarly a process that must commonly begin in sympatry, and produces a patchy arrangement of contact and isolation between diverging taxa (Kay *et al.*, 2011). Fluctuations in geographic range through time may also result in contact and gene flow in some periods, and extensive physical separation

Box 2 Difficulty in distinguishing primary from secondary contact

While speciation with gene flow and allopatric speciation are debated as opposite ends of the spectrum in the geography of speciation, they can be surprisingly difficult to diagnose from patterns of genetic variation (Noor and Bennett, 2010; Cruickshank and Hahn, 2014). In the traditional view of allopatric speciation, adaptive alleles that arise in each emerging species will become differentially fixed, and other loci in linkage disequilibrium with these causal mutations will follow suit (Via and West, 2008). Genetic drift is also expected to result in differential fixation of alleles in each taxon, and many loci across the genome are expected to be highly differentiated (Via, 2001). **Figure B2(a)** shows an example of allopatric speciation. The lower panel represents genetic differentiation with selected loci (solid black line) and other regions throughout the genome (dashed black line). Alternatively, if two taxa emerge in the face of gene flow, adaptive alleles are expected to become highly differentiated, while the rest of the genome exhibits weak patterns of differentiation (lower panel **Figure B2(b)**). This dichotomous view rests on two assumptions: (1) allopatric divergence will be complete before the establishment of secondary contact ('stable coexistence of discontinuous taxa'), and (2) many shared polymorphisms at the initiation of speciation will be lost during the allopatric phase. Secondary contact followed by even small amounts of gene flow may homogenize nonadaptive loci (gray dashed line and arrows), and shared polymorphisms that remain from the progenitor population may also resemble the pattern expected for divergence with gene flow.

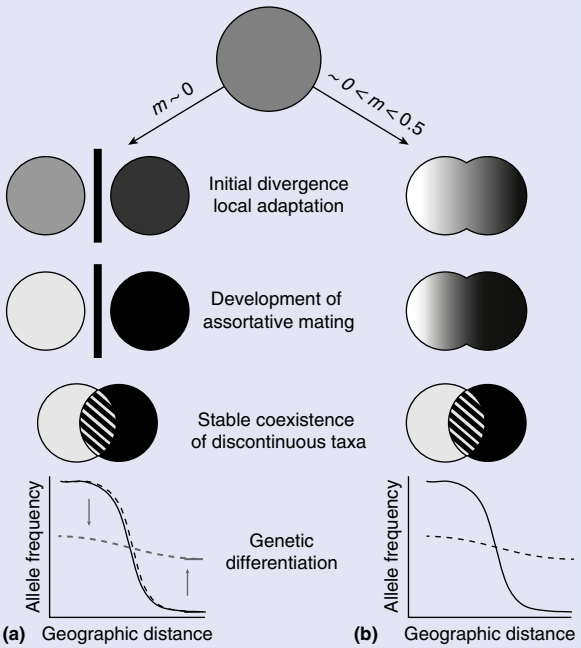

Figure B2 Primary and secondary contact between diverging lineages may produce similar genomic signatures. (a) Two taxa become highly differentiated in allopatry and are maintained after secondary contact despite hybridization. Allopatric divergence impacts all loci simultaneously, but secondary contact results in homogenization of neutral regions of the genome (arrows and dashed lines), while selected loci remain differentiated (solid line). (b) Two taxa arise via divergence with gene flow. Selected loci show strong levels of differentiation (solid line), while neutral loci will be more freely exchanged (dashed line).

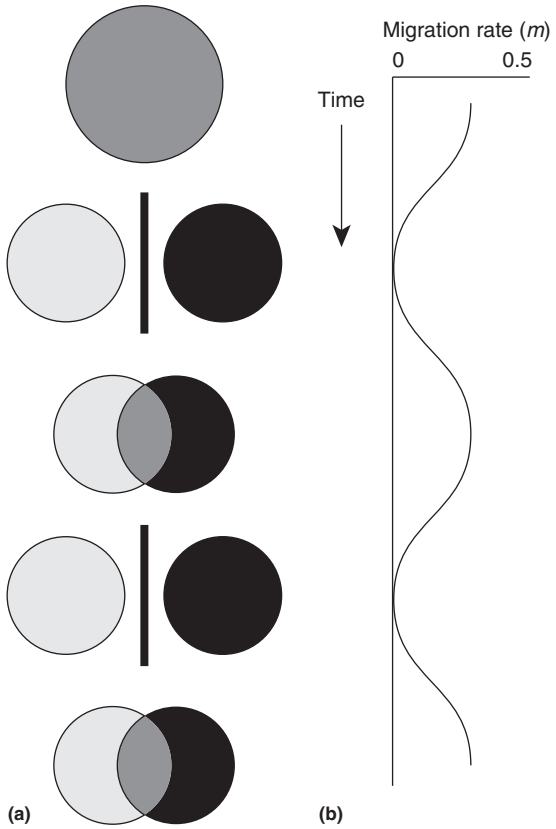

Figure 4 Fluctuating geographic context of speciation. (a) Through time physical barriers may arise and recede repeatedly during the speciation process. (b) Migration rates fluctuate as physical barriers rise and fall. These cases represent a mosaic of allopatric and sympatric factors influencing the potential for divergence.

in others (Butlin *et al.*, 2008; **Figure 4**). While these cases fit into the category of divergence with gene flow, physical separation also plays a key role.

Sources of Geographic Isolation

Given the importance of spatial separation to both purely allopatric speciation and many presumed cases of speciation with gene flow, it is instructive to examine the source of spatial separation in examples besides vicariance. Indeed, while Mayr and others refer to geographic isolation as a purely external factor, the degree to which geographic features impact gene flow is the result of the interaction between biological traits of organisms and the landscape they are found in. For example, the biology of organisms may determine dispersability, which can be highly variable across species. Therefore, whether reaching an isolated habitat represents a rare long distance dispersal event or a frequent stopover may depend on intrinsic characters as well as extrinsic factors. Similarly, the geographic ranges that organisms occupy represent the simultaneous outcome of historical, biological, and geographic factors (Endler, 1982; Thorpe *et al.*, 2008). Therefore, studies of speciation have much to be gained by examining the interaction between biology and geography.

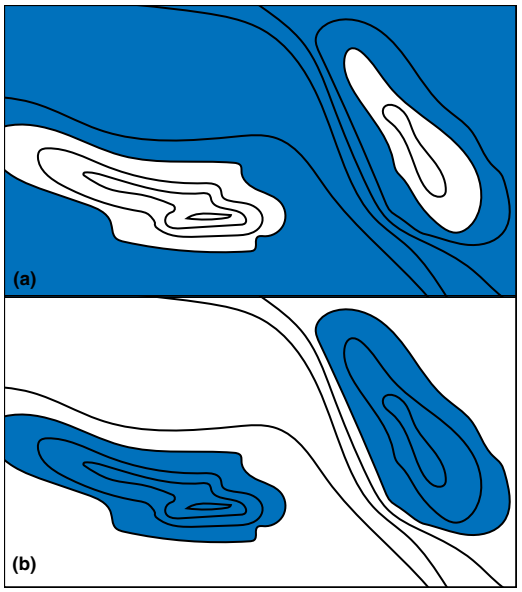

Figure 5 Niche conservatism leads to geographic isolation. (a) A hypothetical topographic map of a region with species range indicated in blue. (b) During periods of climatic warming, niche conservatism leads to range contraction of the previously contiguous species into higher elevations. Thus, retaining niche characteristics can lead to an increase in geographic isolation when suitable habitat becomes spatially constricted.

Niche conservatism

Niche conservatism refers to the notion that ecological niches are likely to experience phylogenetic inertia, and change somewhat slowly (Wiens and Graham, 2005). While it may seem paradoxical to consider how a lack of change in the niche can impact divergence between species, niche conservatism is hypothesized to impact the initial development of allopatry in some cases (Kozak and Wiens, 2006). Climatic fluctuation may change the arrangement of suitable habitat across a landscape over time, resulting in variation in the interconnectedness of habitats. If populations retain their ecological tolerances via niche conservation, they may migrate in order to seek conditions consistent with their retained niche. This can potentially result in increases in allopatric separation between previously connected populations. For example, in mountainous regions, contiguous lowland populations could be driven to higher elevations during periods of climatic warming, producing allopatric separation (**Figure 5**). Although biological differences must accumulate between populations to initiate speciation (Sobel *et al.*, 2010), geographic isolation via niche conservatism may play an important role in facilitating subsequent divergence.

Ecogeographic isolation

In direct contrast to niche conservatism, adaptations to the abiotic or biotic environment may produce shifts in the ecological tolerances of populations, leading to niche divergence (**Figure 6**). Shifts in ecological niche between lineages may have concurrent impacts on the geographic distribution of taxa when alternate suitable habitat is spatially disjunct across the landscape (Sobel *et al.*, 2010). The reduction in gene flow that

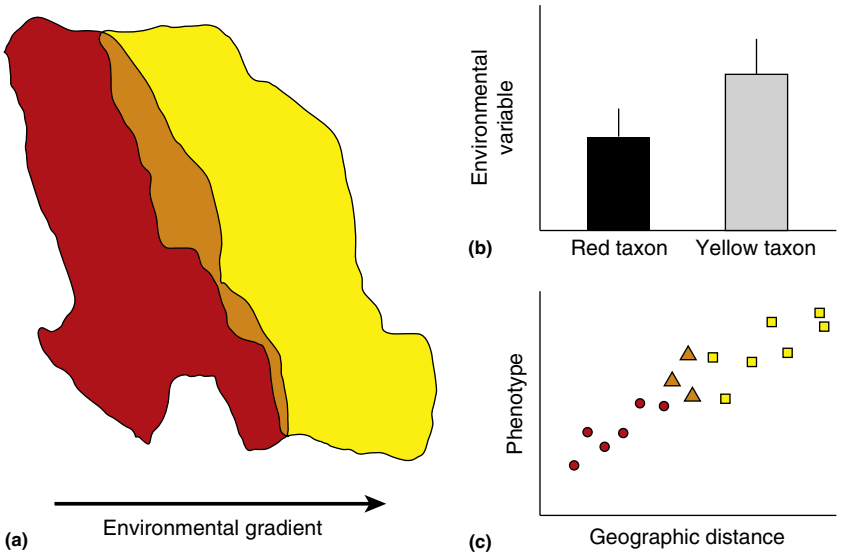

Figure 6 Ecogeographic isolation as a result of niche divergence. (a) Species distribution modeling shows a nonrandom arrangement of suitable habitat for a red and yellow sister taxon pair along an environmental gradient. Ecogeographic isolation is strong but incomplete with a hybrid zone formed where the ranges meet. (b) Environmental variables exhibit significant differences at sites occupied by the two taxa. (c) Ecophysiological phenotypes vary across space, verifying the presence of ecogeographic isolation.

occurs by this process is referred to as ecogeographic isolation (Ramsey *et al.*, 2003; Sobel *et al.*, 2010). The phenotypes involved can be thought of as so-called 'magic traits' from the point of view of divergence with gene flow (Servedio *et al.*, 2011), because local adaptation may simultaneously produce multiple forms of reproductive isolation including assortative mating, immigrant inviability (Nosil *et al.*, 2005), and extrinsic postzygotic inviability (Hatfield and Schluter, 1999).

Ecogeographic isolation can be measured in a variety of ways. In one approach, species distribution modeling (SDM) can be employed to predict biological differences between taxa, and ecogeographic isolation is estimated from the overlap and exclusivity of predicted suitable habitat for each taxon (Sobel, 2014). These models are built using a combination of remote sensing data of environmental conditions and population collection records from museums or herbaria (Elith and Leathwick, 2009). As such, SDMs can be used to reveal differences in the environmental conditions experienced by a pair of taxa, but it does not directly measure ecological differences between them (Warren *et al.*, 2014). The most powerful approach to assess ecogeographic isolation is therefore to measure biological differences between emerging taxa that impact geographic distributions, either by reciprocal transplant experiments (Angert and Schemske, 2005) or via variation in ecophysiological traits (Sheth and Angert, 2014). Local adaptation and clinal variation are well documented in a variety of organisms (Clausen *et al.*, 1940). Therefore, it is perhaps not surprising that ecogeographic isolation appears to be strong in the groups that have been examined (Sobel, 2014).

Conclusions

The topic of the geography of speciation has been marked by fierce debate over whether full geographic isolation is required to initiate the process. The history of the debate goes back at least to Darwin, and survives to this day. We now have empirical examples and theory that suggest the conditions for speciation with gene flow may sometimes be satisfied in nature; however, we will probably never know what frequency of speciation events that occur in allopatry or in contact. However, even in our most compelling cases of speciation with gene flow, geographic isolation may continue to play a crucial role. Therefore, speciation studies are likely to benefit from continued focus on the processes that produce spatial separation, and the topic of the geography of speciation is far from outliving its utility.

See also: Ecological Speciation and Its Consequences. Phylogeography. Species Concepts and Speciation

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Speciation, Sexual Conflict and

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Glossary

Chase away model The evolution of exaggerated secondary sexual characteristics through sexually antagonistic coevolution.

Interlocus sexual conflict Occurs when the expression of a locus in one individual compromises the fitness of its mate with expression of different loci acting to counteract the negative effects. One possible outcome is sexually antagonistic coevolution.

Intralocus sexual conflict Occurs when the same combination of alleles exert opposite effects on fitness when expressed in a male versus a female.

Reproductive isolation Any factor that restricts gene flow to produce independently evolving populations that, in the extreme, yields distinct species. Barriers may act prior to mating (premating isolation), after mating but prior to zygote formation (premating, prezygotic isolation (PMPZ), gametic isolation), or after zygote formation (postzygotic isolation).

Sexually antagonistic coevolution A possible outcome of interlocus sexual conflict whereby selection on one sex favors reproductive traits that induce a fitness cost on its mates. Selection on the mate, in response, favors evasive adaptations to reduce the negative effects, leading to a cycle of male and female trait evolution.

Sexual conflict Differing optimal reproductive strategies of sperm donor and recipient individuals that induces opposing selective pressures on the sexes.

What is Sexual Conflict?

The kernel of the idea that those traits shared between the sexes can experience different fitness optima in each sex – sexual conflict – traces to Darwin (1871), and yet reproduction was, nevertheless, widely viewed as an entirely cooperative endeavor prior to the 1970s. As we now recognize, the fitness interests of mating partners are seldom harmonious in all aspects of the 'economics' of reproduction (Parker, 1979), and instead, male and female reproductive interests often conflict. The fundamental root of conflicting interests between the sexes lies in the production of different sized gametes (anisogamy): smaller male gametes (sperm) essentially parasitize the larger, resource-rich female gametes (eggs) (primordial sexual conflict: Parker *et al.*, 1972; Parker, 1978). This asymmetry in gametic investment in reproduction sets the stage for further layers of conflict to arise between males and females in any traits that elevate the reproductive success of one sex at the expense of the other. Students of sexual selection now apply the term 'sexual conflict' to these disparities between the sexes in their fitness interests, the resulting selective pressures, and evolutionary responses (Parker, 1979; Arnqvist and Rowe, 2005).

Sexual conflict is widespread across taxa (Arnqvist and Rowe, 2002), with conflict over parental investment taking many forms, including mating rate (Rice and Holland, 1997; Holland and Rice, 1998; Rice *et al.*, 1998), sperm selection by females (Ball and Parker, 2003), and epigenetic control of development (Rice *et al.*, 2012). These antagonistic interactions between the sexes contrast with exaggeration of secondary sexual traits through other forms of sexual selection that involve female preference and male–male competition. Rapid nonantagonistic trait evolution mediated by Fisher's (1930) runaway selection model or Zahavi's (1975) handicap 'good genes' hypothesis can contribute to interpopulation divergence and the evolution of reproductive isolation. Likewise, evolutionary responses to sexual conflict also can promote trait exaggeration, via the chase away model involving sexually antagonistic coevolution (Holland and Rice, 1998), to produce population divergence and speciation in a manner potentially even more potent than other modes of sexual selection (reviewed in Gavrilets, 2014). Distinguishing sexual conflict from other forms of sexual selection, however, remains a challenge (Rowe and Day, 2006).

Sexual conflict generates sexually antagonistic selection that discriminates among heritable variation in male traits and in female traits (Rice, 1984), whereby selection favors one trait value to maximize the reproductive success of one sex and a different trait value for the other sex. For example, more numerous matings typically will improve male reproductive success disproportionately greater than female reproductive success because female reproductive output often is limited by total offspring production, independent of the number of matings (Bateman, 1948). Consequently, selection will favor alleles that confer on males a propensity to mate numerously and promiscuously, as well as favoring alleles that enable females to engage in few, select 'high-quality' matings. This tension can lead to the evolution of mating rates that are suboptimal from the point of view of one or both sexes, or can instigate coevolutionary arms races in traits that influence the balance of sexual costs and benefits for each sex (Arnqvist and Rowe, 2005). The roles of which sex inflicts and receives disproportionate fitness costs depend on the organism and its stage in the coevolutionary process, sometimes with females inflicting higher fitness costs on males (e.g., sexual cannibalization of male mantids: Lelito and Brown, 2006) or vice versa (e.g., traumatic insemination of female bedbugs: Stutt and Siva-Jothy, 2001). However, sexual conflict need not necessarily result in harmful behavior, nor is harm a required feature for sexually antagonistic coevolution to occur (see Rowe *et al.*, 2005; Lessells, 2006).

The vocabulary employed for concepts in sexual conflict are tailored toward organisms with distinct and separate sexes

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(gonochorism or dioecy: see Schärer *et al.*, 2014). However, sexually antagonistic selection also occurs in hermaphroditic mating systems, which provided some of the first observations and insights into sexual conflict (Charnov, 1979). Recently, Schärer *et al.* (2014) defined and clarified the nuances of sexual conflict theory in hermaphrodites in order to bring attention back to them as powerful examples of sexual conflict, coming from simultaneously hermaphroditic organisms in which an individual expresses both sexes at the same time, such as the 'love dart' in snails (Koene and Schulenburg, 2005), as well as from sequential hermaphrodite species in which a single individual changes from one sex to the other over time (reviewed in Abbott, 2011). Given that hermaphroditism is taxonomically widespread, with ~70% of animal phyla and >90% of plant genera containing hermaphrodite representatives (Renner and Ricklefs, 1995; Jarne and Auld, 2006), neglecting sexual conflict in hermaphrodites could impede progress toward deciphering general principles and implications of sexual conflict for the production of biodiversity (Schärer *et al.*, 2014). The dual connections of evolutionary transitions in reproductive mode with speciation itself (Goldberg and Igić, 2012) and with shifts in the cost-benefit structure of sexual conflict suggest that consideration of both of these factors simultaneously might prove fruitful in discerning the driving forces in species diversification.

Despite the prevalence of circumstances like multiple mating and sex chromosomes that are conducive to sexual conflict across organisms (Charlesworth, 1991; Zeh and Zeh 1996, 1997; Baur *et al.*, 1998; Michiels *et al.*, 1998; Jennions and Petrie, 2000), sexual conflict is unlikely to provide a prominent selective pressure in several key types of systems. In particular, organisms with reproductive modes dominated by asexuality and parthenogenesis, extremely high self-fertilization, and true monogamy should severely limit the extent to which sexual conflict can contribute to evolutionary change. For example, species with true lifelong genetic monogamy, such that mating with other individuals is not possible for either sex, display completely codependent reproductive success for the two sexes and so escape most sexual conflict (Wedell *et al.*, 2006); any strategy that decreases partner fitness will simultaneously reduce self-fitness, and consequently be opposed by selection. The extreme rarity of monogamy in animals has led to doubts about the existence of this ideal in nature (Birkhead, 1997; Wedell *et al.*, 2006; Hosken *et al.*, 2009b), although asexual and selfing reproduction arise commonly in both plants and animals (Jarne and Charlesworth, 1993).

How Can Sexual Conflict Promote Speciation?

Speciation results from the accumulation of genetic divergence between populations, which fosters the evolution of reproductive isolation. As populations evolve independently, incompatibilities amass and manifest as mismatches in mating behaviors or morphologies, ecological preferences, postmating gametic interactions, or as fitness deficiencies in genetically admixed, hybrid individuals. Sexual selection on mating traits can drive them to diverge rapidly from preexisting genetic variability, but the distinctiveness of such daughter populations may be temporary as they are susceptible to subsequent fusion (Servedio and Bürger, 2014). Similarly, while extrinsic genotype-by-environment interactions can represent a powerful selective pressure to restrict gene flow, transience of environmental conditions can collapse multiple nascent species back into a single evolutionary unit (Seehausen *et al.*, 2008; Futuyma, 2010). While such ecological speciation is most typically framed in terms of divergent local adaptation (Rundle and Nosil, 2005), modulation of the strength of sexual selection by ecological conditions could influence population divergence under sexual conflict in a similar way under some circumstances (Seehausen *et al.*, 1997). By contrast, a permanent reproductive barrier to enforce restricted gene flow evolves when population divergence involves new, derived alleles that create genetically intrinsic reproductive isolation by way of reduced viability or reproductive success of hybrid offspring, for example, via Dobzhansky–Muller incompatibilities affecting development or behavior (Dobzhansky, 1937; Coyne and Orr, 2004; Gavrilets, 2004). Rapid coevolution that results from sexually antagonistic selection provides an intuitively appealing way to explain how trait divergence can foster the production of new reproductively isolated populations or maintain restricted gene flow between nascent species that originated from other evolutionary circumstances.

Sexually antagonistic coevolution has the potential to provide a more potent and rapid source of interpopulation divergence compared to other forms of intersexual selection such as female mate-choice or assortative mating (Panhuis *et al.*, 2001; Kirkpatrick and Ravigné, 2002). A perpetual arms race between the sexes that follows from sexually antagonistic coevolution can bring about rapid and potentially limitless evolutionary change (Parker, 1979), for example, through iterated female evolution of resistance adaptations in response to male persistence adaptations. By comparison, other forms of intersexual selection typically involve an evolutionary response in female preference favoring an exaggerated male trait rather than resistance to an exaggerated male trait. The evolutionary tethering of the expression of female preference to those male traits that provide an indirect or direct benefit to females leads to trait responses constrained within the scope of females' ability to perceive them (Fisher, 1930; Berglund *et al.*, 1996). By contrast, female evasion of male traits under antagonistic coevolution, rather than trait tracking, creates more opportunity for the evolution of novelty and, subsequently, population divergence in allopatry (Gavrilets, 2000; Gavrilets *et al.*, 2001; Martin and Hosken, 2003) or even in sympatry (Gavrilets and Waxman, 2002). Sexually antagonistic coevolution also predicts the evolution of multiple male traits under a wide range of conditions (Arak and Enquist, 1995; Gavrilets *et al.*, 2001) and a greater number of genetic trajectories for the sexes and populations to pursue, resulting both in rapid exaggeration and diversification (Gavrilets, 2000). In some cases, such sexual traits could be co-opted for ecological innovation and provide the basis for divergent natural selection and so indirectly contribute to the origin of new species (Bonduriansky, 2011).

It is straightforward to see how gene flow may be disrupted in early stages prior to mating or in later stages after zygote formation: easily observable traits like species-specific

courtship songs in birds (Grant and Grant, 2010) and sterile hybrids in *Drosophila* (Masly *et al.*, 2006) provide compelling and clear-cut bases of reproductive isolation. By contrast, reproductive barriers that occur midway through the reproductive process at the gametic level – following mating, but prior to zygote formation, termed postmating, prezygotic reproductive (PMPZ) barriers – can involve phenotypically subtle but reproductively crucial divergence, owing to coevolution between the gametes or between the female reproductive tract and the sperm or components of the seminal fluid (Eady, 2001). As we discuss the ways that sexually antagonistic selection can participate in the speciation process, one must consider the potential for evolution of reproductive isolation to manifest through any of these phases: premating barriers, PMPZ incompatibilities, and postzygotic isolation.

The Dual Genetic Bases of Sexual Conflict

Two genetic pathways can produce sexual conflict, whereby sex-specific selection can occur between different loci (interlocus conflict) or within a locus (intralocus conflict). Interlocus conflict can induce an evolutionary arms race between the sexes, while intralocus conflict results in an evolutionary tug-of-war for each sex to reach their own fitness optimum. Both modes may derive from either simple (one- or two-locus systems) or complex (polygenic, quantitative genetic) genetic architectures. Below we differentiate between the two types of sexual conflict and discuss how each can facilitate, or restrain, the process of speciation.

Interlocus Conflict and Speciation

Conflict over the outcome of male–female interactions that are mediated by genetic factors encoded with different loci is known as interlocus sexual conflict. Conflicts between the sexes can occur over a diversity of traits, with commonly considered traits including mating rate, parental investment, remating behavior, and female reproductive rate. Different traits encoded by loci with sex-biased expression in each sex enable males and females to reach their sex-specific fitness optima in a given intersexual context (Rice *et al.*, 1998). As the intersexual context changes, interlocus conflict can drive continuous selective pressure for allelic replacement at the interacting loci in both sexes (Rice and Holland, 1997). Such recurrent conflictual selection will potentially spur a sexually antagonistic coevolutionary arms race between one set of loci that confers benefits to one sex at the expense of the other sex, and a distinct set of loci that control responding traits in the alternative sex. If given the opportunity for a male trait and female response to coevolve under sexual conflict, 'unresolvable evolutionary chases' might result, whereby neither sex reaches their optima (Parker, 1979). Under such conditions, sexually antagonistic coevolution can promote rapid evolutionary change (Parker, 1979).

Such a perpetual arms race that involves multiple loci promotes divergence between separated populations that evolve along independent evolutionary trajectories of trait change and counter-change (Rice, 1996; Rice and Holland, 1997; Holland and Rice, 1998; Rice *et al.*, 1998; Chapman *et al.*, 2003). When new mutations arise with sex-limited expression at sexually antagonistic loci subject to interlocus conflict, selection in one sex will favor their fixation and the same allele will be selectively neutral when it occurs in the other sex. Consequently, iterated over time, sex-limited selection can lead to repeated fixation of new sexually antagonistic alleles. Allopatric divergence accrues between populations as their trait evolution traces independent genetic trajectories, with coevolution driving distinct genotypic solutions to ongoing sexual conflict involving sex-biased adaptations and counter-adaptations. Because any source of evolutionary divergence, especially divergence involving new derived mutations (Orr, 2005), will increase the probability of genetic incompatibilities arising that could impede gene flow between populations, an interlocus genetic basis to sexual conflict can facilitate speciation (Rice *et al.*, 1998).

A perpetual arms race between the sexes represents just one possible evolutionary outcome of interlocus sexual conflict predicted by theory (reviewed in Gavrilets, 2014). Another possibility is the accumulation of many alleles at loci expressed in each of the sexes (Gavrilets and Hayashi, 2005; Härdling and Bergsten, 2006; Hayashi *et al.*, 2007; Härdling and Karlsson, 2009), that can potentially lead to sympatric speciation (Gavrilets and Waxman, 2002; Hayashi *et al.*, 2007). Other evolutionary outcomes of interlocus sexual conflict can generate elevated genetic variation in the population, but not facilitate formation of new species (Gavrilets, 2014), such as evolution toward an equilibrium (Kondoh and Higashi, 2000; Gavrilets *et al.*, 2001; Kimura and Ihara, 2009), and cyclic evolutionary dynamics (Gavrilets *et al.*, 2001; Haygood, 2004). Additionally, stochastic perturbations like genetic drift have the potential to switch the attracting evolutionary regime expected by deterministic theory (Gavrilets and Hayashi, 2005; Hayashi *et al.*, 2007). An alternative outcome to sexually antagonistic coevolution is that females can evolve indifference to male traits rather than resistance, and in extreme cases may select against these traits (Rowe *et al.*, 2005). The indifference that females express to male traits could halt the coevolutionary process, thus preventing further divergence that would otherwise facilitate the speciation process (Rowe *et al.*, 2005).

Intralocus Conflict and Speciation

Intralocus sexual conflict, as a mode of sexually antagonistic selection, favors alternative alleles at the same locus differentially between the sexes and generally acts as a force promoting maintenance of genetic variation (Rice and Holland, 1997; Parker and Partridge, 1998). In intralocus sexual conflict models with a one-locus two-allele genetic system, the strength of selection determines the evolutionary outcome: whether a population reaches an evolutionary compromise between the sexes or, instead, the population reaches an equilibrium that optimizes the fitness of only one sex (reviewed in Gavrilets, 2014). With a polygenic trait subject to intralocus sexual conflict owing to a sex-by-genotype interaction, the same combination of alleles exert opposite effects on the fitness of each sex (Fisher, 1930; Lande, 1980). The resulting conflicting

selection acting to produce an optimal phenotype in males thus, collaterally, impedes selection for the optimal phenotype in females (Rice, 1996). This scenario results in an evolutionary tug-of-war in which neither the male nor female trait value might obtain their sex-specific optima, owing to the evolution of an intermediate phenotype for both sexes and maintaining genetic variation for the trait in both sexes.

Importantly in the context of speciation, this tendency of intralocus sexually antagonistic selection to maintain polymorphism, rather than to drive individual alleles to fixation, will retard the accumulation of divergence between populations. Consequently, we should expect this genetic architecture of sexual conflict to impede, or at least not facilitate, the speciation process. However, sexual antagonism via intralocus conflict might not be able to maintain genetic variation effectively at more than a very small number of loci (Turelli and Barton, 2004), suggesting that the scope for intralocus conflict to constrain divergence might have been overstated in extrapolations of single-locus models unless epistasis among intralocus conflict loci is pervasive (Arnqvist *et al.*, 2014). Intralocus conflict also can impede divergence between populations by interfering with coevolutionary dynamics mediated by interlocus conflict (Pennell and Morrow, 2013). Nevertheless, intralocus sexual conflict could promote speciation when one sex experiences sufficiently strong directional selection, as it could drag traits expressed in the other sex across sex-specific maladaptive troughs of fitness, potentially fostering formation of new species (Bonduriansky and Chenoweth, 2009). Also, interestingly, epistasis among loci that each experience intralocus conflict can lead to 'equity effect' allelic fixation at different loci that could contribute to population divergence if different allele combinations fix in different populations (Arnqvist *et al.*, 2014), though it is unclear whether secondary contact of divergent populations might simply resurrect the sexual conflict rather than contribute to reproductive isolation between them.

Resolutions to intralocus sexual conflict can evolve that alleviate the selection pressure for maintenance of alternative alleles at a locus that confer sex-limited costs and benefits. Specifically, translocation mutations of loci to a sex chromosome, regulatory mutations that induce sex-limited expression, gene duplication that permits each gene copy to evolve to sex-specific fitness optima, or genetic imprinting can all provide evolutionary routes to eliminate intralocus conflict (reviewed by Bonduriansky and Chenoweth, 2009; Stewart *et al.*, 2010). The consequences of such evolutionary solutions to intralocus sexual conflict, however, might then themselves foster the evolution of reproductive isolation, as the resulting genetically decoupled male and female trait evolution respond independently to selection (Parker and Partridge, 1998; Pennell and Morrow, 2013). For example, when the evolution of sex-specific gene expression resolves intralocus conflict to create sexual dimorphism in a trait like body size, further selection on dimorphism can drive diversification whereby the dimorphic population can access and exploit previously inaccessible resources (De Lisle and Rowe, 2015). Moreover, as the sexes evolve independently, exaggeration of a male trait to an extent that it compromises female fitness could then spark a sexually antagonistic coevolutionary arms race via interlocus conflict. For example, genes with different expression between males and females (sex-biased genes), especially for paralogous copies of duplicates, could represent the resolution of intralocus sexual conflict (Wyman *et al.*, 2012; Ingleby *et al.*, 2015), as there can be rapid turnover in sex-biased gene expression (Harrison *et al.*, 2015). In this circuitous manner, the resolution of intralocus sexual conflict might ultimately predispose populations to evolution by interlocus conflict and lay the genetic groundwork for interpopulation divergence (Pennell and Morrow, 2013).

In turn, the independent evolution of separated populations for such evolutionary resolutions to sexual conflict, involving unique genetic causes, would provide the raw substrate for forming genetically intrinsic reproductive incompatibilities through Dobzhansky–Muller interactions in hybrid individuals (Orr, 1995). As a by-product of hybrid genetic incompatibilities, female choosiness in selecting mates might promote premating isolation under secondary contact that could subsequently evolve to an exaggerated extent by reinforcement or reproductive character displacement (Servedio, 2001). Because sexually antagonistic genes might contribute much of the genetic variation in fitness within a population, these loci offer plausible substrates for divergent selection to generate intrinsic incompatibilities between emerging species (Gavrilets, 2014).

How Can the Genetic Architecture of Sexually Antagonistic Selection Impact Evolutionary Responses?

Interlocus conflict provides a more effective potential source of population divergence and reproductive isolation than does intralocus conflict (Gavrilets, 2014). Such disparity between these two genetic modes of sexual conflict arises because of the tendency for intralocus selection to maintain polymorphism, rather than to drive allelic fixation, and for interlocus conflict to generate coevolutionary arms races between alleles with sex-biased expression at different loci. These conclusions ought to hold true regardless of the simplicity (one or two loci situations) or complexity (polygenic, quantitative genetic situations) of the genetic architecture of sexually antagonistic loci (Gavrilets, 2014), with the caveat that polymorphism maintenance via intralocus conflict for polygenic traits might not be stable in the absence of epistasis (Turelli and Barton, 2004; Arnqvist *et al.*, 2014). Whether sexually antagonistic selection operates primarily on standing genetic variability or on new mutations also represents a critical determinant of the potential for sexual conflict to generate irreversible constrictions on gene flow between populations. Responses to selection will always be more rapid when genetic differences are immediately available, meaning that trait divergence can accumulate especially quickly. But such rapid shifts in trait means from selection on additive genetic variation may easily be undone (Servedio and Bürger, 2014). The persistence of divergent populations as genetically distinguishable entities requires that barriers to hybridization are not leaky, and it is genetically intrinsic reproductive incompatibilities from new mutations that are more likely to enforce restricted gene flow even in the face of mating between nascent species, owing to irreversible hybrid dysfunction. Given that new mutational input might be

a rate-limiting step, even for conflict loci for which selection greedily favors novel alleles, it remains a challenging problem to assess the importance of distinct contributors to the genetic architecture of sexual conflict in the accretion of enduring divergence.

What Signatures of Sexual Conflict Reveal Its Influence on Speciation?

It remains a pernicious challenge to discriminate evolution by sexual conflict from other modes of sexual selection (Rowe and Day, 2006), making it especially difficult to demonstrate empirically an association with speciation. Compelling examples of rapid genetic changes in experimental populations owing to sexual conflict nevertheless do not generally make direct links to diversification and the accumulation of reproductive isolation (e.g., mating rates in *Drosophila*: Rice, 1996; Holland and Rice, 1999; Pitnick *et al.*, 2001a,b; Rice *et al.*, 2005). Despite a few examples illustrating proof of principle (but see Martin and Hosken, 2003; Hosken *et al.*, 2009a), given the inability of laboratory evolution to mimic natural conditions (Arbuthnott *et al.*, 2014) and other studies failing to demonstrate divergence coupled to reproductive isolation (e.g., Wigby and Chapman, 2006; Gay *et al.*, 2009; Gagnon and Turgeon, 2011), it remains unclear empirically how broadly antagonistic coevolution might apply to the speciation process.

An alternative to experimental evolution for inferring processes of male–female coevolution and their influence on speciation is to conduct interpopulation or interspecies crosses and examine traits indicative of reproductive isolation, such as mating rate, offspring production, and survivorship (Parker and Partridge, 1998; Andrés and Arnqvist, 2001). Genital structures evolve most rapidly and divergently by intersexual selection as genitalia typically differ, even between closely species (Arnqvist, 1998, reviewed in Hosken and Stockley, 2004). In particular, the male genitalia of *Drosophila* varies across the genus (Bachli and Vilela, 2002), within the *Drosophila melanogaster* species subgroup (Yassin and Orgogozo, 2013), and even within a natural population (Andrade *et al.*, 2009). Sexually antagonistic coevolution can explain why male genitalia are divergent between some species of *Drosophila* (Kamimura and Mitsumoto, 2012), but not all (see Eberhard and Ramirez, 2004). For example, females of *Drosophila yakuba* have coevolved structures (cavities with sclerotized platelets) to minimize infection risk caused by wounding with sclerotized spikes on the male genitalia (Kamimura, 2012), in contrast to females from the sister species *Drosophila santomea* that lack these cavities, as they have coevolved with males with rounded genitalia (Kamimura, 2012). Interspecies matings revealed enhanced mating costs from the risk of microbial infection, owing to genital mismatch and indicating sexually antagonistic coevolution within each species. Similarly, interspecies matings of *Caenorhabditis* nematodes revealed dramatic fitness costs, manifesting as sterilization and premature death to maternal nematodes (Ting *et al.*, 2014). Gametic barriers to fertilization define a potent contribution to reproductive isolation between *Caenorhabditis* species, mediated by heterospecific male sperm that induce the harmful effects by displacing conspecific sperm and by mislocalizing within the maternal gonad and to nonreproductive tissues (Ting *et al.*, 2014). The findings implicate a role of sexually antagonistic coevolution whereby female resistance adaptations to aggressive sperm evolved along different trajectories in different species. However, it remains unclear whether conflictual selection might have occurred during speciation per se or after other drivers of reproductive isolation had already created 'good species.' Moreover, caution must be taken in interpreting interpopulation crosses, as some outcomes are not necessarily unique to or required from sexually antagonistic coevolution (Rowe *et al.*, 2003).

Comparative phylogenetic analysis provides yet another avenue to evaluate a role for sexual conflict in species diversification. One such analysis identified higher species richness in clades with polyandrous compared to monandrous species, as a proxy for sexual conflict (Arnqvist *et al.*, 2000). Despite being consistent with a role for sexual conflict in promoting speciation, additional and more fine-grained analyses are warranted that might better distinguish conflict from other modes of sexual selection, incorporate quantitative measures of reproductive isolation (Rabosky and Matute, 2013; Yukilevich, 2013), and that exploit recent advances in partitioning speciation and extinction rates on phylogenies (FitzJohn, 2010).

How Might Sexual Conflict Hinder Speciation?

Speciation represents a compelling possible evolutionary outcome of sexual conflict and sexually antagonistic coevolution (see Lessells, 2006; Gavrilets, 2014). Sexual conflict could instead hinder speciation, however, and increase the risk of extinction (Rowe and Day, 2006; Rankin *et al.*, 2011). Sexual conflict could elevate extinction rates by reducing the mean fitness of populations (Doherty *et al.*, 2003; Rowe and Day, 2006), which can also occur when unresolved intralocus conflict impedes genetic divergence (Lande, 1980; Bonduriansky and Chenoweth, 2009). Populations can also be driven to extinction when sexual conflict over mating is a 'tragedy of the commons,' whereby traits favored by antagonistic selection are simultaneously beneficial to individuals and costly to the group (Hardin, 1968; Rankin *et al.*, 2007). For example, in the pursuit of increasing mating rates, excessive harassment by males might directly compromise female survival, creating a male-biased population that feeds back in turn until the population collapses (Rankin *et al.*, 2011; Grayson *et al.*, 2014). A possibility worthy of further investigation is to what extent sexual conflict might promote both speciation and extinction, perhaps contributing to more rapid species turnover but little to net diversification.

Conclusions

The prevalence of sexual conflict across the tree of life, combined with its capability to propel rapid evolutionary change, motivates the logic that sexually antagonistic selection might play a creative role in the speciation process or in the maintenance of nascent species' integrity. However, the genetic architecture that underlies sexual conflict (intralocus vs.

interlocus conflict, few vs. many loci, standing variation vs. new mutation) can drastically alter the implications regarding the potential for and permanence of population divergence. Evolutionary responses to antagonistic selection can, in some circumstances, retard population divergence, increase the likelihood of population extinction, or contribute to post-speciation enforcement of restricted gene flow instead of providing the causative feature to species origination. More generally, it remains difficult to confidently ascribe a causal role to sexual conflict in the speciation process, despite the conceptual attractiveness of this idea, making especially important all future mechanistic studies of sexual conflict at premating, gametic, and postzygotic levels in the evolution of reproductive isolation.

See also: Ecological Speciation and Its Consequences. Hermaphrodites. Reinforcement. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Sexual Conflict. Sexual Selection, Theory of. Speciation, Sexual Selection and. Species Concepts and Speciation

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Speciation, Sexual Selection and

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Glossary

Antagonistic coevolution Coevolution in which the evolutionary interactions between two parties (two sexes or two species) impose costs on each other because of different evolutionary interests. The metaphor of an arms race is often used to describe the process and its outcomes.

Assortative mating Mating between individuals that are similar in a trait or set of traits, such as size assortative mating in which large males mate with large females and small males with small females. Also used to indicate preferential mating between individuals of the same species over individuals of other species.

Cryptic female choice Female choice that occurs after copulation.

Fisher's runaway sexual selection A model of sexual selection conceived by R.A. Fisher to explain the exaggeration of both male display traits and female preferences in the absence of benefits to females. Genetic correlation between male trait and female preference creates continual evolutionary exaggeration of both until the reproductive benefit of the exaggerated male trait is balanced by the cost of producing it.

Good genes sexual selection A form of sexual selection in which females obtain genetic benefits, or 'good genes,' from mating with particular males.

Mate preference The selection of mates based on criterion values of specific trait(s). Preference influences the propensity of individuals to mate with certain phenotypes.

Mating trait The secondary sexual traits involved in mating. These typically include display traits and competitive traits for males, and mate search and preference for females.

Reproductive character displacement refers to the greater difference of reproductive traits in regions of sympatry between two species than are seen in regions of allopatry.

Reproductive isolation Speciation occurs via the evolution of isolating barriers, which are characteristics of organisms that keep individuals in one population from exchanging genes with other populations. Reproductive isolation can occur by preventing individuals of separate species from mating (premating isolation) or by selecting against hybrids (postmating isolation).

Sexual conflict The occurrence of conflicting evolutionary interests and optimal strategies for reproduction between the two sexes, including aspects of reproduction such as mating rate.

Sexual isolation A form of premating isolation in which the choosy sex of one population or species (usually female) is less likely to accept members of the other population or species as mates.

Sexual selection Variation among individuals with different trait values in the number of mates acquired and in overall reproductive success, measured as the number of offspring produced. Intersexual selection involves choosiness by one sex for mates of the other sex based on trait values (often called female choice). Intrasexual selection involves competition within a single sex for access to the other sex, frequently through contests (often called male competition).

Signal A trait modified by selection to convey information and to influence the behavior of individuals receiving it. Signals can be in various modalities, including visual, auditory, olfactory, or tactile. Male display traits are signals.

Speciation The process by which one or more species evolves from another via genetic changes and the evolution of mechanisms that restrict gene flow.

Introduction

Background

The idea that *sexual selection* can contribute to *speciation* is an old one, going back more than 150 years at least to Darwin's (1871) book *The Descent of Man, and Selection in Relation to Sex*. The hypothesis is a natural outcome of the observation that in many taxa, the traits that differ most strikingly between closely related species are secondary sexual traits, and these same traits appear to hinder mating between species. Many sexual traits evolve because of their role in attracting and choosing mates or in garnering the resources necessary to do so and thus, sexual selection is at the root of this evolutionary change. As long ago as 1930, Fisher articulated the conditions under which sexual selection could cause speciation (Fisher, 1930) but the topic was then largely ignored while speciation research focused on the role of geography (Mayr, 1942) and then on natural selection (Schluter, 2000). The hypothesis was revived in a seminal paper by Lande (1981). In that work, Lande formalized Fisher's verbal model with a theoretical model of how coevolutionary change between female preference and male sexual traits could 'runaway' to ever more exaggerated forms. *Fisher's runaway sexual selection* has hence become the starting point for much of the theoretical work on sexual selection and speciation. In principle, the runaway exaggeration of both male mating traits and female preferences for those traits can easily lead to differences among allopatric populations because of the arbitrary nature of sexual traits (they are not adaptive with respect to natural selection). This means they are free to vary in many directions and likely to differ between populations (Turelli *et al.*, 2001). If populations subsequently

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encounter one another, they are unlikely to mate because males in one population express trait values that females in the other population do not find attractive. This increases *sexual isolation*, a key component of *reproductive isolation* and thought to be one of the primary barriers isolating species in many taxa (**Figure 1**; West-Eberhard, 1983).

Empirical Case Studies Offer Support

Diverse in-depth studies have shown that sexual selection does contribute to speciation in many taxonomic groups. These empirical studies focus on the evolutionary mechanisms by which sexual selection causes speciation in particular groups of taxa, using observational data and manipulative experiments to test how sexual selection contributes to the process of speciation. For example, compelling evidence from neotropical frogs shows that populations differ in mating traits because of divergent sexual selection (where females prefer different traits causing sexual selection to act in different directions on males), and both genetic differentiation and reproductive isolation increase with greater difference in mating traits (Boul *et al.*, 2007). Another less direct line of evidence is when sexual traits differ substantially between species that are ecologically similar, such as that found in jumping spiders on Arizona mountaintops (Masta and Maddison, 2002), or *Laupala* crickets in Hawaii (Mendelson and Shaw, 2005). An outstanding question is whether these case studies represent a general pattern or are special in whether and how sexual selection acts to cause speciation.

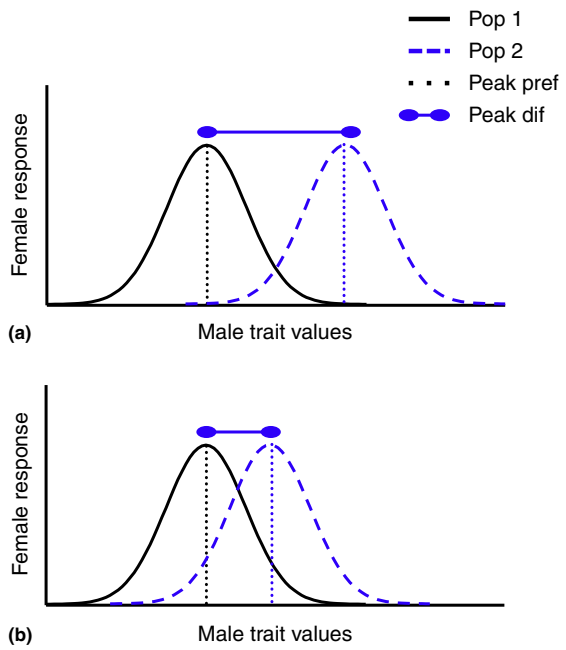

Figure 1 Female mate choice arises from underlying preferences for particular trait values. These can be described with preference functions, which consider how female sexual response depends on male trait values. The figure shows preference functions for two populations, which differ in the value of male trait preferred. Only where preference functions overlap is mating expected between two populations. (a) The populations differ substantially in trait values preferred (large peak dif). Low overlap means females from both populations will accept few males from the other population as mates, resulting in high sexual isolation. (b) The populations differ only a little in trait values preferred (small peak dif). High overlap means females from both populations will accept many males from the other population as mates, resulting in low sexual isolation.

Comparative Studies Are Equivocal

However evidence from comparative studies is much more mixed. These correlational studies explore the patterns of diversity and ask whether taxa in which sexual selection is present or strong have more species than those where sexual selection is absent or weak. Some studies report evidence in support (Owens *et al.*, 1999; Arnqvist *et al.*, 2000; Seddon *et al.*, 2013), others find no correlation (Gage *et al.*, 2002; Morrow *et al.*, 2003; Huang and Rabosky, 2014), and still others suggest that sexual selection may increase extinction rates more than speciation rates (Doherty *et al.*, 2003). Comparative analyses are done to test broad scale and general patterns, so if they succeed in doing so, then the equivocal results cast real doubt. In fact, these mixed results from comparative studies are a major reason why the hypothesis that sexual selection causes speciation is controversial (Panhuis *et al.*, 2001). However, comparative analyses typically use proxies for sexual selection (e.g., sexual size dimorphism or sexual dichromatism) and for speciation (e.g., species richness or number of species) and those proxies may be inexact or do not fully capture the action of sexual selection. These studies may then miss or gloss over real patterns. Recent work suggests that a refocus on the extent to which sexual selection differs among taxa will reveal cleaner answers than the historical focus on the strength of sexual selection (Rodríguez *et al.*, 2013). This makes sense, because most hypotheses suggest differences in mating traits are what causes isolation between species. Therefore, large differences in female preference are likely to cause large differences in male mating traits, whereas strong sexual selection may just speed up the rate of change (Rodríguez *et al.*, 2013).

Theory Is Equivocal

Results from theoretical work are likewise mixed. Theoretical work also focuses on generality, stripping away many biological details to reveal the basic principles and conditions where sexual selection will and will not facilitate speciation. A number of theoretical models strongly support a role of sexual selection in speciation. In some cases this is because of how environments affect the ability to detect male traits, related to sensory drive, as describe below (Higashi *et al.*, 1999; Kawata *et al.*, 2007). In other cases because antagonistic coevolution of male traits driven by *sexual conflict* leads to divergence (Gavrilets, 2000). However, other theoretical models suggest sexual selection is just as likely to hinder the process as facilitate it (Kirkpatrick and Nuismer, 2004; van Doorn *et al.*, 2004; Servedio, 2011). A landmark paper shows that Fisher's runaway on its own is unlikely to cause speciation, and that other mechanisms of sexual selection (and other theoretical approaches) are required (Servedio and Bürger, 2014). A few other models that incorporate *good genes*

sexual selection and which show that sexual selection can cause speciation are discussed below in the section 'Good genes'.

Sexual Selection versus Natural Selection

An additional controversy is over whether sexual selection can cause speciation on its own, or requires complementary natural selection. Proponents of sexual selection alone argue that because sexual selection is often far stronger than natural selection (Hoekstra *et al.*, 2001) and targets mating traits that have a direct role in determining whether gene flow occurs, it is likely to cause greater evolutionary divergence in mating traits and stronger reproductive isolation than natural selection (Svensson *et al.*, 2006; Boul *et al.*, 2007). Proponents of the counter argument acknowledge that greater evolutionary divergence may occur when both natural and sexual selection act in concert (Blows, 2002). This has the added bonus of generating differences in both mating traits to increase sexual isolation, and ecological traits to allow species to coexist (Kraaijeveld *et al.*, 2011). An important way to link sexual and natural selection and foster speciation is when females prefer traits that are also involved in divergent ecological adaptation (Podos, 2001; Servedio, 2004), essentially preferring the males that are well adapted to local environments (Welch, 2003; van Doorn *et al.*, 2009). This is related to the idea of magic traits, which also link mate choice to adaptation and makes speciation more likely. Of course, sexual and natural selection may oppose one another, which would retard divergence and impede reproductive isolation. This controversy requires further work to resolve.

How Sexual Selection Causes Speciation

Premating Isolation

Sexual selection should be especially important in the evolution of sexual isolation because of its importance in determining which individuals mate. Sexual isolation arises when males and females of different populations or species do not mate with each other, even when given the opportunity. Several forms of sexual selection could be involved in causing *mate preferences* and *mating traits* to diverge between populations. Focusing on the mechanisms of sexual selection causing speciation takes the study from descriptions of pattern to experimental tests of the process, giving far greater insight into the evolutionary process than correlational studies can provide.

Sensory drive

Sensory drive is a process that causes evolutionary change in communication systems – both in the sensory mechanisms needed to detect and discriminate communication *signals*, and in the structure of the signals themselves (Endler, 1992). The environment in which communication takes place affects the transmission of signals, selecting for those that transmit with minimal interference and degradation by habitat. Environments also select on properties of sensory systems to enable detection of predators, prey, and social partners (including mates). Even small differences in habitat can have substantial effects, causing both mating signals and sensory systems to adapt to local conditions, which leads to divergence between populations that live in different habitats (**Figure 2**). A natural outcome of this process is sexual isolation (Boughman, 2002). Speciation occurring due to this form of sexual selection has strong support from multiple taxonomic groups and studies.

Among the best worked out examples are African cichlid fish, where sensory drive on color perception, color preference, and color signaling is implicated in generating tremendous numbers of species – several hundreds in the African rift lakes of Victoria, Tanganyika, and Malawi (Seehausen *et al.*, 2008). Water color is the key aspect of the environment that mediates communication between male and female cichlids over mating. Differences in color perception are mediated by genes called opsins that influence how well specific wavelengths of light are detected and discriminated. Fish that live in clear shallow water express different opsins in their retinas than fish that live in red-shifted deep water, which leads females to prefer different color signals in their prospective mates. Water color also affects color and brightness contrast, which in turn affects signal detectability. Signals contrast when they are either different in color or different in brightness from the water color, and this contrast makes them easy to detect, especially when female eyes have evolved in response to water color as well. Males of the species found in the shallow clear water are blue and these females see blue well and prefer it, whereas males of the species found in deep red-shifted water are red and those females see red well and prefer it. Such divergent perception, preference, and signaling causes sexual isolation, as evidenced by color based *assortative mating* and genetic differentiation between the species (**Figure 2**).

Sensory drive is not limited to visual signals, because habitat also influences the transmission of other modalities, such as hearing and acoustic signals. A nice example of this is comparing rainforest birds that live in different types of forest (Tobias *et al.*, 2010). Species that live in bamboo forests produce songs with structure distinct from that of their close relatives that live in upland forest. The authors find that the pitch and pace of songs differs, with birds that live in dense bamboo singing songs of lower pitch, more complex structure, and faster pace than birds living in upland forest. This is consistent with predictions of sensory drive, because the song structure produced should resist degradation by the local habitat. Because song is important to mate choice it may also play a role in sexual isolation, although that has not been tested.

Good genes

A few studies have explored ways in which good genes sexual selection can contribute to sexual isolation. Good genes sexual selection occurs when females prefer males possessing alleles with high fitness (good genes), which makes females' choice of mates adaptive. Females typically use traits that correlate with genetic quality, or indicator traits, to identify those males. Two distinct mechanisms underlie this idea. The first is that the genetic benefits females obtain from their choice of mates is likely to depend on the environment – for example, obtaining genes for large offspring body size may be beneficial when resources are abundant and competition is fierce, but be costly

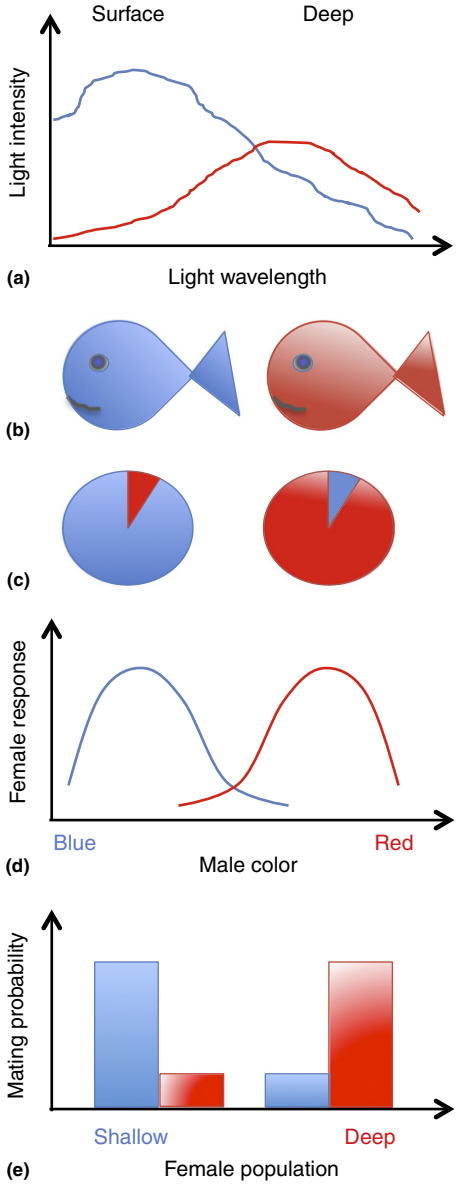

Figure 2 Sensory drive can cause sexual isolation as a by-product of adaptation to local signaling environments. Differences in signaling environment are likely to cause divergence in signals because of how they transmit in different environments; in sensory systems because of how environments affect detection of predators, prey, and mates; and in mate preference because it is affected by sensory abilities. The example shown is for water color and effects on fish diversification. On the left are data for a shallow water population and on the right for a deep water population. (a) Water color changes with water depth because of the way water attenuates light. The color spectrum in shallow water is bluish, whereas the spectrum in deep water is reddish. The curves shown are how light intensity varies across the light spectrum for surface and deep environments. (b) Male fish display colors that match the dominant water color, such that males are blue in shallow water and red in deep water. (c) Opsins expressed in the eyes of fish in shallow water consist predominately of the blue sensitive type, whereas opsins in shallow water are predominately red sensitive, making fish see those particular colors well. The pie charts show the frequency of each opsin type in the eyes of fish from shallow and deep water. (d) Female fish prefer different colors because of their sensitivity to different wavelengths of light, thus favoring blue males in shallow and red males in deep water. The curves show preference functions indicating how female response depends on male color. (e) The combination of color preference and male color leads to sexual isolation between shallow and deep dwelling fish. Females from shallow water are likely to mate with blue males but not red males, and the reverse is true from females from deep water. The bars show the probability of mating with blue males (blue bars) and red males (red bars) for females in shallow and deep populations.

when resources are scarce. This is termed as context dependent benefits of choice (Welch, 2003). It means that females in populations or species that live in different environments may have preferences for different male traits because females will acquire for their offspring a different set of alleles that are favored in that particular environment. Thus, the genes that are 'good' vary with environment, and preferences should evolve in concert in order to reap the genetic benefits of choice (Qvarnström, 2001).

The second, related way arises from the condition dependent expression of mating traits. Only males in good condition express high values of mating traits such as bright color, large ornaments, and vigorous courtship, because males in poor condition lack the energetic and physiological resources to do so (Kodric-Brown and Brown, 1984). Female preferences are predicted to evolve to favor these condition dependent traits (Schluter and Price, 1993; Rowe and Houle, 1996). The way this connects to speciation is that, local males who are well adapted to the local environment because they possess locally good genes, will be in good physical condition and express high values of mating traits, making them attractive to females (**Figure 3**). Foreign males from another population with different environmental conditions will not be well adapted to the local conditions, will be in poor condition, and express low trait values. Thus, with preferences for condition dependent traits, females will prefer males from their own population who are locally adapted, selecting against males who have migrated from populations in other environments because their poor condition means they express unattractive mating traits. This should increase sexual isolation even if female preferences do not differ between the populations (van Doorn *et al.*, 2009). This mechanism seems likely to be important in the context of climate change, because it is expected to result in range movement (Hickling *et al.*, 2005; Hitch and Leberg, 2007), potentially making this a widespread phenomenon.

Sexual conflict

Sexual conflict arises because males and females achieve high reproductive fitness in different ways. The consequence is that when females are at their fitness peak, males are not. Therefore, when females evolve to their fitness peak, this exerts selection on males to evolve traits that increase male fitness. The reverse is also true, that when males evolve to their fitness peak, this exerts selection on females. This tug of war results in continual *antagonistic coevolution* between the sexes. The direction of evolutionary change is not determined by environment (contrary to sensory drive), but instead, by the interactions between the sexes. Sexual conflict may easily cause

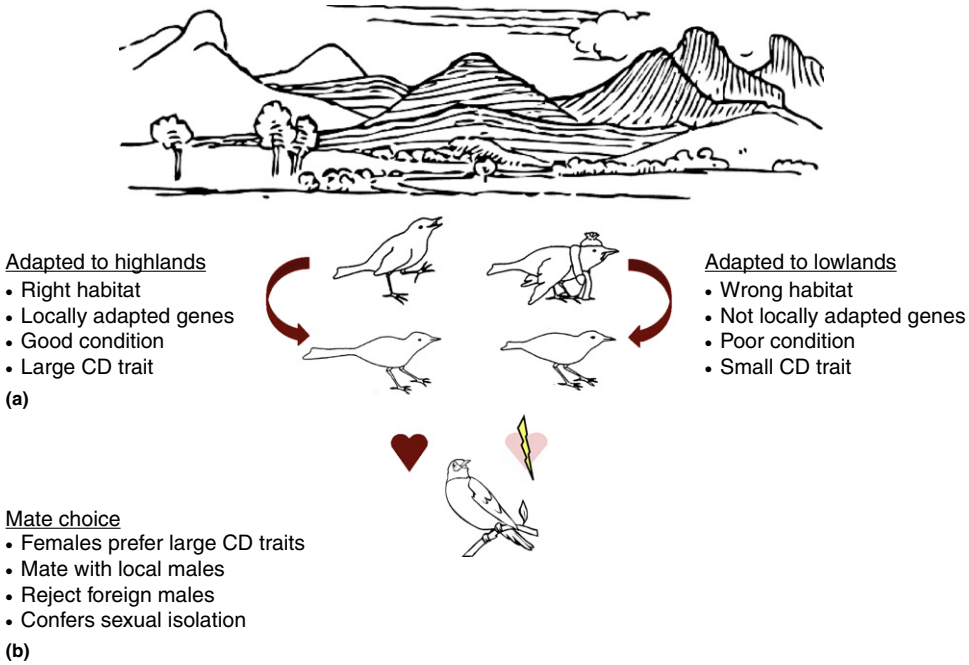

Figure 3 Condition dependent traits and preferences can lead to sexual isolation. (a) Long tail feathers and bright colors are condition dependent traits. Both highland and lowland males in their native habitat possess the genes that confer local adaptation, thus they are in good condition and have long tails. However, if lowland males migrate to the highlands they will not be locally adapted and are likely to be in poor condition. As a consequence, they will have short tails (a condition dependent trait). Thus, condition dependent traits function as indicators of local adaptation. (b) Females in both highland and lowland populations prefer long tailed males. Therefore, females are likely to reject foreign males who have immigrated because their poor condition gives them unpreferred trait values, or short tails. This results in sexual isolation between highland and lowland populations, simply due to condition dependent trait expression and preference for condition dependent traits.

allopatric populations to evolve in different directions. Subsequently, if populations come into contact males and females from the two populations are not likely to mate, increasing sexual isolation, and may even have reproductive incompatibilities, increasing postmating isolation. There is some theoretical support (Gavrilets, 2000), some comparative support (Arnqvist *et al.*, 2000), and some empirical support (Martin and Hosken, 2003) for sexual conflict increasing overall reproductive isolation. The route to speciation through sexual conflict is not completely clear however. Antagonistic coevolution leads to divergence and possibly speciation when females evolve resistance to male traits because this selects for exaggerated male traits to overcome resistant females, which perpetuates the coevolutionary cycle. If instead females evolve indifference to those male traits or even evolve discrimination against them, sexual isolation would contribute little to speciation. Other studies suggest that speciation is the least likely manner in which to ameliorate the conflict between the sexes (Rowe *et al.*, 2005); therefore, the role of sexual conflict in speciation requires further work.

Postmating Isolation

Sexual selection affects not only premating or sexual isolation, but also postmating isolation. Although much less studied than sexual selection on premating isolation, nonetheless, a few studies suggest this can occur through multiple avenues, including *cryptic female choice*, heterospecific sperm doing poorly in competition with conspecific sperm, hybrids having poor mating success, and even genetic incompatibility because of rapid evolution for genes that underlie reproductive traits (Birkhead and Pizzari, 2002).

Cryptic female choice and sperm competition

Cryptic female choice could contribute to postmating isolation in a few ways, because hybrid males are not likely to be preferred by females, but instead represent low-quality mates. In some species females can eject sperm from unpreferred males after copulation but before fertilization (Dean *et al.*, 2011), and it stands to reason that females would be especially likely to eject heterospecific sperm. Females can also alter their investment in offspring and thus, lower the survival and fitness of offspring from unpreferred partners (Burley, 1988; Sheldon, 2000; Loyau *et al.*, 2006). Competition among males for fertilization can also contribute to postmating isolation. When females mate with multiple males, the sperm of those males compete to fertilize the female's eggs, known as sperm competition (Parker, 1970; Birkhead and Pizzari, 2002). Studies have shown that conspecific sperm often have higher fertilization rates than heterospecific sperm. This can be caused by conspecific sperm just being better at fertilizing conspecific eggs (Price *et al.*, 2001), or by females mediating sperm competition and preferentially using sperm from conspecifics (Tyler *et al.*, 2013).

Sexual selection against hybrids

Even if all these other mechanisms fail so that hybrids are formed and survive to adulthood, hybrid males are likely to

BOX 1

A few studies suggest that occasionally, mating with heterospecifics can be adaptive. This occurs when there is little or no intrinsic postmating isolation, such that hybrid offspring are likely to survive, and when the heterospecific species has traits or alleles that are beneficial in particular environmental circumstances. A well-known example is in spadefoot toads, which breed in ephemeral ponds. When water levels are low, ponds may dry out before tadpoles can metamorphose, favoring rapid development. In these conditions, females from the slow maturing species *Spea bombifrons*, can benefit from mating with rapidly maturing *Spea multiplicata* because their hybrid offspring are more likely to reach metamorphosis (Pfennig, 2007).

have secondary sexual traits that are unusual combinations of trait values or intermediate to the pure species that produced them. Sexual selection against hybrids is a natural extension of sexual selection within species because females often evolve strong preferences for particular trait values. Given that females have often evolved to be quite selective in which males are acceptable as mates, their mismatched or intermediate phenotypes are likely to make hybrid males unattractive to females of both pure species, who instead find conspecific males more attractive. Thus, sexual selection acts against these hybrids, which have low mating success and thus low reproductive fitness. This has been seen in hybrid flycatchers (Svedin *et al.*, 2008), in hybrid butterflies (Naisbit *et al.*, 2001), and in hybrid sticklebacks (Vamosi and Schluter, 1999). It can contribute substantially to overall reproductive isolation, for example, comprising 25% of the total postmating isolation in flycatchers (Svedin *et al.*, 2008; but see **Box 1** for counter examples).

Female Choice and Male Competition

Most of the work to date focuses on how sexual selection generated from female choice contributes to speciation, but sexual selection also arises from males competing with each other for mating opportunities. Increasingly, scientists are asking whether and how male competition contributes to speciation. The most commonly considered mechanism for male competition in speciation is that if males are more likely to be aggressive to males who are similar to them, males with rare phenotypes will escape most aggression. This creates frequency dependent disruptive selection that favors divergence (Seehausen and Schluter, 2004; Qvarnström *et al.*, 2012). Essentially, males who have average phenotypes receive the most aggression, and males with rare phenotypes (at the extremes of the phenotypic distribution) the least. The general pattern is similar to how competition for food resources favors rare types which then generates disruptive selection (Dieckmann and Doebeli, 1999). Empirical tests of this idea are mixed; however, with some studies showing this effect is insufficient to cause speciation (Dijkstra *et al.*, 2007). Perhaps more promising is the idea that it's not rareness per se, but that certain combinations of phenotypes are successful in male competition, and that there is more than one way to beat your rivals (Keagy *et al.*, 2015). For example, large and aggressive males can win contests, but so can brightly colored and quick males. Therefore, two different types can have high fitness through male competition. Which males win competitive contests may also vary with habitat, suggesting that male competition can lead to divergent selection between habitats (Lackey and Boughman, 2013).

A related idea is the role that male competition can play in *reproductive character displacement* when two formerly allopatric species come into contact (Grether *et al.*, 2009). Males who are sufficiently different are not recognized as competitors, thus avoiding the costs of competitive interactions. For example, several species of damselflies differ in whether male wings are clear, black, or ruby spotted. Males direct aggression to other males who share their wing color pattern, so when two species are similar, they 'waste' aggression on heterospecific males. Much better is to reserve energy and time for driving off only conspecific males who are truly competing for conspecific females. This selects for differences between species in sympatry, resulting in reproductive character displacement to facilitate recognition of competitors (Anderson and Grether, 2010).

Male competition and female choice are the two halves of sexual selection, which interact to determine the overall direction and strength of sexual selection. Whether they work in concert to favor the same divergent set of phenotypes or in contrast to favor different sets of phenotypes will affect how much sexual selection contributes to overall diversification and isolation. Little theoretical work has been done to investigate this, but one model found that when the traits that confer success in male competition are also attractive to females, divergence and sexual isolation are enhanced (van Doorn *et al.*, 2004).

Learned Mate Choice and Speciation

Recent work is highlighting the potential for learning to foster speciation, although it can sometimes hinder the process (Verzijden *et al.*, 2012). Mate preferences can be either genetically encoded or acquired by learning, and the traits used to attract mates can also be either genetic or learned. Mate preferences can be learned through imprinting, where juveniles learn the phenotypes of parents, and choose mates later in life who match those parental phenotypes. This is fairly well known in birds, where for example, great tits raised by foster blue tit parents in the wild, grew up to sing like blue tits and preferred mating with blue tits (Slagsvold *et al.*, 2002; Johannessen *et al.*, 2006). Imprinting is less well understood in other taxa, but may be widespread. For example, in sticklebacks, females raised by foster fathers imprint on traits that are targets of divergent natural selection, thereby learning to prefer mates of the foster father species over conspecifics and causing increased sexual isolation (Kozak *et al.*, 2011).

Mate preferences can also be learned through experience with potential mating partners. Learning can be involved for both sexes, but males and females will often differ in what is learned. Studies show that females often learn to prefer familiar phenotypes, which should promote sexual isolation (Kozak and Boughman, 2009). When mate preferences are learned, either through imprinting or prior experience, speciation is promoted (Verzijden *et al.*, 2005; Servedio *et al.*, 2009), because such learning enhances the fidelity with which

females choose mates like themselves. This has the effect of keeping the ecological and mating traits together, enhancing divergence and maintaining trait-preference correlation.

Males may modify courtship behavior based on experience, courting familiar phenotypes based on positive responses from conspecific females, and avoiding unfamiliar phenotypes based on negative responses from heterospecific females, as was shown for *Drosophila* (Dukas, 2008). Both of these effects should facilitate speciation. However, when the traits males use to attract mates are learned, such as birds learning song, this often inhibits speciation because learning disconnects the phenotype from genotype. For example, if a young male finch learns the song of a heterospecific, he will be unattractive to conspecific females and more likely to mate with heterospecifics. This is likely to lead to hybridization, and undermine sexual isolation.

Sexual Selection and Speciation in Plants

Sexual selection occurs not only in animals but also in plants (e.g., Bernasconi *et al.*, 2004). Although the particulars are different, the principles are shared. For example, both animals and plants attract potential mates through elaborate display: in animals through exaggerated secondary sexual traits such as plumes or song that potential mates find attractive, whereas in plants this may be through elaborate floral traits such as petal color or nectar rewards that potential pollinators find attractive. These pollinators are essential to actual mating in outcrossing plants. Another shared mechanism is competition to fertilize ovules: in animals through sperm competition and in plants through pollen competition. Male traits may influence success in sperm and pollen competition (e.g., how quickly sperm/pollen reach the ovules, or how many sperm/pollen grains are transferred), and may thus experience strong sexual selection through male competition. Females may not be passive in this process, and may influence which sperm/pollen fertilize their eggs through cryptic female choice.

To what extent does sexual selection in plants contribute to speciation? Some high profile cases have provided evidence that floral traits which differ between species will attract different pollinators or cause variation in pollen placement, resulting in pollinator isolation (reproductive isolation mediated by animal pollinators, which is akin to sexual isolation in animals) (e.g., Schemske and Bradshaw, 1999; Bradshaw and Schemske, 2003; Ramsey *et al.*, 2003). Such pollinator isolation can create substantial barriers between species and recent work finds this barrier is strong in a number of systems (Baack *et al.*, 2015). Whether this mechanism is solely responsible for speciation is questioned in a review by Kay and Sargent (2009), who suggest that although pollinator isolation occurs, it is often combined with other isolating mechanisms. Their arguments are reminiscent of the debate on whether sexual selection alone or in combination with natural selection is more powerful and/or more likely to cause speciation. Further work will be needed to resolve these questions, but it seems that multiple barriers contribute in almost all systems studied to date; not surprising given that speciation is a complex process that may unfold over long periods of time. Pollen competition can also contribute to reproductive isolation when conspecific pollen are more likely to fertilize ovules than heterospecific pollen. This is termed conspecific pollen precedence, and can occur if conspecific pollen tubes grow more rapidly or germinate at higher rates than heterospecific pollen, as seen in several plant systems, such as *Iris* (Carney *et al.*, 1996), *Helianthus* (Rieseberg *et al.*, 1995), and *Mimulus* (Ramsey *et al.*, 2003). Cryptic female choice can contribute to these patterns as well, if female traits have evolved to mediate competition between conspecific and heterospecific pollen, thus favoring the production of conspecific offspring (Baack *et al.*, 2015). Moreover, heterospecific seeds may be selectively aborted, a process similar to differential allocation in animals (Sheldon, 2000). These mechanisms may be widespread, given that about half the plant taxa investigated by Lowry *et al.* (2008) show strong pollinator isolation and/or pollen competition, and pollinator isolation is one of the strongest barriers. Thus, sexual selection may be a key mechanism generating reproductive isolation in plants, and deserves more intensive study.

Conclusion

Given the many ways in which sexual selection can contribute to the process of speciation and its power to cause evolutionary change, it almost certainly plays a role in generating the diversity we find around us. Exactly what that role is, and under what conditions speciation requires sexual selection to proceed are areas of very active research.

See also: Ecological Speciation and Its Consequences. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Speciation, Sexual Conflict and

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Speciation-with-Gene-Flow

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Glossary

Divergence hitchhiking The process by which physical linkage to a gene under divergent selection increases genomic divergence for adjacent regions along a chromosome. Divergence hitchhiking creates a localized region of reduced gene flow, enhancing the potential to maintain or accumulate differentiation at linked sites, both neutral and those under selection.

Divergent selection Selection that acts on genetically based traits in different directions between two populations, including the special case where selection favors two extremes within a single population (i.e., disruptive selection).

Dobzhansky–Muller incompatibilities Genetic incompatibilities that evolve between populations or closely related species without going through an adaptive valley. It requires changes in two or more loci that cause reduced fitness within hybrids. For example, if an ancestral population is homozygous at two loci (AABB) and divides into two populations, a new allele arises and fixes at one locus in one population (population 1 = aaBB), but is incompatible with another new allele that arises at the second locus in the second population (population 2 = AAbb). When combined in hybrids, these incompatibilities cause reduced fitness in the hybrids (AaBb).

Genome hitchhiking The process by which genetic divergence across the entire genome is facilitated, even for loci unlinked to those under selection, by a global reduction in average genome-wide gene flow caused by selection.

Genome-wide congealing A process associated with the combined effects of genes throughout the genome during adaptive divergence that results in a rapid and nonlinear transition from one ancestral genome into two distinct genomes and one population into two reproductively isolated species.

Host races Ecologically divergent, partially reproductively isolated, sympatric populations of plant-feeding insects, the presumed initial stage in speciation-with-gene-flow.

Linkage disequilibrium (LD) The nonrandom association of alleles at two or more loci arising from physical proximity of genes on a chromosome (linkage) and/or by strong epistatic interactions of linked or unlinked alleles that affect the fitness of an organism more strongly than either allele alone (epistasis).

Parapatry Two or more populations or species (or other taxonomic unit) whose geographic ranges partially overlap or are immediately adjacent to each other. In parapatry, populations or species may exchange genes to some degree.

Primary contact When two or more divergent evolutionary lineages (i.e., populations or species) evolve differences while in geographic contact.

Recombination In eukaryotes, a process by which a piece of DNA is broken and then joined to a different piece during meiosis via crossing over which leads to the offspring having different combinations of alleles from those of their parents.

Reinforcement A process by which natural selection favors the increase of reproductive isolation between two evolutionary divergent populations that geographically overlap, either in primary or after secondary contact, in response to the production of maladapted hybrids.

Reproductive isolation Barriers to gene exchange between populations or species is called reproductive isolation.

Secondary contact Recent geographic overlap of two or more divergent evolutionary lineages (i.e., populations or species) after a period of geographic isolation, which allowed for some degree of differentiation to occur from a common ancestor.

Selection–recombination antagonism A term that describes how recombination breaks up associations between selected loci related to ecological adaptation and loci causing reproductive isolation related to assortative mating, impeding genetic divergence across the genome and constraining speciation-with-gene-flow.

Sympatry Two or more species (or other taxonomic unit) that geographically overlap in the same locality where taxa have the opportunity to exchange genes (i.e., gene flow).

Overview: The Major Issues of Speciation-with-Gene-Flow

Speciation occurs as genetically based barriers to gene flow evolve and cause previously interbreeding populations to become reproductively isolated from one another (Mayr, 1963). One of the biggest debates about speciation concerns whether populations can diverge into new species when they geographically overlap and are connected by gene flow (termed speciation-with-gene-flow; Berlocher and Feder, 2002; Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007). Generally speaking, speciation-with-gene-flow includes situations in which populations overlap entirely in their ranges (sympatric) or only partially (parapatric; **Figure 1**). It also includes cases in which populations evolve in the face of continuous gene flow and have always been in contact (primary contact) or have experienced periods of geographic isolation (allopatry; **Figure 1**) and episodes of subsequent gene flow (secondary

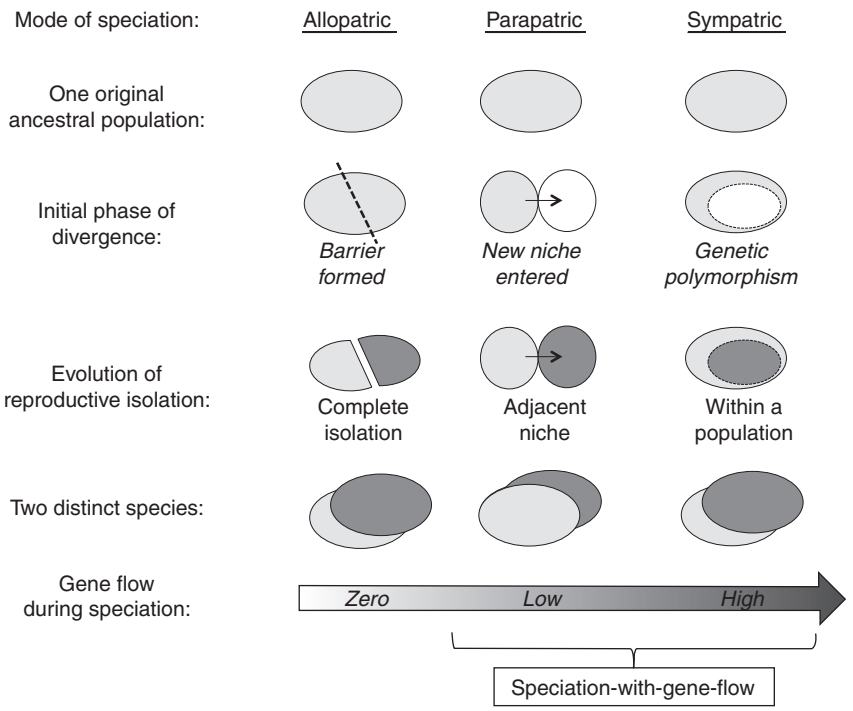

Figure 1 Different geographic scenarios of speciation highlighting the opportunity for gene flow during the speciation process.

contact). The key issue is whether additional reproductive isolation can evolve that culminates in speciation when gene flow is occurring between populations.

The most contentious question concerning speciation-with-gene-flow is whether the process can occur *de novo* in populations in primary contact (termed sympatric speciation; Coyne and Orr, 2004). However, there has also been considerable debate about whether additional prezygotic reproductive isolation (i.e., barriers to gene flow acting before zygotes are formed) can evolve between populations in secondary contact (termed reinforcement; Noor, 1999; Ortiz-Barrientos *et al.*, 2004). In this latter case, a degree of post-zygotic reproductive isolation (i.e., genes causing the inviability or sterility of hybrids) has accumulated between populations during the period when they were geographically separated. Once they reestablish contact and hybridize, selection could then act to directly favor traits that reduce the degree that individuals from the different populations interbreed because of the reduced fitness of their offspring. Although sympatric divergence and reinforcement may not be as common as other forms of speciation, accumulating evidence over the last 20 years (see below) has increasingly shown that these processes can occur and are not as uncommon as once thought.

History

The debate about speciation-with-gene-flow dates back to Benjamin Walsh, a colleague of Charles Darwin, who proposed that plant-eating insects may undergo speciation without geographic isolation (i.e., in sympatry) by shifting and adapting to new host plants (Walsh, 1864). The key to Walsh's hypothesis of speciation-with-gene-flow is that strong divergent selection imposed by different habitats or environments acts as an ecological barrier to gene flow and initiates speciation. Populations cannot simultaneously adapt to the contrasting selection pressures imposed by different habitats to be jack-off-all-trades generalists. Traits that result in individuals surviving better or garnering more mates in one environment have detrimental consequences in other habitats (i.e., fitness tradeoffs exist). Consequently, migrants moving between habitats or hybrids between individuals from different populations do not thrive compared to resident parental types. Thus, an ecological reproductive barrier is generated that, if strong enough, can result in speciation.

The Selection–Recombination Antagonism

An important consideration that has been argued to constrain speciation-with-gene-flow is called the 'selection–recombination antagonism' (Felsenstein, 1981). While divergent selection can create alternate combinations of genes adapted to different habitats, migration and hybridization between individuals from different populations, coupled with subsequent recombination during meiosis, can break these suites of genes apart, homogenizing populations and impeding progress toward speciation. As a result, it has been argued that strong divergent selection must act on genes closely physically linked together in the genome affecting different aspects of ecological adaptation (e.g., for plant-eating insects, loci encoding traits for performance and choice of a specific host plant) in order for speciation-with-gene-flow to proceed. Such strong selection

may be rare in nature and genes effecting ecological adaptation may only fortuitously, and not commonly, be closely linked in the genome.

Given these considerations, some evolutionary biologists have argued that divergence-with-gene-flow may be rare in nature compared to allopatric speciation (Mayr, 1963; Futuyma and Mayer, 1980; Coyne and Orr, 2004). The reason is that geographically isolated populations can evolve independently and unopposed by gene flow. Thus, there is no constraint imposed by the selection–recombination antagonism. Because hybrids do not form, diverged combination of genes that form in allopatry are not broken down. Moreover, divergent and directional natural selection, sexual selection for mating preferences, and even random genetic drift can all contribute to population differentiation in allopatry. In comparison, divergent ecological selection is the primary process initiating speciation-with-gene-flow in primary contact. Post-zygotic isolation due to 'intrinsic' genomic incompatibilities between genes (aka Dobzhansky–Muller incompatibilities) is more likely to evolve in allopatry. In this case, new mutations may be favored by natural selection in the genetic background of the population they arise in. However, if these populations were to ever come back into secondary contact and hybridize, the mutations may interact negatively and cause inviability or sterility when mixed for the first time with those of the other population. It is generally thought that intrinsic reproductive isolation cannot evolve readily during the initial stages of speciation-with-gene-flow because migration and interbreeding between populations would immediately expose the negative fitness effects of incompatibility causing mutations in hybrids, resulting in their rapid elimination. Thus, the presence of Dobzhansky–Muller incompatibilities between geographically overlapping and hybridizing populations would indicate either (1) a case of secondary contact, or (2) that substantial 'extrinsic' ecological reproductive isolation has already accumulated in primary contact to allow for intrinsic post-zygotic to evolve.

Evidence for Speciation-with-Gene-Flow: The Apple Maggot Fly

The greater number of ways that reproductive isolation can evolve and the reduced importance of linkage for divergence when populations are geographically isolated imply that speciation is easier in allopatry than in the face of gene flow. However, this should not be taken to indicate that speciation-with-gene-flow cannot happen and is exceedingly rare. Indeed, just as Benjamin Walsh had predicted over 100 years earlier (Walsh, 1864), empirical evidence began to accumulate in the 1980s that ecologically divergent, sympatric host races of plant-feeding insects, the presumed initial stage in non-allopatric speciation, could form in the face of gene flow (Berlocher and Feder, 2002; Dres and Mallet, 2002).

One example argued by Walsh (1867) and later by Bush (1969) to be a prime example of speciation-with-gene-flow is the apple maggot fly, *Rhagoletis pomonella*, which historically infested the fruits of native hawthorne trees (*Crataegus* sp.), but a genetically distinct host race has evolved in the last 150 years to infest apples, *Malus domestica* (Bush, 1966; Bush, 1969). *Rhagoletis* flies infest the fruits of many different species of host plants from many different plant families. Host fruits are typically available for a discrete window of time over the growing season and each fly species having one generation per year (Dambroski and Feder, 2007). Adult flies meet exclusively on or near the host fruits to mate, females lay eggs into the host fruit, larvae consume the fruit, then burrow into the soil to pupate, and enter a pupal diapause that lasts until the following year. Thus, phenological matching of fly to host plant fruiting is critical to fly fitness. Over 25 years of genetic and field studies have demonstrated that *R. pomonella* flies from apple and hawthorne trees represent partially ecologically reproductively isolated host races, the hypothesized initial stage of speciation-with-gene-flow (Filchak *et al.*, 2000). There is no evidence for intrsinic, non-host-related reproductive isolation between the host races (Linn *et al.*, 2004) and mark-recapture studies have determined that flies migrate between apples and hawthorne trees at a gross rate of ~4–6% per generation (Feder *et al.*, 1994). Thus, gene flow has been continuous between the host races since their origin and geographic isolation (allopatry) can be discounted as a factor contributing to the build up of genomic divergence (Feder *et al.*, 2013). Diapause and differential fruit choice are two major host-related adaptations ecologically reproductively isolating apple and hawthorne flies (Feder *et al.*, 1997; Linn *et al.*, 2003). Apple varieties that are commonly infested by *R. pomonella* flies produce fruit about three weeks earlier than hawthorne trees during the late summer or early autumn (**Figure 2**). Associated with this ecological difference, the apple fly race has evolved earlier emergence times and changes in diapause phenology. As a result, apple and hawthorn flies mate at different times of the season and display a degree of allochronic isolation. In addition, hawthorne flies generally prefer the odor of the

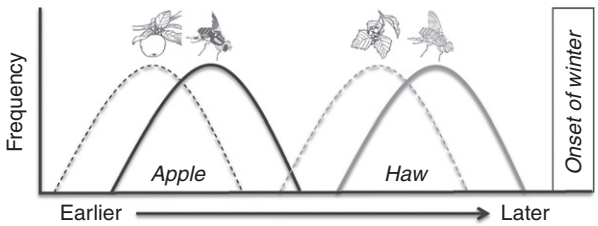

Figure 2 A textbook example of speciation-with-gene-flow: the ancestral hawthorn (gray lines) and recently derived apple-infesting (black lines) host races of the tephritid fruit fly *Rhagoletis pomonella*. *Rhagoletis* flies infest the fruits of many different species of host plants from many different plant families. Host fruits are typically available for a discrete window of time over the growing season (dashed lines) and each fly species (solid lines) having one generation per year. Adult flies meet exclusively on or near the host fruits to mate, females lay eggs into the host fruit, larvae consume the fruit, then burrow into the soil to pupate, and enter a pupal diapause that lasts until the following year. Thus, phenological matching of fly to host plant fruiting is critical to fly fitness and divergent selection between populations on different trees is strong enough to drive speciation in the face of gene flow. Illustration credit: *R. pomonella* – Chris Brown; host plants – Erin Kelso. Redrawn from Egan, S.P., Ragland, G.R., Assour, L., *et al.*, 2015. Experimental evidence of genome-wide impact of ecological selection during early stages of speciation-with-gene-flow. Ecology Letters 18, 817–825.

volatile compounds emitted from ripe hawthorne fruit and use this as a cue to find and orient to hawthorne trees while avoiding apples, whereas the reverse is true for apple flies (Linn *et al.*, 2003; Forbes *et al.*, 2005). Because adults mate on host fruit, the differences in host preference translate directly into mate choice and prezygotic reproductive isolation between hawthorne and apple flies. Recent work comparing allele frequency changes across the genome induced by a selection experiment with allele frequency differences observed between sympatric haw and apple host races in nature demonstrated that natural selection affects large fractions of the genome during divergence (Egan *et al.*, 2015).

Other Textbook Examples for Speciation-with-Gene-Flow

Evidence from other plant-feeding insects (e.g., goldenrod gall flies, leaf beetles, and treehoppers; Abrahamson and Weis, 1997; Wood, 1980; Funk, 2010; reviewed by Berlocher and Feder, 2002; Dres and Mallet, 2002), from parasites attacking specialist insects, as well as from other animals and plants (e.g., cichlid fish and palm trees; Barluenga *et al.*, 2006; Savolainen *et al.*, 2006; reviewed by Bolnick and Fitzpatrick, 2007), further strengthened the case that taxa could diverge in sympatry in the face of gene flow. The finding that reinforcement could promote the evolution of additional reproductive isolation between populations following secondary contact, for example, between *Drosophila* fruit flies (Noor, 1999), has also led to a greater appreciation that all of the genetic differences contributing to speciation need not arise in allopatry. Finally, other examples of ecologically based speciation that appear to involve differences not wholly evolved in allopatry, such as stickleback fish, lake whitefish, pea aphids, and sunflowers (reviewed by Feder *et al.*, 2013), have also helped shift debate from the feasibility of speciation-with-gene flow to how prevalent it is in nature and what are the most important factors promoting and constraining the process.

New Theoretical Insights

At the same time that empirical evidence was accumulating for speciation-with-gene-flow, new theoretical insights widened the conditions under which reproductive isolation could evolve in sympatry and parapatry (Dieckmann and Doebeli, 1999). For example, if assortative mating is based on habitat preference rather than differentially choosing mates in a common mating pool or if the same allele causes assortative mating in both populations, then the selection–recombination antagonism can be alleviated (Felsenstein, 1981; Servedio *et al.*, 2011). Moreover, when divergent selection occurs and migration is not completely random between populations, a degree of disequilibrium can be established between performance genes even in the absence of genetically based habitat choice and physical linkage among loci.

Other arguments have been made that 'divergence' and 'genome' hitchhiking can increase the effectiveness of divergent selection and facilitate the evolution of reproductive isolation in the face of gene flow (Feder and Nosil, 2010). In divergence hitchhiking, selection on an already diverged gene reduces the effective migration rate locally for regions of the genome around the selected locus. This makes it easier for new divergently selected mutations to 'hitch' a ride with the diverged gene and become established because they are not opposed by as high a rate of gene flow as implied by the gross migration rate of individuals between populations. Genome hitchhiking occurs when several genes are diverged in the genome. In this case, the collective effect of multifarious selection on these genes results in a global, genome-wide reduction in effective migration rate. As a result, new mutations, even those not under strong divergent selection, can begin to accumulate across the genome without being tightly physically linked to already diverged loci (Feder *et al.*, 2012).

We caution that recent results should not be interpreted as meaning that population divergence is easier in sympatry or parapatry than allopatry. However, for many organisms that experience their environments on fine spatial and temporal scales, divergence in the face of some gene flow may be fairly common and not as limited by theoretical considerations, as previously thought. Moreover, even if speciation is not primarily initiated in sympatry or parapatry, many populations may not completely diverge and speciate in allopatry. Mixed modes of speciation in which populations experience periods of allopatry interspersed with secondary contact and gene flow may be frequent. Indeed, in certain cases like sticklebacks and *Rhagoletis* flies, genetic variation that arose in the past in allopatry may be present at high standing levels in extant populations to help fuel rapid divergence-with-gene-flow when new ecological opportunity presents itself (Schluter, 2000; Schluter, 2001; Feder *et al.*, 2003; Bolnick, 2011).

Next-Generation Sequencing: Testing the Role of Genome Structure in Speciation-with-Gene-Flow

New empirical and theoretical findings have shifted the emphasis of researchers from testing whether speciation-with-gene-flow can occur (it can) to investigate in more detail the conditions and factors facilitating the process. In this regard, a focal point of study investigates the role that genome structure may play in making speciation-with-gene-flow more or less likely (Seehausen *et al.*, 2014), which has been enabled by advances in next-generation DNA sequencing. When there is no limit to the number of mutations and traits under strong divergent selection, in principle, speciation will progress relatively unimpeded, even in the face of gene flow. Here, genome structure is not imperative because the direct effect of selection on individual mutations is sufficient for them to establish and differentiate populations. However, when this is not the case, which may be typical, then divergence and genome hitchhiking take on increased significance and genome structure can be an important consideration for speciation-with-gene-flow (Feder *et al.*, 2012).

Next-generation sequencing has helped enable this line of enquiry by allowing genomic differentiation to be characterized between populations at varying stages of the speciation process, thus, providing a means to test for patterns of divergence consistent with hitchhiking. It is generally thought

that speciation-with-gene-flow will generate a heterogeneous pattern of genetic differentiation across the genome between diverging populations (Nosil *et al.*, 2009). Regions that contain genes under divergent selection and tightly linked neutral markers will show differences, while the remainder of the genome will tend to be homogenized by gene flow. This has given rise to a metaphor of 'genomic islands of divergence,' where a genomic island is any gene region which exhibits significantly greater differentiation than expected under neutrality (Turner *et al.*, 2005). Divergence hitchhiking may be important for allowing differentiation to build from these initially isolated islands to successively encompass more of the genome (Via and West, 2008; Via, 2012). Thus, divergence hitchhiking predicts that populations very early in the speciation-with-gene-flow process should display only a few regions of exceptional divergence in genome scans (Feder *et al.*, 2012). Additional mutations contributing to ecological adaptation and reproductive isolation should subsequently accumulate almost exclusively in these genomic islands, as population progress further in speciation, until they eventually spread to cover the entire genome. Recent theoretical analyses have suggested, however, that windows of reduced effective migration around selected sites may be narrower than has been argued (Feder and Nosil, 2010), implying that while divergence hitchhiking may provide a boost in certain circumstances, the process is not essential for speciation-with-gene flow. An exception may be chromosomal inversions and translocations, which can decrease recombination rates across larger stretches of the genome and, thus, may qualitatively increase the potential for divergence hitchhiking to occur compared to collinear regions where gene order is the same along chromosomes (Noor *et al.*, 2001).

In contrast, genome hitchhiking predicts that not just a few strongly selected genes, but many more weakly selected loci across the genome, are often and principally involved in divergent ecological adaptation (Flaxman *et al.*, 2013). When a critical threshold number of these genes accumulate to reduce effective migration genome-wide, populations will show a rapid, nonlinear transition from comparatively low to high levels of adaptive genetic divergence, and between locus linkage disequilibrium. Reproductive isolation essentially becomes an increasing characteristic of the genome rather than of individual genes, as the genomes of populations congeal into the alternate adaptive entities we recognize as species. Hence, genome hitchhiking predicts that many genes across the genome will be found to show low level differentiation in early stages of speciation-with-gene-flow followed by a marked and dramatic increase in levels of divergence between populations that have moved into the genome-wide-congealing phase of the process (Flaxman *et al.*, 2014).

Genome scans in a range of organisms, including *Timema* walking sticks (Soria-Carrasco *et al.*, 2014), *Heliconius* butterflies (Nadeau *et al.*, 2012), *Anopheles* mosquitos (Lawniczak *et al.*, 2010), *Rhagoletis* fruit flies (Michel *et al.*, 2010), *Gasterosteus* threespine sticklebacks (Jones *et al.*, 2012), *Coregonus* lake whitefish (Gagnaire *et al.*, 2013), and *Helianthus* sunflowers (Scascitelli *et al.*, 2010), have revealed extensive genetic differentiation between populations even at the early stages of ecological divergence. These taxa may therefore have the capacity to undergo genome-wide congealing during speciation-with-gene-flow (Flaxman *et al.*, 2014). Manipulative transplant and selection experiments on key conditions or traits known to differentially affect natural populations of *Rhagoletis* (Michel *et al.*, 2010; Egan *et al.*, 2015) and *Timema* (Soria-Carrasco *et al.*, 2014) also imply that numerous loci having moderate to weak effect relative to gene flow contribute to divergent ecological adaptation. Also, population comparisons of butterflies have revealed what looks like nonlinear increases of divergence through time (Nadeau *et al.*, 2012). However, much further research is required to confirm that divergence hitchhiking and genome-wide congealing resulting from the consequences of numerous small effect genes are important genome-level processes facilitating speciation-with-gene-flow.

Conclusion

New research on speciation-with-gene-flow is currently yielding novel insights into the origins of biodiversity. This understanding is being forged from accumulating evidence from nature on speciation-with-gene-flow involving many different forms of life in combination with next-generation DNA sequencing of the genome. By comparing taxa at varying stages in the speciation continuum from partially isolated races to near completely separated taxa, progress is being made in characterizing how genes are arrayed and collectively diverge during speciation. This research will indicate whether different stages of the speciation-with-gene-flow process exist, how different genome structures evolve and are influenced by divergence being initiated in sympatry versus parapatry versus allopatry, and how genome structure affects the potential for further population divergence through processes such as divergence and genome hitchhiking. The study of speciation-with-gene-flow has therefore progressed from research primarily documenting the process to analyses of genome-level processes facilitating population divergence.

See also: Ecological Speciation and Its Consequences. Genetic Variation in Populations. Hybrid Speciation. Reproductive Isolation, Prezygotic. Speciation Continuum. Speciation Genomics. Speciation, Geography of. Species Concepts and Speciation

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Species Concepts and Speciation

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Introduction

Sexual recombination and mutation often produce continuous distributions of traits in a population (Galton, 1889; Wright, 1931; Fisher, 1958). However, as students of nature can confirm, continuity of form often breaks down as populations move through environmental gradients (Barton and Hewitt, 1985), migrate over geographical obstacles such as rivers and mountains (Mayr, 1942b), or coexist with related organisms that compete for resources or mates (Darwin, 1859; **Figure 1**). Although these circumstances immediately suggest that evolutionary forces like natural and sexual selection as well as genetic drift cause the decoupling of variation within and between populations (Coyne and Orr, 2004), the biological meaning of the resulting morphological gaps is less obvious.

Understanding the origins of discrete phenotypes among populations is one of the most fundamental and enduring problems in evolutionary biology; it not only underpins our understanding of the origins and maintenance of biodiversity, but also forms the conceptual basis of the species problem. This article provides a brief introduction to our current understanding of what species are and how they originate. It discusses the species problem (Mallet, 1995; Coyne and Orr, 2004; Lowry, 2012) and reviews the relationship between gene flow and the origin of new species, while discussing the likelihood of speciation under different geographical settings (Dobzhansky, 1940; Futuyma and Mayer, 1980; Gavrilets, 2000; Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007). This article also describes the various forms of reproductive isolation that accumulate as species originate, and it highlights some integrative questions about the speciation process that require further development.

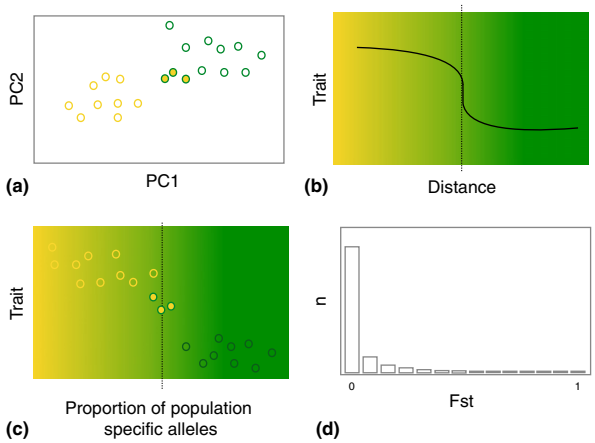

Figure 1 Phenotypic variation in nature is discontinuous. The principles of genetics suggest that sexual recombination should create a continuous distribution of phenotypes. However, careful examination of trait variation suggests that organisms cluster into groups of well-defined morphologies. (a) Morphological clusters often arise as populations colonize contrasting habitats (yellow and green landscape). (b) Traits may cline or abruptly step into different morphospaces. Alleles, or linked variants, responsible for the trait difference sort into each habitat. (c) Individuals found in one habitat contain a large fraction of such alleles (yellow circles), whereas individuals found in the other habitat contain alternative alleles responsible for their specific phenotypic value (green circles). In (a), (c) a few individuals may have mixed fractions of specific alleles from the two populations as shown with the green circles filled with yellow. How striking the proportions of mixed and non-mixed genotypes reflect the relative strength of divergent selection and homogenizing gene flow in a given trait. (d) Frequency distribution of Fst values between two populations. Because this process likely reflects the early stages of speciation, genomic differentiation between populations is likely to be low and to reflect similarity due to ancestral polymorphism, although in some cases can reflect homogenization by gene flow.

The Nature of Species

The origin of new species can be understood in two major ways. On one hand, speciation is the set of processes that create morphological and genotypic discontinuity in populations. Darwin was the first to provide a meaningful explanation for this process by suggesting that natural selection is responsible for the phenotypic gaps seen in nature (Darwin, 1859). These gaps arise from the elimination of unfit individuals with intermediate phenotypes, which leads to the origin of forms often distinguished by multiple traits and by their inability to occupy each other's niche. More recently, these ideas have been extended to include the formation of genotypic gaps between populations, or the formation of genotypic clusters in sympatry (Mallet, 1995). Although these 'Darwinian species' are probably common, many could be short-lived given that environmental conditions can be unstable over long periods of time, and the homogenizing effects of gene flow can often overpower the differentiating effects of natural selection.

On the other hand, species originate when interbreeding groups of individuals can no longer reproduce with other such groups (Dobzhansky, 1937, 1940; Mayr, 1942a, 1963). In this view, reproductive gaps, and not necessarily morphological ones (e.g., sibling species are nearly identical), define biological species, and neither environmental fluctuations nor hybridization can eliminate the genetic differences that have accumulated between them. (Coyne and Orr, 2004; Rieseberg *et al.*, 2006). These two major solutions to the species problem, Darwinian and biological species, have helped us understand how natural selection and other evolutionary forces play a role in the origin of biodiversity. However, the two solutions assign a different emphasis to the role of evolutionary forces: while Darwinian species arise by divergent natural or sexual selection during primary contact, many different evolutionary forces acting in a variety of geographic

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Box 1 Speciation and gene flow

Sympatric and parapatric speciation, as well as reinforcement of reproductive isolation, are difficult because gene flow antagonizes the forces that create divergence (such as drift and natural selection). Gene flow can be present since the beginning of sympatric and parapatric speciation (primary contact), or during the completion of allopatric speciation (when populations come into secondary contact). Although primary contact may never lead to divergence of populations, and secondary contact can fuse them, theoretical models suggest that strong divergent selection, physical linkage between genes responsible for adaptation, and stable genetic associations between prezygotic and postzygotic reproductive isolation can facilitate speciation with gene flow in both cases. Sympatric and parapatric speciation are likely to always lead to the origin of Darwinian species, but perhaps less likely to the origin of biological species.

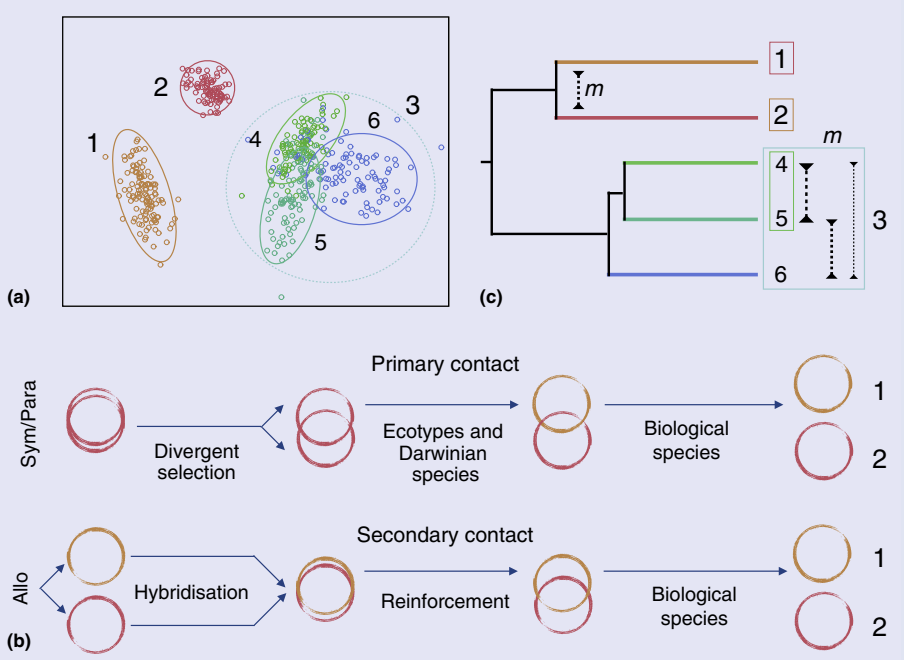

Figure Box 1 Speciation with gene flow during primary and secondary contact. (a) Populations showing strong associations between phenotype and habitat often form clearly defined clusters in multivariate trait space (clusters 1 and 2), and are likely to be reproductively isolated. (b) However, some populations may partially overlap in morphology, either because they are in the early stages of speciation, or because they have come back into contact after a previous period of allopatric divergence (clusters 4–6). Natural selection can resolve these stages and lead to the formation of populations clearly separated by complete extrinsic or intrinsic reproductive isolation. (c) Examination of variability in multiple genetic markers is expected to reveal the history of their divergence, although patterns of gene flow can distort relationships amongst populations. *m* represents migration rate between populations, and its strength is denoted by the thickness of the inverted arrows connecting a population pair.

settings (including secondary contact) can fuel the origin of biological species (**Box 1**; **Figure 2**).

Different from its sexual counterparts, asexual organisms are difficult to sort into these views (Holman, 1987; Cohan, 2002, 2006; Barraclough *et al.*, 2003; Cohan and Koeppel, 2008; Tang *et al.*, 2014). However, given that natural selection often favors single asexual genotypes that replace the entire population in a given habitat, asexual species can be better understood in terms of their adaptations, and perhaps as genotypic clusters suited to particular environments. This is because complete replacement of genetic variability leads to a correlation between genotype and phenotype, and thus to organic discontinuity. In groups of organisms where both sexual and asexual reproductions is rampant (agamic complexes; e.g., Krak *et al.*, 2013), organic discontinuity does not arise, suggesting that the origin of species is intimately related to the mode and prevalence of sexual reproduction in any given system.

Reproductive Isolation

Speciation studies investigate the evolution of genotypic clusters and of biological species. In other words, they seek to understand how divergent natural and sexual selection create the morphological and genotypic gaps of Darwinian species, and how reproductive isolation evolves and leads to the irreversible formation of biological species. Studying the origin of genotypic clusters and biological species often requires performing ecological (Hatfield and Schluter, 1999; Rundle and Schluter, 2004; Rundle and Nosil, 2005, e.g., Thibert-Plante and Hendry, 2009), behavioral (e.g., Noor, 1995; Noor *et al.*, 2001; Moehring *et al.*, 2006) (depending on the organism), and genetic experiments between populations that grow suboptimally in each other's habitat (e.g., Lowry *et al.*, 2008b; Lowry and Willis, 2010), fail to recognize heterospecific partners (Ortiz-Barrientos *et al.*, 2004), or fail to produce fertile or viable hybrid offspring (Presgraves *et al.*, 2003;

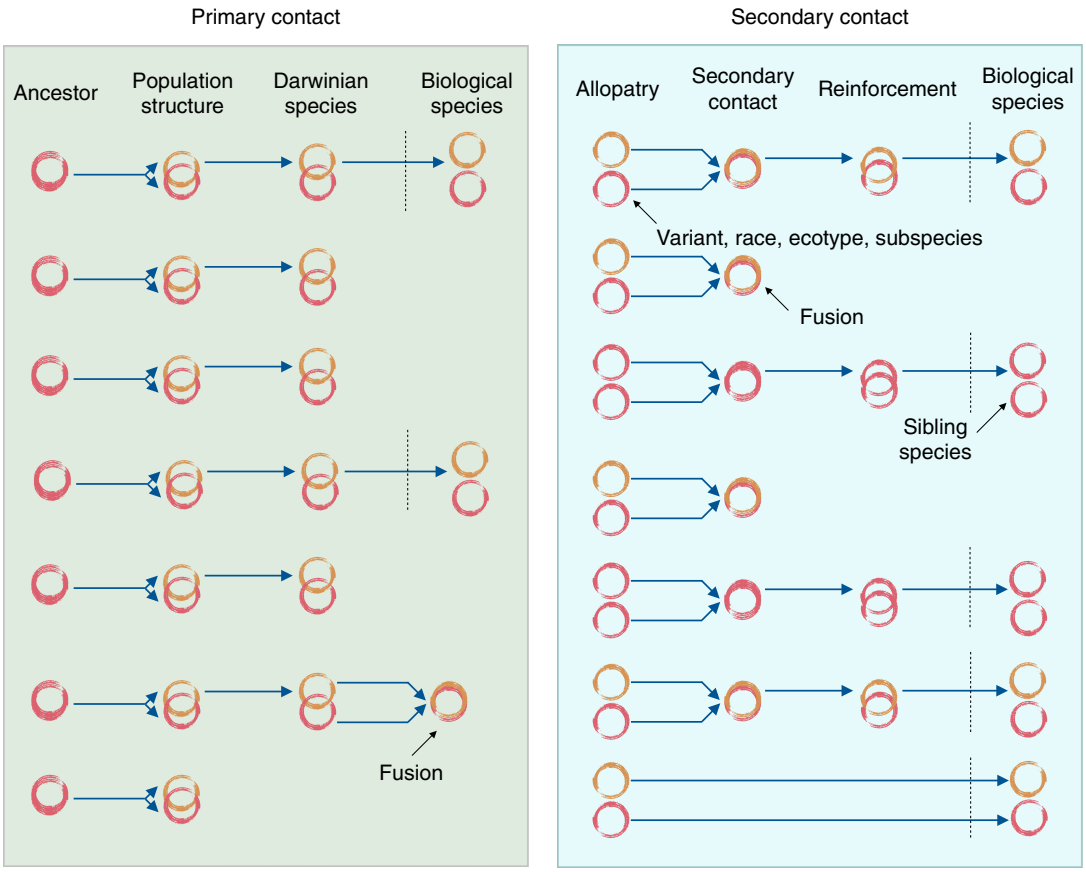

Figure 2 Primary and secondary contact speciation, and the distribution of speciation events. The left panel shows primary contact speciation, where divergent natural selection, mainly leads to the creation of Darwinian species, and less often to the formation of biological species. The transition from Darwinian to biological species might occur only if there is either the right genetic architecture for linking extrinsic and intrinsic reproductive isolation or gene flow fully ceased between Darwinian species so intrinsic isolation can evolve unimpeded by homogenizing gene flow. Fusion and lack of divergence are also possible outcomes. The right panel shows secondary contact speciation, where previously allopatric populations come into contact and either fuse, remain separated by a hybrid zone, or complete the speciation process via reinforcement of reproductive isolation. Allopatric populations might have differentiated via natural selection (either divergent or uniform) or drift, and the outcomes of speciation do not necessarily lead to morphologically distinct species (i.e., sibling and cryptic species). Dotted vertical line represents the moment at which speciation becomes irreversible.

Presgraves, 2010a; Moyle, 2007). Despite the differences between Darwinian and biological species, these experiments on speciation address whether reproduction works as effectively between populations as it does within populations, and whether the kind of reproductive isolation that evolves is dependent on the ecology of the organism (see Sobel and Chen (2014) for a recent discussion on how to measure reproductive isolation). To understand the genetic, behavioral and ecological basis of speciation, the various forms of reproductive isolation that arise when populations become new species must first be described (Sobel *et al.*, 2010).

Reproductive Isolation *before* the Formation of Heterospecific Zygotes

Prezygotic reproductive isolation evolves when traits facilitating access to mates and gametes diverge between populations. Well-known prezygotic isolating barriers include the evolution of flowering time differences in plants (e.g., Hall and Willis, 2006; Savolainen *et al.*, 2006), the evolution of female preferences for male songs in insects and frogs (Blair, 1974, e.g., Littlejohn and Watson, 1985; Pfennig, 2003; Pfennig and Pfennig, 2010), and habitat isolation in response to adaptation to contrasting environments in plants (Ramsey *et al.*, 2003; Angert and Schemske, 2005; Sobel *et al.*, 2010; Schemske, 2010; Sobel, 2014; Sobel and Streisfeld, 2015; Baack *et al.*, 2015; Nakazato *et al.*, 2010). Because of the distinction between finding mates, and gametes finding each other after copulation, it is appropriate to call reproductive barriers that affect fertilization but not mating postmating-prezygotic isolation in animals (Price, 1997; Howard, 1999; Servedio, 2001; Matute, 2010b), and postpollination-prezygotic isolation in plants (Baack *et al.*, 2015). A common and important form of postmating-prezygotic isolation is called conspecific gametic precedence: here, mixed loads of pollen or sperm from two populations result in offspring ratios biased toward conspecific over heterospecific individuals (Rieseberg *et al.*, 1995; Price, 1997; Chang, 2004).

Reproductive Isolation *after* the Formation of Heterospecific Zygotes

If heterospecific zygotes form, hybrids might not be as viable and fertile as their diverged parents. Any change in development that reduces viability and fecundity of hybrid individuals represents a form of postzygotic reproductive isolation (Sasa *et al.*, 1998; Price and Bouvier, 2002; Presgraves, 2002; Lijtmaer *et al.*, 2003; Moyle, 2007). However, hybrids may suffer in more subtle ways, like when they fail to mate with other hybrids or parental types even though they are viable and fertile (Noor, 1997). Because the causes could in principle be so vast, the study of hybrid inviability largely relies on genetic experiments that isolate the causal genes of hybrid death, coupled with subsequent experiments that find the normal function of the gene in the parental species (Presgraves *et al.*, 2003; Barbash *et al.*, 2003; Orr *et al.*, 2004; Presgraves, 2007b; Phadnis *et al.*, 2015). The reasons for hybrid sterility are perhaps more restricted and might relate to normal variation found in reproductive genes within populations (e.g., Sun *et al.*, 2004; but see, Masly *et al.*, 2006).

Although hybrids tend to show reduced fitness compared to their parents, this need not be the case. In plants, it is common to find hybrid vigor (Lowry *et al.*, 2008a; Baack *et al.*, 2015), where hybrids show higher fitness than their parents; this can weaken some ecological forms of postzygotic reproductive isolation (see next section) and perhaps divergence during the early stages of speciation. Hybrids can also form new populations in areas where their parents cannot grow and reproduce, and therefore become new hybrid species (Barton, 2001; Greig *et al.*, 2002; Gross and Rieseberg, 2005; Rieseberg and Willis, 2007; Abbott *et al.*, 2013; Baack *et al.*, 2015). Finally, in some cases of primary and secondary contact between populations with adjacent habitats, hybrids can exist in intermediate environments, or ecotones, and form phenotypic and genetic clines that serve as conduits of genetic exchange between the diverged populations (Barton and Hewitt, 1985; Jiggins and Mallet, 2000; Abbott *et al.*, 2013). Whether hybrid zones often resolve in complete reproductive isolation, extrinsic or intrinsic, remains to be empirically explored.

Extrinsic and Intrinsic Reproductive Isolation

Extrinsic reproductive isolation refers to barriers to reproduction that arise from heritable variation that interacts with environmental conditions (Rundle and Whitlock, 2001; Rundle, 2002; Rundle and Nosil, 2005). These barriers manifest only in the habitats of the two diverging populations (**Figure 3(a)**). The opposite is true for intrinsic reproductive isolation, where barriers manifest in any environmental condition where hybrid formation occurs (**Figure 3(b)**; Coyne and Orr, 2004). The genetic changes responsible for intrinsic barriers are also expected to accumulate non-linearly ('snowball') with time (Moyle and Nakazato, 2010; Matute *et al.*, 2010; Orr, 1995), thus making the evolution of biological species difficult to reverse. One key difference between the two forms of barriers is the inexorable involvement of natural selection in extrinsic but not necessarily intrinsic reproductive isolation. Extrinsic barriers are predicted to evolve when divergent natural selection drives traits apart to the extent that any intermediate phenotype would be maladapted in the habitat of the two parents (Rundle, 2002), and any migrant phenotype would fail to survive in the alternative habitat of the other population (Nosil *et al.*, 2005).

Although intrinsic barriers may evolve as a by-product of divergent natural selection acting on traits within populations (Nosil, 2012), genomic conflict and, meiotic drive (Presgraves, 2010a,b), and gene transposition (Masly *et al.*, 2006) can also create hybrid sterility and inviability. Extrinsic barriers are the quintessential feature of Darwinian species, whereas intrinsic barriers are the critical component of biological species as they create irreversibility of divergence between populations. Although the study of these barriers has historically been split between ecologist and geneticists, respectively, modern speciation research aims to integrate the study of both, making it easier to understand the origin of both Darwinian and biological species, and the transitions across the speciation continuum (**Figure 4**; Wu, 2001; Lowry, 2012; **Box 2**).

Questions about Reproductive Isolation

How Important Is Genetic Drift in Speciation?

The role of natural selection and genetic drift in speciation can be easily perceived: divergent natural selection can trigger speciation events or complete them via reinforcement, whereas genetic drift leads to the inexorable accumulation of genetic differences between allopatric populations that create reproductive isolation between populations. Less obvious it is how genetic drift plays a role during colonization of new areas (e.g., via founder effects, Mayr, 1942a; Templeton, 1980; Coyne, 1994; Matute, 2013) or during speciation with gene flow (Via, 2009, 2012; Feder *et al.*, 2012; Nosil, 2012). A founder effect occurs when dramatic reductions in population size restructure the gene pool of a population after colonization of a new area. Some of these changes are expected to alter the mean value of traits causing reproductive isolation between populations, thus leading to what is known as founder speciation. Although some theoretical scenarios suggest that reproductive isolation may evolve directly from changes in population size (Slatkin, 1997; Uyeda *et al.*, 2009), experimental and observational evidence shows that the principal consequence of founder effects, inbreeding, leads more often to extinction than to speciation, suggesting that founder speciation exists in nature but might be rare (Matute, 2013).

Divergence arising from genetic drift is unlikely when populations diverge in the face of gene flow. This is because migration leads to the recombination of parental genomes during meiosis in hybrids and therefore to the eventual homogenization of allelic frequencies between populations (Felsenstein, 1981). However, neutral alleles can accumulate in regions that are recalcitrant to homogenization, which often are under strong divergent natural selection (Nosil *et al.*, 2009). Because of their physical proximity, recombination during meiosis in hybrids cannot decouple the neutral allele from the selected allele. Therefore, the neutral allele diverges together with the selected allele, in a process termed divergence hitchhiking (Via, 2012). Theory also suggests

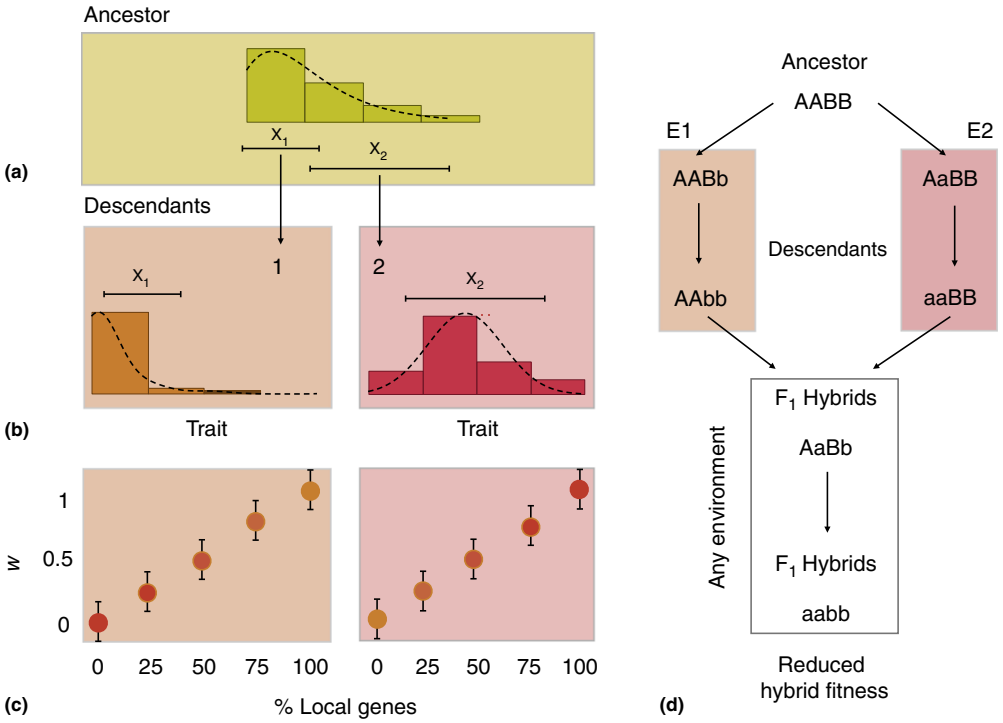

Figure 3 Extrinsic and intrinsic postzygotic reproductive isolation. (a) Trait distribution in an ancestral population that occupies the yellow habitat. The population colonizes two abutting and contrasting habitats where different mean trait values are favored (X_1 and X_2). (b) As divergent natural selection shifts trait means apart, populations start manifesting extrinsic reproductive isolation. (c) The strength of extrinsic reproductive isolation is a function of the proportion of local genes in the individuals exposed to each habitat. Migrants (i.e., 0% local genes) have reduced fitness (W) in the alternative habitats (immigrant inviability). F_1 hybrids, in the absence of dominance show an intermediate fitness response, whereas the two types of backcrosses (25% and 75%) shift rank in fitness across the two environments (note that the backcross type containing 25% of local genes in one environment would contain 75% of local genes in the other environment). This pattern is consistent with the effects of ecology-based divergent natural selection being responsible for the origin of reproductive isolation between populations adapting to contrasting environments. Other forms of selection (e.g., uniform selection) can also create reproductive isolation, but they would not lead to this pattern (see Nosil, 2012 for a review). (d) The evolution of Dobzhansky–Bateson–Muller genetic incompatibilities. An ancestral interaction between loci A and B evolves to fix alternative alleles of one of the loci in two populations found in two different environments; however, natural selection or genetic drift can drive the process. Hybrids between the two populations will experience reduced fitness because the alleles *a* and *b* have not been tested against each other (note that these are only a subset of all fixed differences that accumulate between the two populations).

that unlinked neutral alleles may diverge beyond neutral expectations if natural selection is quite strong, in a process termed genome hitchhiking (Feder *et al.*, 2012).

The effect of selected sites on neutral variability is also problem fundamental to the formation of hybrid zones. Hybrid zones establish when there is a balance between natural selection and migration (Barton and Hewitt, 1985), and this determines the movement of neutral alleles across the zone. The strength of selection creating the hybrid zone is visualized in terms of gradients that reflect how quickly phenotypes and genotypes change per unit of geographic distance (i.e., phenotypic and genetic clines, Harrison, 1993). The movement of neutral alleles across the zone largely depends on how close they are to the selected loci, and on the strength of selection (Barton and Bengtsson, 1986). Strong barriers to gene flow in a hybrid zone can delay the movement of neutral alleles for hundreds of generations, thus facilitating genetic differentiation in the face of gene flow (Barton and Gale, 1993), and possibly the accumulation of additional barriers to gene flow between the populations (Nosil *et al.*, 2009). Whether hybrid zones resolve into irreversible species or not, remains an active question of speciation research (Rudman and Schluter, 2016).

Is There a Genetic Link between Extrinsic and Intrinsic Reproductive Isolation?

Ecology-based divergent natural selection leads to the accumulation of extrinsic reproductive isolation (Schluter, 2001, 2009; Rundle and Nosil, 2005; Schluter and Conte, 2009; Nosil, 2012). Theoretically, hybrids will display intermediate trait values that would render them unfit in parental habitats (Rundle and Whitlock, 2001). Similarly, migrants will fair poorly in each other's habitats (Nosil *et al.*, 2005). Different from extrinsic postzygotic isolation (that relies on additive genetic variance for fitness), intrinsic postzygotic isolation relies on the evolution negative epistatic interactions amongst loci derived from two populations (Orr, 1995; **Figure 3(b)**). As mentioned before, intrinsic reproductive isolation manifests in any environment where hybridization occurs, including the laboratory, and regardless of the evolutionary

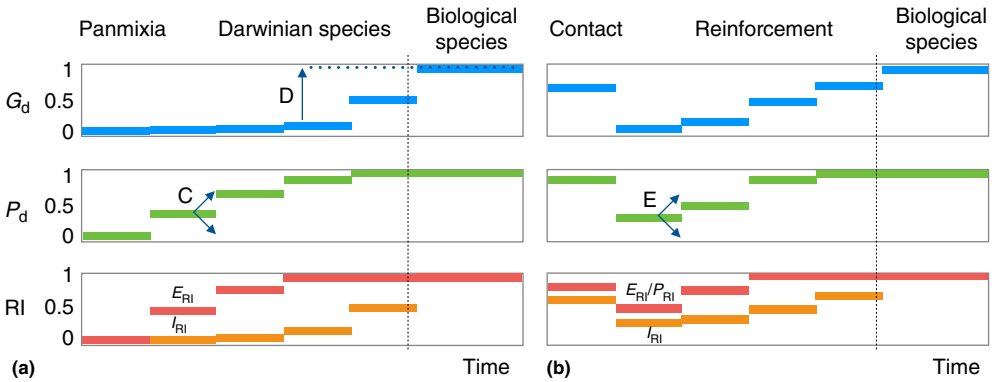

Figure 4 The evolution of genetic and phenotypic differentiation and the accumulation of reproductive isolation between populations. (a) Species may originate in sympatry or parapatry if natural selection is relatively stronger than the amount of gene flow between the two populations. Populations start in panmixia where genetic (G_d) and phenotypic (P_d) differentiation is minimal, and intrinsic reproductive isolation (I_{RI}) is absent. If natural selection is creating differentiation despite gene flow, then extrinsic reproductive isolation (E_{RI}) will become stronger in the system and both migrants and hybrids will be removed from the populations. Darwinian species arise when multiple traits and genes clearly define phenotypic and genetic clusters. Extrinsic reproductive isolation can be quite strong at this stage, thus reducing gene flow to minimal levels and facilitating divergence between populations due to drift and selection. Intrinsic reproductive isolation can now freely evolve without the homogenizing constraints of gene flow, thus creating evolutionary independence and species irreversibility. (b) A similar process occurs when two previously allopatric populations come into secondary contact (but this may be difficult to distinguish if it happened long ago). Levels of genetic and phenotypic divergence as well as the evolution of reproductive isolation are initially moderate, eventually increasing in strength as speciation proceeds. One way in which this can happen is via reinforcement. Here, maladaptive hybridization creates a strong selective pressure for favoring individuals that prefer conspecific over heterospecific individuals. C and E represent the points at which divergent selection favors the evolution of alternative traits, including behavioral ones. D represents the special case in which entire genomes start to diverge dramatically as a consequence of very strong selection acting on many loci (Feder *et al.*, 2013; Flaxman *et al.*, 2014).

forces that drove the fixation of alternative interacting alleles. The negative epistatic interactions that reduce fitness in hybrids are called Dobzhansky–Muller–Bateson (DMB) genetic incompatibilities (Bateson, 1909; Muller, 1942; Dobzhansky, 1934), and they have been the intense focus of genetic research in the last decades (Presgraves, 2010a). Although relatively few genes causing DMB incompatibilities have been identified, it appears that in most cases natural selection and genomic conflict have driven their rapid evolution (Presgraves *et al.*, 2003; Presgraves, 2007a). But, is it possible to reconcile the role of natural selection in creating both forms of reproductive isolation?

One viable scenario that links the evolution of extrinsic and intrinsic reproductive isolation by natural selection is via a special form of divergence hitchhiking, which in this article is referred to as isolation hitchhiking. Under divergence hitchhiking, neutral alleles linked to divergently selected alleles also become highly differentiated between the two diverging populations. This occurs because gene flow cannot breakdown the genetic association between adaptive and neutral alleles that arises from their proximity along a chromosome. Although most neutral mutations will be harmless in hybrids, a fraction of them will be part of a genetic incompatibility, and therefore will create intrinsic reproductive isolation. Isolation hitchhiking therefore refers to the evolution of intrinsic isolation as a by-product of DBM incompatibilities evolving in tight linkage with those genes causing extrinsic reproductive isolation. It remains unclear whether the genetic variability necessary for isolation hitchhiking exists, but recent work in monkey flowers from the genus *Mimulus* suggests that is possible (Presgraves, 2013; Wright *et al.*, 2013).

In the absence of linkage within a chromosome, mechanisms that suppress recombination between these two kinds of mutations could facilitate their genetic association (**Figure 5**). For instance, if a population carries the locally adapted genotype *AABB* and a second population the locally adapted genotype *aabb*, recombinant genotypes are expected to display reduced fitness. Consequently, any modifier allele that reduces recombination between A and B will be favored (Lenormand and Otto, 2000), including chromosomal inversions (Kirkpatrick and Barton, 2006). Once in place, this region of suppressed recombination, which contains the genes responsible for extrinsic postzygotic reproductive isolation, facilitates the accumulation of the alleles responsible for the evolution of DBM incompatibilities and therefore of intrinsic postzygotic reproductive isolation. This mechanism of isolation hitchhiking has an advantage over the linkage model explained before: because some modifiers of recombination, such as inversions, can suppress recombination rates over long stretches of a chromosome, they also increase the mutational availability for the evolution of DBM incompatibilities. Both models predict co-localization of quantitative trait loci for extrinsic and intrinsic forms of reproductive isolation, which should map to regions of suppressed recombination.

Even though suppression of recombination facilitates speciation with gene flow, extrinsic and intrinsic reproductive isolation could be genetically independent, and evolve sequentially under specific circumstances. For instance, intrinsic reproductive isolation may evolve once extrinsic reproductive isolation has reduced gene flow to nil levels (Seehausen *et al.*, 2014). Different from scenarios of suppressed recombination, this would facilitate the accumulation of DMB incompatibilities across most of the genome (**Figure 5**). Note that this

Box 2 Phylogenies, genealogies and reproductive isolation

A major consequence of population differentiation is the sorting of ancestral alleles into independent lineages and thus the eventual formation of groups where allelic ancestry is confined to the lineage and not to other such (**Figure 4**). Achieving this level of lineage-specific allelic ancestry (i.e., monophyly) can take a long time, particularly in large populations where loci can remain polymorphic for many generations. Therefore, the historical relationships of some genes may not reflect the relationships arising from the speciation process (Avise and Wollenberg, 1997; Pagel, 1999; Edwards and Beerli, 2000; Beltran *et al.*, 2002; Brumfield *et al.*, 2003; Leache *et al.*, 2014; Edwards *et al.*, 2016).

The use of phylogenies, where only one individual per type is sequenced, and genealogies, where many individuals per population are sequenced, are some of the most common approaches to establish taxonomic relations across the tree of life. Although phylogenies and genealogies arise from the same principles that create species, the population genetics of ancestral and new mutations make it difficult to create straightforward equivalences between phylogenetic or genealogical species and Darwinian or biological species. However, phylogenies and genealogies can provide insights as to the basic role of speciation versus extinction in creating diversification (Reddy *et al.*, 2012; Rabosky *et al.*, 2013; McGuire *et al.*, 2014; Rabosky, 2015), reveal the tempo of speciation in swift evolutionary processes such adaptive radiations (Rundell and Price, 2009; Gavrilets and Losos, 2009; Agrawal *et al.*, 2009; Rabosky and Glor, 2010; Reddy *et al.*, 2012).

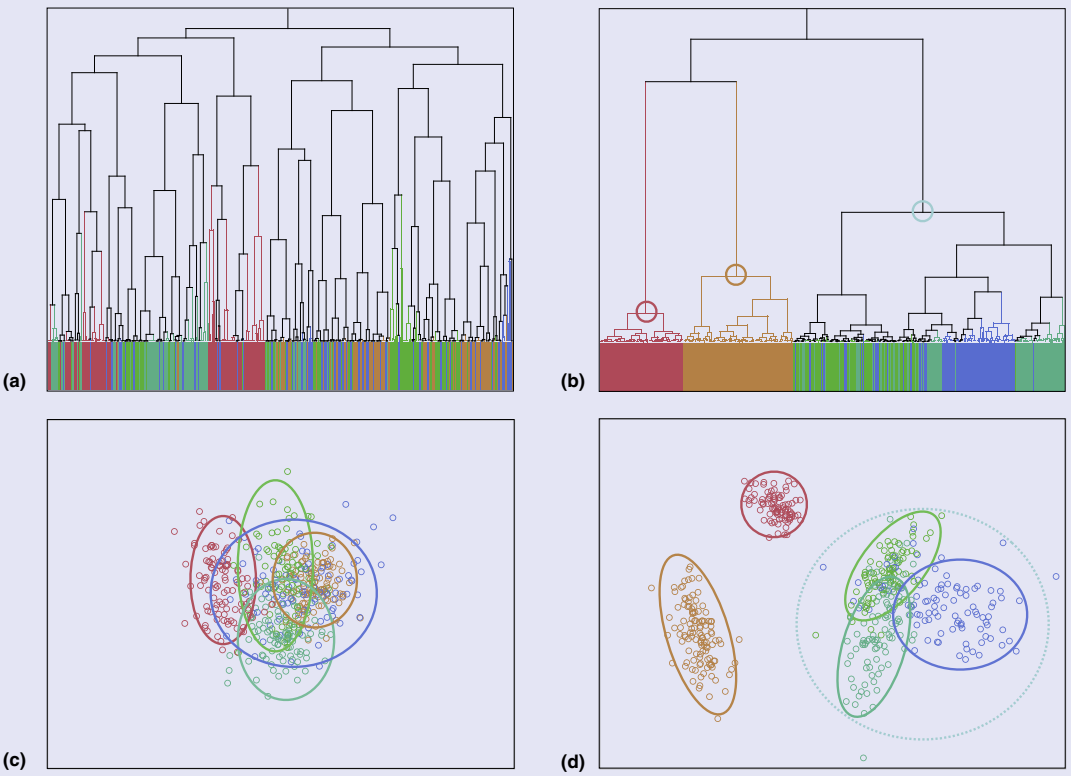

Figure Box 2 Clusters, genealogies, phylogenies, and reproductive isolation. As fewer traits are shared between them and populations become discrete, their phylogenetic relationships also change. For instance, transitions from continuous variation (a), (c) to discontinuous variation (b), (d) can lead to clustering of phenotypic forms that might be associated with particular environmental conditions as shown by the multiple colored sets inside the dotted circle in (d). Nodes in (b) represent where reciprocal monophyly can be observed between biological species (red vs. orange vs. blues and greens) or Darwinian species (green and blue clusters). (d) Also shows that Darwinian species share individuals with similar genotypes and trait values as a consequence of recent common ancestry. Biological species are less affected by incomplete lineage sorting than Darwinian species, but reciprocal monophyly is likely to manifest after speciation has been completed. Reciprocal monophyly, like complete reproductive isolation, is seemingly a definite point in time. However, and different from the biological species, reciprocal monophyly can be affected by incomplete sampling of individuals across the range of an organism.

expectation is also found in allopatric speciation where suppression of recombination is not directly related to the probability of speciation. However, and different from speciation with gene flow, there is no a priori expectation that extrinsic or intrinsic reproductive isolation should evolve first during allopatric speciation.

Can Speciation Repeat Itself?

Across the geographic range of a species, genetic drift cannot create an association between traits and environment, and therefore its role on the evolution of reproductive isolation is unpredictable. In contrast, natural selection can create strong

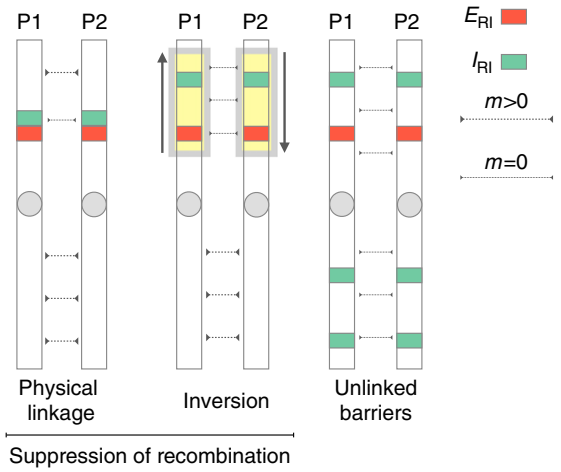

Figure 5 The evolution of extrinsic and intrinsic reproductive isolation during primary speciation with gene flow. Three general models for the isolation hitchhiking of extrinsic and intrinsic reproductive barriers to gene flow between populations. P1 is population one, and P2 is population two. Rectangles and the circle in the middle represent a metacentric chromosome. The green rectangles are locations in the genome containing genes that are members of a DBM genetic incompatibility that reduces fitness in hybrids and creates intrinsic reproductive isolation (I_{RI}). The red rectangles are locations in the genome containing genes under ecology-based divergent natural selection and causes extrinsic reproductive isolation (E_{RI}). The large yellow rectangles and associated arrows represent a chromosomal inversion separating the two populations. The inverted arrows represent varying levels of gene flow along chromosomal regions, *m*, between the two populations. The first model (physical linkage) assumes tight linkage (i.e., little recombination) between the genes responsible for the two forms of reproductive isolation, whereas in the second model (inversion) a chromosomal inversion suppresses recombination between otherwise unlinked loci responsible for the two forms of reproductive isolation. Model three (unlinked barriers) assumes that gene flow stopped across the entire genome and now is free to evolve by both genetic drift and natural selection. In this model, isolation hitchhiking arises after Darwinian species evolved (i.e., extrinsic reproductive isolation reduced gene flow between the two populations completely).

associations between environment and phenotype and thus create repeated patterns of divergence amongst the populations of a species. Parallel speciation is a deterministic case of speciation where ecology-based divergent natural selection leads to the evolution of reproductive isolation repeatedly and independently between populations adapted to contrasting environments (Schluter and Nagel, 1995). Populations inhabiting the same habitat are expected to remain reproductively compatible for longer periods of time compared to populations found in contrasting habitats. Therefore, typical tests of parallel speciation involve phylogenetic approaches that evaluate independence and number of origins, genetic crosses both between populations adapting to the same environment and between populations adapting to different environments, and finally, experiments that link trait variation with variation in reproductive isolation (Ostevik *et al.*, 2012). Although there are several convincing cases of parallel speciation in animals (e.g., in snails (Johannesson *et al.*, 2010); stick insects (Soria-Carrasco *et al.*, 2014); and stickleback fishes (Schluter and Nagel, 1995), there are relatively few putative ones in plants, perhaps suggesting that the consequences of divergent natural selection across kingdoms might be different, or that the right tests have not yet been performed in plants (Ostevik *et al.*, 2012).

Can Reinforcement of Reproductive Isolation Complete the Speciation Process?

Natural selection can complete the speciation process by reinforcing reproductive isolation in response to maladaptive hybridization between two populations (Dobzhansky, 1940). However, this is rather difficult: gene flow can randomize the associations between the genes causing maladaptive hybridization and those causing prezygotic (or extrinsic) reproductive isolation, and thus causing the two populations to fuse. More problematic, as prezygotic isolation spreads in the population, maladaptive hybridization becomes rare, effectively removing the pressure for evolving further prezygotic isolation. As a consequence, reinforcing selection can be self-defeating. Despite these and other seemingly fatal arguments (Servedio and Noor, 2003), recent experimental evidence for reinforcement suggests that it could be quite common in nature (Ortiz-Barrientos *et al.*, 2004; Hoskin *et al.*, 2005; Lukhtanov, 2010; Lemmon and Lemmon, 2010; Matute, 2010a,b; Yukilevich, 2012; Hopkins, 2013; Nosil, 2013; Pfennig and Rice, 2014).

Models of reinforcement illustrate how the antagonism between gene flow and natural selection can be tipped in favor of natural selection and therefore speciation. Felsenstein (1981) used a simple model where alleles at two loci, B and C, controlled fitness in two habitats. In one population the fittest genotype was *bc* and in the other one it was *BC*. Hybrids between them, *bC* and *Bc*, had low fitness. A third locus, A, controlled the mate preferences for *A* or *a* individuals. Although recombination between the loci B and C would favor speciation, but recombination between A and BC would antagonize it. The reason was simple: recombination between B and C would help restore the fittest genotypes, whereas recombination between A and BC would lead to preference for mates that would not be fit in the habitat where choice took place. Because the A locus had two preference alleles, these models are referred to as two-allele models for the evolution of mate preferences. Two-allele models suggest that speciation with gene flow would be difficult unless mechanisms suppressing recombination between A and BC evolved, or recombination between these A and the BC loci were unnecessary.

Felsenstein (1981) suggested that a single allele for mate preferences at the A locus could provide a solution. The mate preference would act as an enhancer locus of a previously present preference, or as an assortative mating locus that would favor mating between conspecific individuals. For instance imagine two groups of individuals that fail to see each other well and often misidentify members of each group. If they were both given glasses that enhanced the ability to distinguish group members, the exchange of the glasses (i.e., the same allele) would enhance the ability to correctly identify the identity of each individual. These models are

called one-allele models for the evolution of assortative mating, and recombination in the ABC system does not hamper speciation. Theoretical models, as well as the empirical evidence for speciation by reinforcement have been recently reviewed in plants and animals (Hopkins, 2013; Ortiz-Barrientos *et al.*, 2009).

Is Recombination Suppression Necessary for Speciation with Gene Flow?

Suppression of recombination may sometimes favor divergence during speciation (**Figure 5**). In the Felsenstein model, suppression of recombination would favor mating between individuals that survive well in the habitat where the mate choice took place. Recently, work on speciation with gene flow has revealed that chromosomal inversions play an important role in speciation by suppressing recombination between loci contributing to various forms of reproductive isolation. For instance, Noor *et al.* (2001) suggested that inversions would lock DBM genetic incompatibilities into place thus preventing their elimination from the population. The consequences were simple but powerful: first, inversions would maintain maladaptive hybridization levels over long periods of time, thereby facilitating the evolution of prezygotic isolation until the completion of speciation. Second, inversions would create conditions for genetic associations between loci responsible for prezygotic and postzygotic isolation, thus alleviating the antagonism between recombination and speciation by natural selection. A related model, proposed by Rieseberg (2001) suggested that inversions could amplify the effects of reproductive isolating genes by extending their effects over larger regions of the genome. More recently, it has become clear that allelic modifiers of recombination, or those alleles responsible for natural variation in recombination within species, can also play a role in during the early or late stages of speciation with gene flow by preserving favorable allelic combinations in each locally adapted population (Ortiz-Barrientos *et al.*, 2016). However, suppression of recombination might be unnecessary in several models of speciation (Ortiz-Barrientos *et al.*, 2016). For instance, if divergent natural selection is very strong since the early stages of speciation, the frequency of maladapted hybridization would be low and the conditions for the evolution of suppressed recombination would not occur.

What are Speciation Genes?

Studies of reproductive isolation have attempted to isolate the genes responsible for reproductive isolation. However, the very definition of what a speciation gene is makes it difficult to agree how many speciation genes have been found, and whether they reveal patterns of speciation (Rieseberg and Blackman, 2010; Presgraves, 2010a,b). Under the view that speciation is the evolution of reproductive isolation, it is clear that genes whose alleles contribute to some form of reproductive isolation can be considered a speciation gene (Nosil and Schluter, 2011). On one hand, there are genes contributing to DBM genetic incompatibilities, and therefore to the evolution of intrinsic reproductive isolation (hybrid sterility and inviability). On the other hand there are genes responsible for local adaptation and the evolution of extrinsic reproductive isolation (immigrant inviability and extrinsic postzygotic isolation), and those responsible for gametic recognition, for male–female interactions, and mate choice. In this sense, the list of speciation genes that have been discovered is perhaps larger than anticipated; yet they still have not clearly revealed whether there are special categories of speciation genes.

Questions about the contribution of a particular gene to speciation are similar to those applied to measurements of reproductive isolation. It would be important to know when the speciation gene arose, and whether or not speciation genes create full or weak reproductive isolation (Nosil and Schluter, 2011; Coyne and Orr, 2004). In relative terms, a speciation gene causing strong reproductive isolation might see its effects reduced to a very small magnitude if it arose very late in the speciation process. In a similar fashion, a gene whose expression is late during the life cycle of the organism (e.g., hybrid sterility), might also contribute relatively little to total reproductive isolation if genes expressed earlier during development (e.g., genes responsible for seed germination in plants) already produced high levels of isolation between populations.

Why are There Species?

The fundamental principles of population genetics determine the patterns of genetic variation in nature. However, it is less clear how they lead to the emergence of species. Darwinian species are perhaps best understood in terms of the action of disruptive and ecology-based divergent natural selection (Mallet, 1995), whereas biological species ultimately rely on the evolution of DBM genetic incompatibilities (Coyne and Orr, 2004). These proximate causes form the basis of the research program to understand the origin of species but they do not answer the question as to why there are species and not just a continuum of diversity in nature. As discussed by Maynard Smith and Szathmary (1995), one putative answer is that genetic variation occupies a discontinuous ecological and geographical space. As individuals colonize and adapt to such conditions the complex interrelation amongst genes leads to the emergence of trade-offs that make it difficult to occupy other environments. In this sense, the reason for the existence of species leads to the natural formation of Darwinian species. However, as it has been discussed before, ecological divergence need not be the last step of the formation of new species and therefore other features of the world (in addition to environmental and geographic heterogeneity) must explain the existence of both Darwinian and biological species.

Sexual recombination and mutation are perhaps the missing ingredients, which incidentally are also the major sources of genetic variation in nature. A combination of heterogeneous landscapes and sexual recombination of new mutations might inexorably lead to the formation of both Darwinian and biological species (Coyne and Orr, 2004). This is because, over time, new genetic variation sorts into new niches via the action of both drift and natural selection. However, some of this variation is incompatible with the occupation of specific areas across space and environment (the evolution of extrinsic reproductive isolation), and sometimes in an irreversible way

(the evolution of intrinsic reproductive isolation). Biological species sometimes may not need to evolve in this environmental grid, but in sexual selection grids, or neutrally evolving grids. In each one of these grids, phenotypes sort into clusters via different evolutionary forces, mostly in allopatry, and then genetic incompatibilities evolve leading once more to the production of irreversible biological species and the facilitation of coexistence in sympatry.

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See also: Ecological Speciation and Its Consequences. Founder Speciation. Reinforcement. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Speciation, Chromosomal Rearrangements and. Speciation Continuum. Speciation-with-Gene-Flow

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Species Concepts: Viral Quasispecies

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Glossary

Combination therapy A treatment consisting in the simultaneous administration of two or more drugs.
Complementation within mutant spectra The increase in virus progeny production by gene products supplied by different members of the same mutant spectrum.
Consensus sequence The one that at each position has the residue (nucleotide or amino acid) which is most abundant at the same position in the set of aligned sequences.
Defective viral genome The one that cannot complete a replication cycle, or that completes it with a substantially lower efficiency than the corresponding standard virus.
Error catastrophe Loss of genetic information due to an excess of mutations.
Error threshold An average error rate above which a virus cannot maintain its infectivity.
Fitness The replicative capacity of a virus mutant, population or isolate, measured relative to a reference mutant, population or isolate of the same virus.
Interference In the quasispecies context it means the capacity of a viral mutant or population to inhibit the replication of higher fitness genomes. Interference by a mutant spectrum is the converse of complementation within a mutant spectrum (see term above).
Lethal defection Loss of viral infectivity mediated by a class of defective genomes termed defectors.
Lethal mutagenesis Extinction of a virus due to an excess of mutations.
Master sequence The highest fitness genome in a viral quasispecies.
Monotherapy Treatment with a single drug.
Mutant spectrum (also termed cloud, distribution, or swarm) The ensemble of mutants that constitute a viral quasispecies.
Mutation frequency The average number of mutations per nucleotide in a mutant spectrum.
Mutation rate The frequency of occurrence of mutations during replication of a viral genome.
Rate of evolution Increase in the number of mutations in a viral genome population as a function of time. Generally the mutations are counted in the consensus sequence.
Viral quasispecies A distribution of related genomes subjected to genetic variation, competition, and selection, and that act as a unit of selection.

Introduction: Theoretical and Experimental Quasispecies

The term viral quasispecies refers to a population structure of RNA viruses whose major feature is the presence at any given time of multitudes of viral mutants that can approach a dynamic equilibrium when the population size is large and the environment constant. In practice, a true equilibrium is not attained because population size is finite and environments are variable. The absence of population equilibrium favors the unpredictable change of frequency of multitudes of dynamically arising and decaying mutants that altogether offer a rich substrate for virus adaptability. The quasispecies concept had two independent origins, one theoretical, coming from the field of biophysics; and another experimental, coming from molecular biology. The theoretical quasispecies were founded by Manfred Eigen in a pioneer mathematical study that addressed the evolution of molecules that replicated with the regular production of error copies (Eigen, 1971). The theory was completed by Manfred Eigen and Peter Schuster, and its objective was to develop a model for self-organization and adaptability of primitive replicons that are thought to have been at the origin of life on earth (Eigen, 1992, 2013; Eigen and Schuster, 1979). In this treatment, the replicating molecules are organized around a dominant or most fit (with maximum replicative capacity) sequence termed the 'master' sequence (terms defined in the Glossary). The mutants around the master sequence are ranked according to fitness, with higher fitness corresponding to molecules with a sequence closer to the master. The information conveyed by the mutant distribution is lost if the average error rate during replication exceeds a threshold value. Violation of the error threshold leads to loss of information. Quasispecies was initially formulated as a deterministic theory, meaning that the mutant distributions were of infinite size in equilibrium, and the evolutionary outcomes predictable. This was a mathematical necessity amply accepted in the formulation of theoretical models. Extensions of the original quasispecies theory to the more realistic situations of finite populations in variable fitness landscapes have been developed (as recent review, see Domingo *et al.*, 2012). These extensions approximate quasispecies to real biological entities that often display a stochastic (i.e., with an unpredictable chance component) rather than deterministic (predictable) behavior.

The experimental origin of quasispecies was the discovery that populations of the RNA bacteriophage Qβ were composed of complex spectra of mutants rather than a defined virus with a precise nucleotide sequence, as it was thought at a time at which a virus isolate was synonymous with a nucleotide sequence. The estimate was that in populations of bacteriophage Qβ the subset of genomes without mutations (what could be

considered the master or more abundant sequence) did not amount to more than 15% of the total; the rest were mutants (Domingo *et al.*, 1978). This observation on the population structure of bacteriophage Qβ was in striking agreement with the main precepts of quasispecies theory (Domingo *et al.*, 2001, 2012; Eigen and Schuster, 1979). The fact that RNA virus (and many DNA virus) populations consist of dynamic (continuously changing) mutant distributions has been amply confirmed by application of new ultra-deep sequencing techniques that have greatly facilitated analyses of viral populations; the more deeply mutant spectra are analyzed the more dramatic their complexity turns to be (Acevedo *et al.*, 2014; Domingo *et al.*, 2012; Lauring and Andino, 2010). No exceptions to a quasispecies genomic structure have been found among the RNA viruses. Mutant clouds replicate in humans infected with HIV-1, influenza virus, hepatitis C virus (HCV), or Ebola virus. Pathogenic and nonpathogenic RNA viruses are quasispecies (Holland, 2006). Presently, quasispecies are defined in virology as dynamic collections of different but closely related genomes subjected to a continuous process of genetic variation, competition and selection, and that act as a unit of selection (Domingo *et al.*, 2012).

Implications of the Quasispecies Population Structure

There are two major classes of consequences of viral populations existing as quasispecies: (1) of a theoretical nature regarding how should viruses be defined, and (2) of a practical nature derived from the mechanisms by which viruses adapt to changing environmental conditions, and ensuing difficulties for viral disease prevention and treatment (Holland *et al.*, 1982).

With regard to the definition of virus, the main departure of quasispecies from previous views is that the 'wild type' can no longer be considered a genome with a defined sequence, of the type currently cataloged in data banks. Instead, a wild type is a distribution of related but different sequences, that is, a rather indeterminate average of related sequences. This is an important conceptual novelty since the term 'wild type' still used in many microbiology and virology textbooks has only an evanescent meaning in that it should be defined for each isolate, for each clone and even for each experiment in laboratory designs. The connection among sequences of the same replicative unit is often established by mutation, but it can be also made by recombination and segment reassortment in the case of viruses with a segmented genome. Quasispecies theory is compatible with any mechanism of genome variation fueling the genetic change for competition and selection to act on any mutant or mutant ensemble.

A second implication that pertains to the way viruses should be defined originates from internal interactions that are frequently established among components of the same mutant spectrum, and the influence of such interactions in virus behavior. The first experimental result that pointed at intrapopulation interactions was that individual biological clones isolated from a population of bacteriophage Qβ displayed lower fitness than the total population from which the clones were isolated (Domingo *et al.*, 1978). The same result was obtained with biological clones of vesicular stomatitis virus (Duarte *et al.*, 1994). Other observations both in cell culture and *in vivo* on the collective behavior of viral quasispecies are summarized in **Table 1**, and have been reviewed (Domingo *et al.*, 2012; Lauring and Andino, 2010). Thus, mutant spectra that are generated during viral replication are not merely an

Table 1 Evidence of collective behavior of viral quasispecies

Virus	*Observation*	*References*
Qβ, VSV	Entire populations display higher replicative fitness than individual clones from the same population	Domingo *et al.* (1978) and Duarte *et al.* (1994)
VSV	A mutant spectrum can suppress replication of a variant of superior fitness	de la Torre and Holland (1990)
PV	In live poliovirus vaccines the attenuated virus can suppress minority virulent virus	Chumakov *et al.* (1991)
FMDV	Populations selected to be resistant to polyclonal antibodies endure a fitness cost, and they can suppress high-fitness variants	Borrego *et al.* (1993)
LCMV	Nonpathogenic LCMV can suppress expression of pathogenic LCMV and disease manifestation	Teng *et al.* (1996)
FMDV	Presence of memory in viral quasispecies in the form of minority subpopulations. Memory affects the response to repeated selective pressures	Ruiz-Jarabo *et al.* (2000) and Briones and Domingo (2008)
FMDV	Mutagenized viral RNA can interfere with infectious RNA	González-López *et al.* (2004)
LCMV	A class of defective LCMV termed defectors can suppress infectivity of standard LCMV	Grande-Pérez *et al.* (2005) and Martin *et al.* (2010)
PV	The mutant spectrum can suppress inhibitor-resistant mutants	Crowder and Kirkegaard (2005)
PV	Viral quasispecies can complement a specific mutant allowing its penetration into the central nervous system	Vignuzzi *et al.* (2006) and Pfeiffer and Kirkegaard (2005)
MV	Two mutants together (but not each individually) mediate membrane fusion	Shirogane *et al.* (2012)

Abbreviations: FMDV, foot-and-mouth disease virus; LCMV, lymphocytic choriomeningitis virus; MV, measles virus; Qβ, *Escherichia coli* bacteriophage Qβ; PV, poliovirus; VSV, vesicular stomatitis virus.

aggregate of mutants as a result of mutation-selection (the traditional Wright–Fisher view of population genetics) in which the mutants coexist independently of the others. Rather, the population is an aggregate whose composition is influenced not only by mutation-selection acting on the dynamically arising variants, but also by the acquisition of new phenotypes through interactions among mutants (Shirogane *et al.*, 2012). There is a sort of 'coevolution' of population structure and selection forces, as if intrapopulation interactions modified the environment. In other words, and in agreement with the initial quasispecies theory, in viral quasispecies an ensemble of mutants, not the individual components, is the target of selection. In some cases, however, the behavior of individual mutants may prevail over the ensemble. Part of the current research on viral quasispecies aims at distinguishing those properties of mutant spectra that depend on individualities from those that depend on collectivities, with the objective of better understanding viral population dynamics and viral disease mechanisms, to design more effective antiviral strategies.

Quasispecies as Reservoirs of Phenotypic Variants

Mutant spectra (also termed mutant distributions or clouds) are a rich reservoir of genetic and phenotypic variants, as can be intuitively gathered from examining **Figure 1**, and considering that an individual infected with an RNA virus can attain 10^{10} to 10^{12} (in some cases even more) potentially infectious particles at any given time during an acute infection (about one billion pages filled with the genomes related to those depicted in **Figure 1**). Mutations can impact viruses in different ways. A mutation is considered neutral when it does not have a significant effect in the biology of the virus harboring it. If the mutations depicted in **Figure 1** were neutral, the behavior of a virus would be independent of their presence. It turns out that a large proportion of mutations (difficult to tell how many, but probably the majority) are not selectively neutral. Many mutations are deleterious to various extents, and they will be either eliminated or their frequency reduced as a result of negative selection. However, some mutations present in a mutant spectrum have the potential to rescue the virus from a selective constraint, for example, to overcome an immune response (neutralizing antibodies or cytotoxic T cells), or an antiviral agent administered to control the infection.

Very few out of the entire repertoire of mutations will be adequate to confront a specific constraint, but the accumulation of mutations in a large mutant spectrum endows the ensemble with the capacity to respond to many constraints. The virus population size, also referred to as viral load, is an important determinant of virus adaptability (Domingo and Perales, 2012). Host antibody responses or inhibitors administered during antiviral treatments constitute selective constraints that are easy to recognize and to quantify (**Table 2**). Viruses in the multiple intracellular and extracellular stages of their life cycles face multiple selective constraints that are not easy to identify. Some, however, are essential to ensure short-term and long-term virus survival in nature. Changes in host cell tropism and host range, or variations in virulence that favor virus spread among host populations are examples of essential variations that occur regularly during virus spread. A common feature to the mechanisms of response to most selective constraints is that they often require one or a small number of mutations in the virus genome to be operational. In other terms, quasispecies distributions, with the likely prevalence of single and double mutants over multiple mutants, are the ideal object for selection of relevant traits to occur. From a historical perspective, this should not be surprising since viruses probably exist because they have evolved means to respond to such constraints. Any virus that would require 100 precisely located mutations in its genome to overcome an antibody response would have been extinguished long ago by the types of immune systems we see today. Viruses are confined to one or a few hosts, and they are amenable to classification, because the number of mutations needed to infect other types of phylogenetically distant hosts is not within reach of their mutant spectra. Thus, except in rare cases (e.g., large evolutionary jumps through recombination) phenotypic flexibility is confined to traits whose modification requires a limited number of mutations.

Exploration of Sequence Space, Viral Fitness, and Viral Load

Nucleotide sequence space means the number of genomic sequences available to a replicating entity, be it a virus or a cell. The term was first used by John Maynard Smith for amino acid sequences, and extended by Manfred Eigen and colleagues to nucleic acids and to viral evolution (Eigen and Biebricher, 1988). The theoretical sequence space available to a virus is a huge quantity; for a 10 000 nucleotide genome it is calculated as $4^{10\,000}$ (4, number of different types of nucleotides to the power of the length of the viral genome). Only a minority of the theoretical sequence space can be occupied by a virus because most of the sequences have no biological meaning since they fail to encode the regulatory and coding regions with functional sequences at the required genomic positions. The key issue here is that transitions among minority sequences compatible with representing a viral entity can give rise to viral forms with altered properties. There are continuous movements in sequence space in components of RNA viral quasispecies, even those components that represent minority subpopulations present at low frequency (Tsibris *et al.*, 2009). Such movements are fueled by high-mutation rates (in the range of 10^{-3} to 10^{-5} mutations produced per nucleotide copied (Domingo *et al.*, 2012)), and guided by fitness differences. Fitness is relative to the environment. A pathogenic human virus can be attenuated by extensive replication in cell culture. By increasing its fitness in cell culture as a result of repeated passage, a virus may lose its fitness for humans. Fitness and virulence are not necessarily directly correlated (Herrera *et al.*, 2007 and references therein), but for some pathogenic viruses fitness, population size, and population heterogeneity are relevant to disease progression. Viral load has long been recognized as a predictive parameter in clinical virology: the higher the load of a pathogenic virus in an infected individual the higher the probability of disease

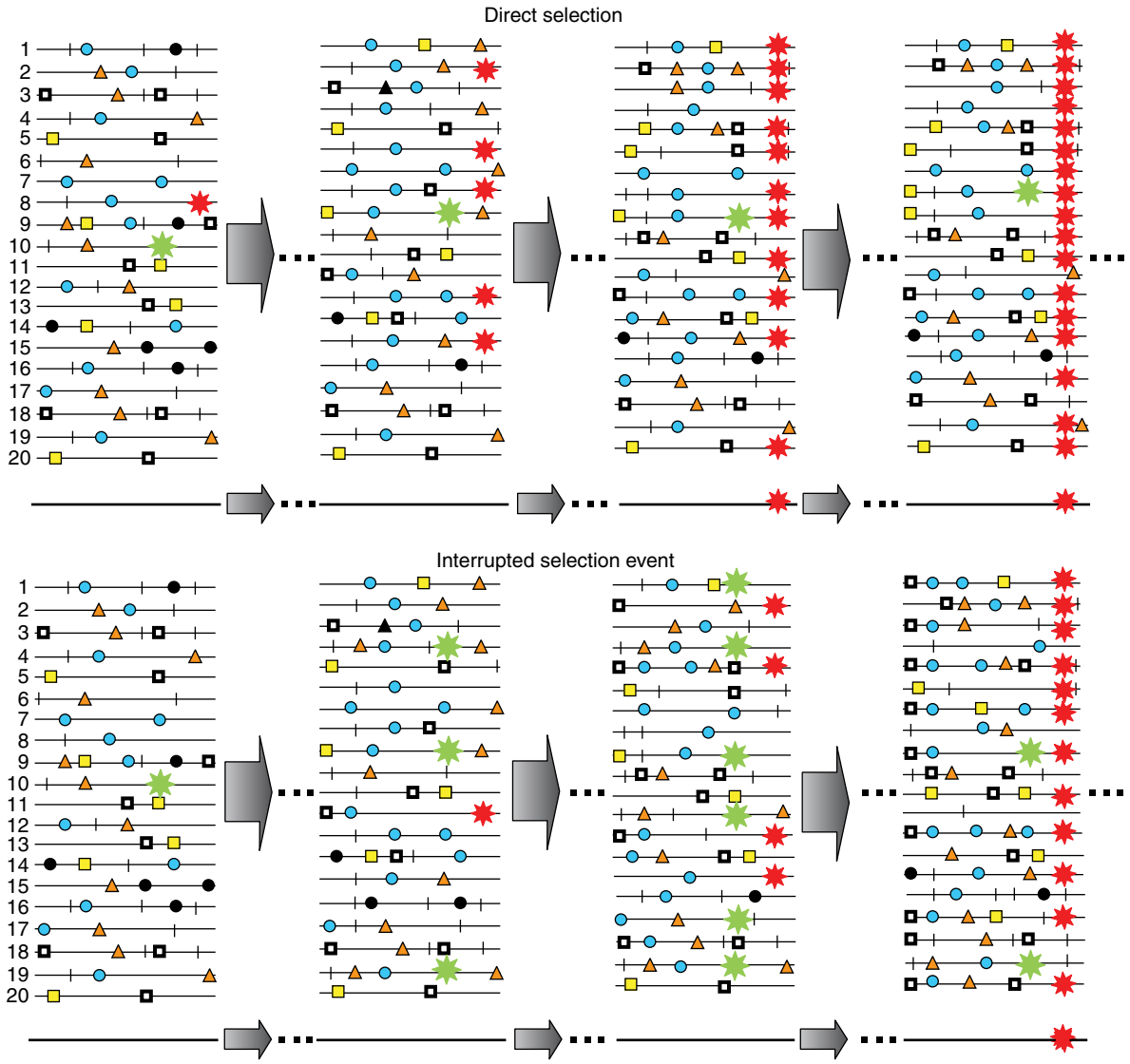

Figure 1 The complexity of viral mutant spectra, and selection dynamics. Individual genomes are depicted as horizontal lines, and mutations as different symbols on the lines. Below each mutant distribution, the consensus sequence is also represented by a horizontal line. In the top series direct selection of genomes harboring the mutation represented by the red asterisk occurs and the mutations becomes part of the consensus. In the bottom series, an interrupted selection of the mutation depicted by a green asterisk is represented. Because of the continuous dynamics of appearance of new mutations, the mutation that was in its way to dominance is overgrown by a newly arising mutation (red asterisk) that finally becomes part of the consensus. Application of deep sequencing is revealing multiple unsuccessful or interrupted selection events in viral populations (see text).

progression. The connections between replication rate, exploration of sequence space, and viral fitness with regard to viral disease potential have been reviewed (Domingo *et al.*, 2012). In simple terms, the process by which quasispecies dynamics plays its role in viral pathogenesis and persistence can be expressed as follows: rapid replication and high-mutation rates imply efficient exploration of sequence space for fitness gain; fitness gain optimizes replication in the considered environment, with the consequent increase of viral load and further exploration for adaptability; variant sequences able to replicate in new environments become more likely when the repertoire of sequences increases (**Figure 2**). Fitness can also be a determinant of multidrug resistance, as suggested by studies with HCV in cell culture (Sheldon *et al.*, 2014). Thus, the dynamics that allow the replicative optimization of a virus may also endow it with phenotypic pluripotency, further supporting the selective advantage of quasispecies behavior. Once the implications of quasispecies for viral infections were understood, clinicians realized how important it is to limit the viral load by initiating treatment as soon as a disease is diagnosed (for AIDS treatment, the title of an article by David Ho illustrates this point (Ho, 1995)). Thus, despite being seemingly of a theoretical nature, the conceptual body derived from quasispecies theory impinges on problems of a practical nature, particularly therapeutic designs, as summarized next.

Table 2 Some types of selective internal and external constraints recognized to act during a virus life cycle

Type of constraint	*Quantification*	*References*
Virion stability in extracellular environments. Genome packaging constraints	Indirect, through calculations based on structural information or determinations of infectivity following exposure to altered environment (temperature, ionic conditions, etc.)	Mateu (2013) and Ojosnegros *et al.* (2011a)
Different cell types, host species	Difficult to quantify mutants with altered cell tropism or host range, but they can be selected from evolving quasispecies	Baranowski *et al.* (2001), Baranowski *et al.* (2003), Ruiz-Jarabo *et al.* (2004), and Domingo *et al.* (2012)
Neutralizing antibodies	10^{-2} to 10^{-6} is the usual range of frequencies of monoclonal antibody-resistant viral mutants	
Inhibitors used in therapy	Frequencies in the range of 10^{-3} to 10^{-5} depending on genetic and phenotypic barrier, and viral fitness	Domingo (1989), Richman (1996), Domingo *et al.* (2012), and Sheldon *et al.* (2014)

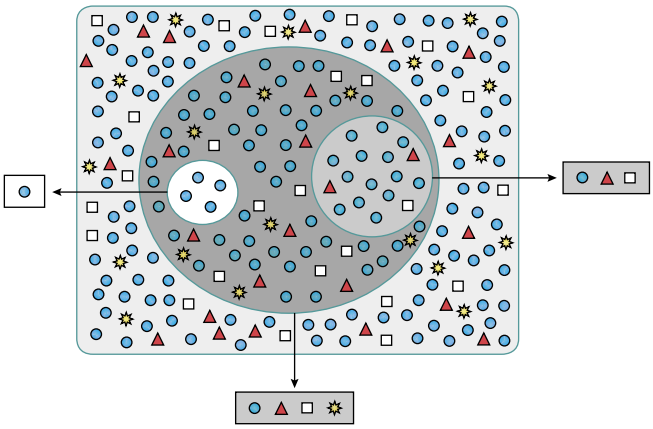

Figure 2 A simplified representation of sequence space and the importance of population size. The large rectangle includes many types of variants, the most relevant highlighted by four symbol types. Depending on the population size (delimited by circles of different size) the repertoire of variants available for selection or other evolutionary events varies (variants depicted in small rectangles). It must be considered that immediately after replication of the variants takes place, a new mutant distribution is formed. Reproduced from Domingo, E., Sheldon, J., Perales, C., 2012. Viral quasispecies evolution. Microbiology and Molecular Biology Reviews 76, 159–216, with permission from the American Society for Microbiology, Washington DC, USA.

Antiviral Designs to Counteract the Adaptive Response of Viral Quasispecies

In pharmacology, only a few decades ago the combined use of several drugs was against basic principles of good practice. Yet, in the last years, the efficacy of combination therapies over monotherapy has become evident in cases in which the agent to be controlled undergoes dynamic adaptive variations derived from the Darwinian mechanisms that are part of quasispecies theory. The need of combination therapies is now amply recognized to treat genetically variable viruses and to control cancer, and it might be also recognized in the future for prion diseases, also subjected to Darwinian dynamics (Domingo *et al.*, 2012; Mas *et al.*, 2010; Ojosnegros *et al.*, 2011b; Weissmann, 2012). Combination therapy is one among several alternative treatment designs that have been suggested or are under investigation to counteract the adaptive potential of viral quasispecies (reviewed in Domingo *et al.*, 2012).

As a general principle, for a mutant virus to be resistant to two or more drugs that target different steps of the virus life cycle, it should acquire at least two or more specific mutations (at least one mutation per each administered drug) to survive the treatment. For basic statistical reasons, the probability of occurrence of two specific mutations in the same viral genome equals the product of the probability of occurrence of each of them separately. Taking as a reasonable estimate a frequency of escape to one drug of 10^{-4}, the frequency mutants resistant to two, three, or four drugs is expected to be of the order of 10^{-8}, 10^{-12}, and 10^{-16}, respectively. Soon a probability is reached whose reciprocal value is lower than the viral population size in the infected organs targeted by the drugs. It is very unlikely that the viral quasispecies reaches a position in sequence that harbors resistance to three or more drugs. Highly active

antiretroviral therapies (HAART) for AIDS are based in this principle; HAART has dramatically decreased AIDS-related deaths, and has converted HIV-1 infection largely into a chronic condition. Procedures to eradicate HIV-1 from the organism are under investigation. Several books and review articles have addressed the problem of drug resistance in viruses in its theoretical, experimental, and clinical aspects (Domingo *et al.*, 2012; Richman, 1996; and references therein).

An antiviral strategy that was developed as a consequence of one of the corollaries of quasispecies theory is termed virus entry into error catastrophe or lethal mutagenesis. As mentioned in the Introduction, the inheritable information conveyed by any replicative system is lost if the error rate increases over a threshold value. In support of this prediction, John J. Holland and colleagues were the first to prove experimentally that mutation frequencies at specific sites of viral genomes could be increased at most by 2.8-fold, and that increases in mutation rates were detrimental for infectious progeny production (Holland *et al.*, 1990; Lee *et al.*, 1997). It is worth noting that despite the recognized fact in that most mutations on well adapted viruses and organisms are likely to be deleterious, the result of Holland and colleagues was not obvious. *A priori*, higher than basal mutation rates might be advantageous. It has been described that mutator bacteria that display 10^2- to 10^3-fold higher mutation rates than their parental populations, have enhanced adaptive capacity relative to their standard counterparts. Also, contrary to the results of Holland and colleagues, the reversion frequencies at several defined sites of the *Escherichia coli lac Z* gene could be increased 10^2- to 10^3-fold by chemical mutagenesis with good survival levels (discussed in Holland *et al.* (1990)). The reason that enhanced mutation rates are detrimental for RNA viruses is that they replicate very close to the error threshold for maintenance of genetic information. If they did not, additional mutational loads would have been beneficial for adaptation. Even if the molecular mechanisms by which viruses cross an error threshold are not the same as implied by the original quasispecies theory, the basic concept and its consequences are very similar (Domingo *et al.*, 2012).

Experiments on virus extinction through increased mutagenesis were pursued by Esteban Domingo, Pedro Lowenstein, Juan Carlos de la Torre, and their colleagues using foot-and-mouth disease virus and lymphocytic choriomeningitis virus as model systems in cell culture and *in vivo* (Eigen, 2002; Grande-Pérez *et al.*, 2002; Ruiz-Jarabo *et al.*, 2003; Sierra *et al.*, 2000) and by Larry Loeb, Jim Mullins and colleagues with HIV-1 (Loeb *et al.*, 1999). These authors coined the term lethal mutagenesis, the expression most used in virology. Important developments have occurred in the theory and practice of lethal mutagenesis, opening new prospects of antiviral designs.

Sequential Antiviral Treatments Based on Lethal Mutagenesis

Initial experiments with HIV-1 and FMDV indicated that combinations of mutagenic agents and non-mutagenic antiviral inhibitors were more effective to extinguish virus than a mutagenic agent alone, particularly when the virus had high-replicative fitness (Pariente *et al.*, 2001; Tapia *et al.*, 2005). In principle, this result was expected from the observations and arguments in favor of combination therapy outlined in Section 'Antiviral Designs to Counteract the Adaptive Response of Viral Quasispecies'. However, experiments by Celia Perales and colleagues in which alternative protocols of administration of

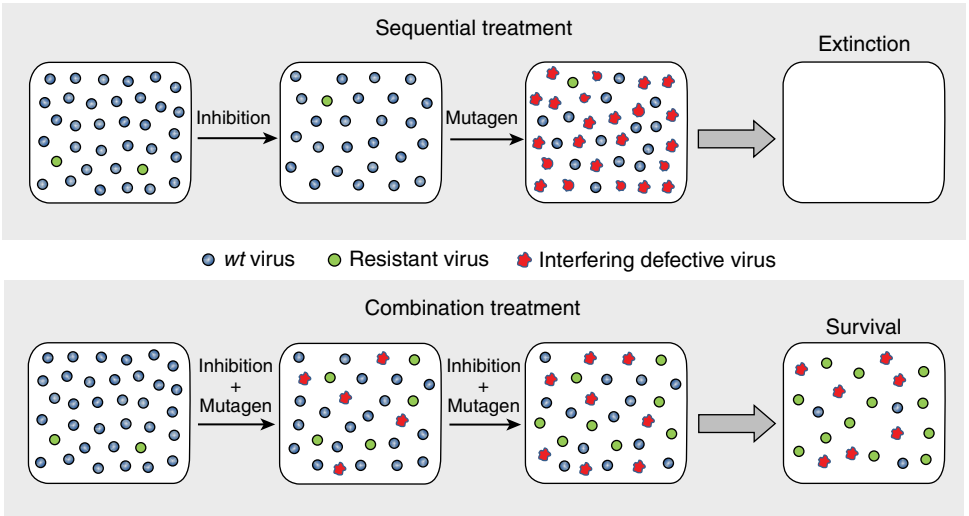

Figure 3 Schematic representation of the effect of a sequential versus combination treatment on a viral population. Top: A viral population contains some inhibitor-resistant mutants (green circles). Administration of an inhibitor diminishes the viral population size prior to a significant increase of inhibitor-resistant mutant frequencies. Subsequent mutagenesis results in the generation of defectors (rough red circles) that interfere with replication of the ensemble and can lead to virus extinction. Bottom: Administration of an inhibitor together with a mutagen may reduce the viral load, inhibit replication of defectors thereby attenuating their interfering activity, and increase the frequency of inhibitor-resistant mutants thus permitting viral survival. The advantage of a sequential versus combination administration to achieve virus extinction depends on the concentrations of inhibitor and mutagen (see text).

mutagens and inhibitors were compared indicated that a sequential administration of an inhibitor first, followed by a mutagen could have an advantage over the corresponding combination or the administration first of the mutagen and then the inhibitor. For each virus–host system there is a range of concentrations of inhibitor and mutagen at which either the sequential or combination administration are more effective (Iranzo *et al.*, 2011; Perales *et al.*, 2012). The advantage of the sequential protocol has been documented with several viruses that use different replication strategies such as FMDV, LCMV, and HCV, and new antiviral protocols are now under investigation (review in Perales *et al.*, 2012; **Figure 3**). Therefore, quasispecies dynamics has practical consequences regarding the design of antiviral strategies, documenting the value of basic research to open practical possibilities.

Conclusions and Prospects

Quasispecies is a theory of molecular evolution adequate to describe the behavior of replicating entities characterized by high-mutation rates. It has found its maximum interest in the interpretation of adaptability of RNA viruses, chiefly with the recognition that populations of RNA viruses consist of complex mutant spectra, as predicted by the theory. Although the term quasispecies applies mostly to RNA viruses, the concept is also valid for other biological entities. Some DNA viruses that are replicated by low-fidelity DNA polymerases display a dynamics comparable to that of RNA viruses. Quasispecies behavior has been also observed with subviral replicons such as viroids and with RNA molecules evolving *in vitro*, or cellular collectivities such as mutator bacteria, bacterial biofilms, or cancer cells. More recently, the Darwinian behavior of prions has been attributed to conformational heterogeneity of the prion protein, with strain modifications ('mutations') being associated with conformational transitions. This is a new development that will require an extension of quasispecies theory to non-replicative systems, in which mutations are based on conformational changes. Thus, quasispecies theory has been extremely important as an experiment-provoking incentive to understand the dynamics of many biological systems (overview in Domingo (2016)).

See also: Coevolution, Bacterial-Phage. RNA Viruses, Evolution of

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Species Trees, Inference of

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Nomenclature

θ The neutral mutation parameter, in diploid organisms equivalent to four times the effective population size multiplied by the mutation rate of the gene in question.
N Effective population size.
μ Mutation rate.
τ A measure of time in coalescent units, usually applied to branches of a species tree.
t The number of generations along a branch of a species tree.

Glossary

Anomaly zone A type of species tree with internal branch lengths that are so short (in coalescent units) that the most common gene tree produced is topologically different from the species tree.
Coalescent unit A unit of time measurement in coalescent theory. When speaking of diploid organisms, usually one coalescent unit is equal to 4N generations, where N is the effective population size and the generation time is specific to the organisms being studied.
Deep coalescence Looking from the present to the past down the branches of a species tree, a situation in which two gene lineages do not coalesce before the next common ancestral species is encountered. Two lineages are said to coalesce deeply when they do not coalesce within the branch designating their common ancestral species. Usually deep coalescence occurs when branches are short in coalescent units.
Effective population size The size of an ideal population whose genetic dynamics are similar to the actual population being studied. An ideal population is one that is experiencing no migration or selection, undergoes random mating and whose genetic variation is neutral. The effective population size of a species is usually smaller than its census size.
Incomplete lineage sorting Looking forwards in time, from the past to the present, a situation in which gene lineages to not come to fixation within a species before that species diverges into two species.
Gene tree A phylogenetic tree of alleles sampled from one or more species.
Multispecies coalescent model A model providing probability distributions of gene trees sampled from phylogenetic trees consisting of multiple species. Both the branch lengths and the topologies of gene trees are predicted by the multispecies coalescent model. These parameters are in turn predicted by the sample sizes of alleles within each species and the topology, branch lengths, and effective population sizes of the species tree.
Single-nucleotide polymorphism (SNP) A DNA polymorphism at a single site or nucleotide. Generally gene trees cannot be constructed from a single SNP but a SNP can designate a bipartition among species or alleles in a dataset.
Species tree A phylogenetic tree of species, each of which consists of alleles at one or more loci.

As outlined in other articles in this section, phylogenetic trees are an essential tool for mapping the history of biodiversity, reconstructing evolutionary events, and depicting evolutionary relationships among species. Additionally, although there continue to be debates about the relative utility of morphological and molecular data in phylogenetic analysis, systematics of extant or recently extinct species in the twenty-first century is largely, although certainly not exclusively, based on molecular data. With the advent of new ways of generating molecular data using next-generation sequencing and other genomic technologies, molecular data increasingly comes in the form of DNA sequence data consisting of many loci, each with multiple single-nucleotide polymorphisms, or SNPs (Lemmon and Lemmon, 2013; McCormack *et al.*, 2013). There has also been a rapid increase in datasets consisting of many individual SNPs, each characterized across a set of populations or species (Peterson *et al.*, 2012). With these new and increasingly large molecular datasets come challenges of handling the stochasticity and randomness inherent in such datasets, especially at the level of the genealogy of genes or DNA sequences. Such stochasticity is a major reason for the advent of so-called 'species tree' methods of phylogenetic inference (Edwards, 2009; Nakhleh, 2013; Szöllősi *et al.*, 2015).

Species tree methods are best thought of as a general framework for handling phylogenetic data from multiple loci. This framework includes methods such as concatenation or supermatrix methods, in which separate loci are concatenated into a single 'supergene' for analysis; in this way, concatenation can be thought of as a special case of the more general models employed in species tree inference (Liu *et al.*, 2015; **Figure 1**). In turn, species trees – being based primarily on models solely involving divergence (isolation) events from common ancestors, can be thought of as special cases of even more general isolation-migration or network models, which assume some level of gene flow between descendant lineages (**Figure 1**). Although currently species tree methods focus exclusively on molecular data, and specifically on DNA sequence and SNP data, in the future, the species tree framework will

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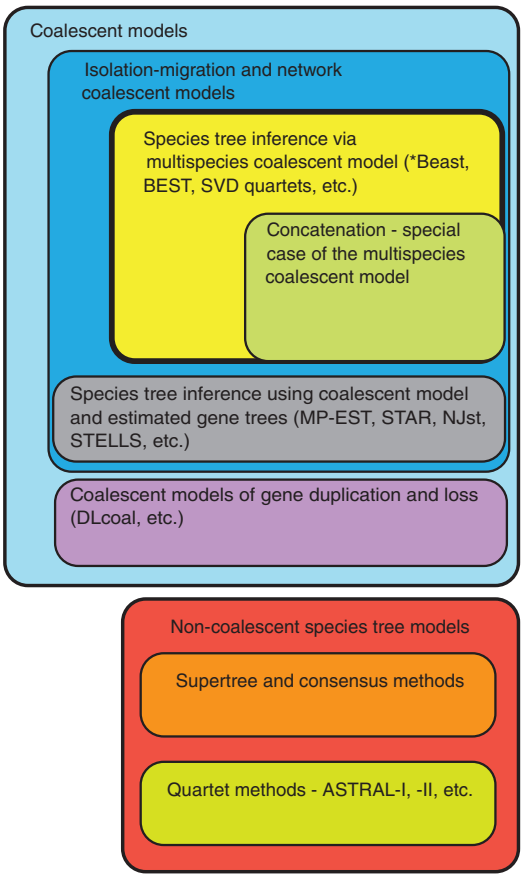

Figure 1 Relationships among coalescent models and species tree methods. This article focuses on models in the yellow box. Species tree methods are viewed as based either on coalescent models or arbitrary models. Species tree methods are seen as specific instances of models that include both isolation and migration, i.e., species tree methods are network or isolation-migration models with no migration among lineages. Concatenation or supermatrix methods are in turn seen as specific instances of species tree methods in which gene trees are identical across genes. With reference to both species trees and concatenation methods, these specific instances are likely to be most often violated in any given dataset. Additional models for gene duplication and loss in multigene families also employ the multispecies coalescent model. Supertree and consensus methods are viewed as non-coalescent methods in so far as they are usually not grounded in any specific biological model. Similarly, species tree methods such as ASTRAL are based on simple heuristics and do not reference coalescent theory directly.

likely include analysis of morphological data and other types of molecular data such as rare genomic events and insertions and deletions. Thus species tree inference can be thought of as synonymous with the general problem of phylogenetic inference, providing a general and inclusive framework for inferring the history of life (Figure 1).

Historical Background to Species Tree Inference

Phylogenetic analysis has undergone major changes in the last few decades. The first widely used phylogenetic methods focused on distance and parsimony approaches in the late 1960s, followed in the early 1980s by maximum likelihood, and Bayesian methods in place by the mid-1990s (reviewed in Felsenstein, 2003; Rannala and Yang, 2008). Each of these updates were driven by the need to handle larger and more complex datasets that better accommodate the statistical properties of the data used to build phylogenetic trees. Species tree methods are no different. In the case of species tree methods, the statistical properties that were ignored by previous methods included the recognition that the different loci used for phylogenetic analysis in multilocus datasets could often have different topologies from one another, even in the absence of complicating factors like gene flow between species or molecular events such as horizontal gene transfer (HGT) or gene duplication (Maddison, 1997; Nakhleh, 2013). The phenomenon known as 'incomplete lineage sorting' (ILS), a major source of gene tree heterogeneity in phylogenetic analysis, was first recognized and the term coined by John Avise in his studies of mitochondrial gene trees in a related field known as phylogeography, which focuses on the history of closely related species and populations within species (Avise *et al.*, 1987). Avise realized that there were cases in which the topology observed and clearly supported by mitochondrial data sometimes conflicted with the pattern of relationships clearly suggested by phenotypic data of the species or populations being studied. Working with Joe Neigel and others in the mid-1980s (Neigel and Avise, 1986), Avise also realized that such conflict did not necessarily have to arise from demographic events such as hybridization between species, but could in principle arise from purely stochastic processes such as genetic drift as species diverge from one another. The variety of sources of discordance between gene trees and species trees was synthesized in a seminal review in 1997 by Maddison (1997), who coined the term 'deep coalescence' to describe the phenomenon of ILS, but looking backwards in time, from the present to the past, rather than from Avise's perspective, which saw the process proceeding 'forwards' from ancestral to descendant populations (Figure 2). Whereas events such as HGT, gene duplication, or hybridization require specific molecular or population forces to be realized, the only criterion for ILS to arise is the finiteness of populations – a criterion which of course applies to every species and lineage in any clade, from bacteria to plants to humans (Edwards, 2009). Only in the imaginary situation when genetic drift is so severe that fixation of ancestral variants occurs instantaneously could ILS be ruled out in principle. Hence ILS is now recognized as the most prevalent of the major sources of incongruence between gene trees and the relationships of species in which those gene trees are embedded. As such it is the most important, but by no means the only, statistical issue that arises when multiple loci are analyzed.

Deconstructing Incomplete Lineage Sorting and Deep Coalescence

A good (nonmathematical) way to understand the phenomena of ILS and deep coalescence is to imagine a field of marbles of two different colors, with each color representing an allele in a single, ancestral population (Figure 2(a)). The two alleles in the ancestral population describe a simple gene tree, one with just

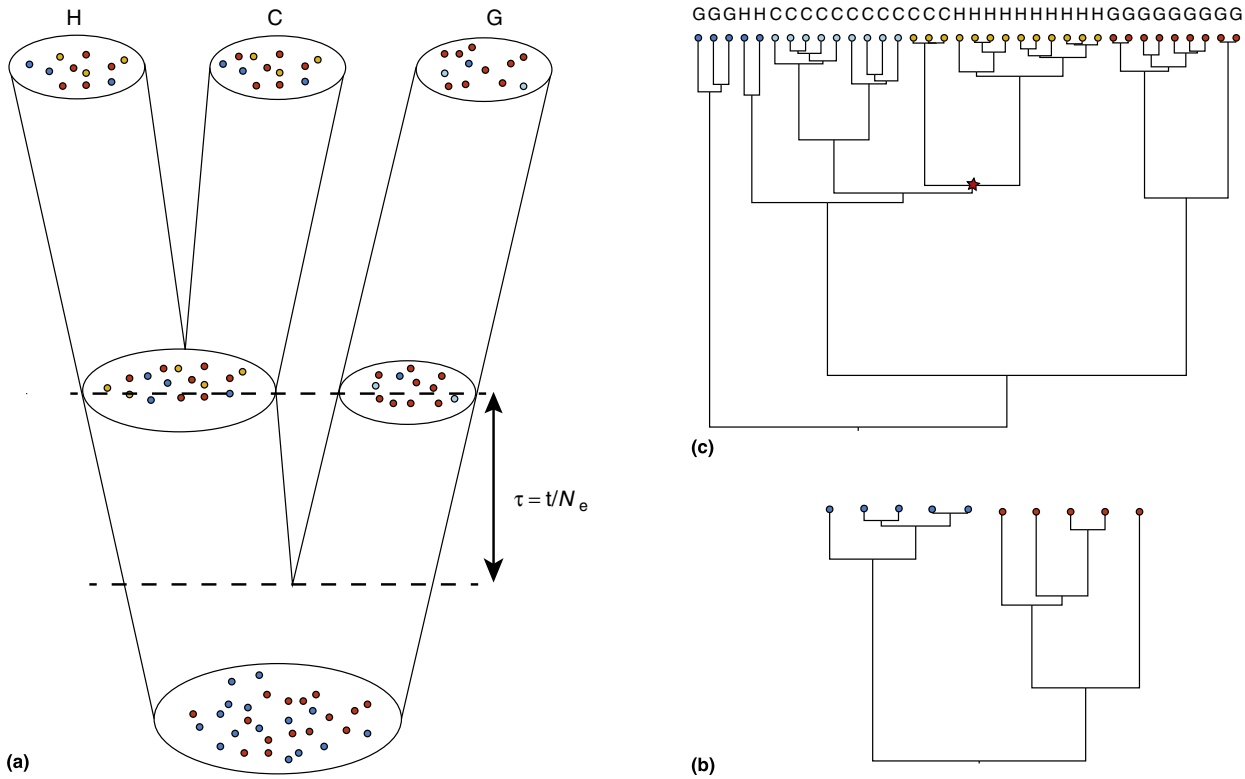

Figure 2 Illustration of the concept of incomplete lineage sorting or deep coalescence. (A) Species tree showing diversification of three lineages from a common ancestor. Alleles present in the common ancestor are retained in all three descendant lineages because of branches that are short in coalescent units. The length of the internode (τ) in coalescent units is indicated. (B) Gene tree of 12 alleles sampled from the ancestral population in A. The coalescent tree was generated using the R package Phybase (Liu and Yu, 2010). The single population used to generate the gene tree had a value of $\theta = 4\ N\mu$ of 0.01. (C) Gene tree of 12 alleles sampled from each of three populations, again using Phybase. The designations for the three species from which alleles are sampled are H, C, and G. Colored balls are qualitative designations of each allele corresponding to general relationships of alleles in the gene tree. The species tree used to generate the gene tree was: ((H:0.00402#0.01, C:0.00402#0.01):0.00304#0.01,G:0.00707#0.01))#0.01. The units of branch lengths of the species tree are substitutions per site and the values of θ appear after the pound sign. This gene tree displays substantial incomplete lineage sorting or deep coalescence. The interspecific coalescent event that occurs closest to the present (indicated by a star) is between alleles sampled from H and C, suggesting the species tree in A according to the method of Takahata (1989).

two lineages designating the two alleles (**Figure 2(b)**). An absolute barrier sundering the ancestral population into two spatially and phylogenetically distinct populations will result in both sets of alleles being found on either side of the barrier. To the extent that the two populations on either side of the barrier represent incipient species that will continue to diverge, the barrier immediately causes population para- and polyphyly of gene lineages to arise, a type of gene tree species tree conflict. The 'conflict' or discordance arises because the two populations no longer display reciprocal monophyly of gene lineages (Neigel and Avise, 1986). The conflict is also evident because we have sampled more than one allele per population. Had we sampled only a single allele per population, no such discordance would arise; either we would sample the same allele on both sides of the barrier, in which case the two alleles are identical, or we sample different alleles, in which case the gene tree simply reflects the historical divergence between the two alleles. In the latter case, the gene tree simply has two tips and no discordance with the 'species' tree is evident.

By the time one of the two descendant populations itself undergoes another population divergence, genetic drift may have caused allele frequencies in the two populations to have diverged and additional mutations may or may not have resulted in additional alleles (**Figures 2(a)** and **2(c)**). The second divergence event and consequential ILS in one of the two ancestral populations proceeds as the first one, with the diversity of alleles being found on either side of the second barrier, and discordance arising if multiple alleles have been sampled. However in this case, even if only a single allele has been sampled from what are now three descendant populations (or species), discordance is now possible. This discordance under three populations can occur if genetic drift prior to the second speciation event has not caused allele frequencies to shift significantly enough so that one or another allele is completely lost from one of the two populations (Pamilo and Nei, 1988). A key result of classical population genetics is that the average conditional fixation time of a new neutral allele in a population is 4N generations, where N is the effective population size of a diploid species with 2 N chromosomes (Kimura and Ohta, 1969). In the parlance of coalescent theory, we might say that the average coalescence time of two neutral alleles in a population is also 4 N

generations (Hudson, 1990). If the time in generations between our two speciation events is less than 4 N, there is a good chance that the alleles in the lineage comprising the internode in the species tree will not have gone to fixation, with the result that polymorphism still exists once the second speciation event comes about. If this is the case, the probability of discordance of the allelic genealogy, even when a single allele is sampled, is substantial and ILS can occur. Even if fixations have occurred, there is some chance that they would have proceeded in such a way as to cause discordance with the species tree. Going backwards in time according to coalescent theory, if the length of the internode between two speciation events is short on the scale of the population size (again, <4 N generations), then there is a higher chance that the two alleles sampled from sister lineages in the species tree will not have coalesced once one reaches the first speciation event, and ILS again is likely. The probability of ILS is not dependent on the absolute length t (in generations) of the internode, but rather on the ratio of t/N_e). Additionally, although a major impetus for species tree theory, ILS is not required for most species tree methods to apply.

Species Trees and the Multispecies Coalescent Model

The above scenario is meant to help novices visualize the causes of ILS. All of the events described above can be assigned probabilities, which are central to species tree inference methods and have their roots in seminal results of coalescent theory. Pamilo and Nei (1988) and Takahata (1989) were among the first to take the rules of coalescent theory as applied to a single population and extrapolate them to calculate probabilities of gene trees sampled from multiple phylogenetically related populations. Rannala and Yang (2003) formulated a likelihood model for gene trees sampled from multiple populations by applying coalescent theory for a single population sequentially to the various branches of a species tree. This work comprised the origins of the multispecies coalescent (MSC) model, the theoretical basis for most methods of species tree inference (Degnan and Rosenberg, 2009). The MSC allows researchers to compute probabilities, and hence likelihoods, of a gene tree or a set of gene trees given a species tree. This likelihood was a conceptually important advance in species tree inference, because it clearly separated the likelihood traditionally used in phylogenetics, namely likelihood of DNA sequence data given a gene tree, from the additional likelihood incorporated in species tree inference, namely the likelihood of a set of gene trees given a species tree (**Figure 3**).

When a single allele is sampled from the tips of a three-species tree, the only relevant parameter for calculating the probability of ILS is the length in coalescent units of the internode between the two speciation events, and probabilities of gene tree topologies can be calculated easily by hand (Wakeley, 2008). When more than three species are involved or when branch lengths of gene trees are specified, then likelihoods of gene trees can be calculated using software packages such as COAL (Degnan and Salter, 2005) or Phybase (Liu and Yu, 2010). The MSC also facilitates easy simulation of gene trees from a species tree (Leaché and Rannala, 2011; Leaché *et al.*, 2014). Programs such as COAL can rapidly calculate entire distributions of gene trees from a species tree with arbitrary numbers of alleles sampled. For example, for a four-tip species tree (say human, chimp, gorilla, and orangutan) with a total of seven alleles sampled (three human alleles, two chimp, one gorilla, and one orangutan), there will be 10 395 distinct rooted gene tree topologies, even though the species tree itself can have only 15 possible rooted topologies. COAL can calculate the probabilities of each of these gene trees as well as the entire distribution. Other programs such as Treefix (Wu *et al.*, 2013) allow more accurate estimation of gene trees given a species tree, and are especially applicable to large numbers of gene trees from multigene families across the genome of a suite of species.

Recent History of Species Tree Inference

The years 2006 and 2007 saw important advances in the development of species tree methods and challenges to the concatenation paradigm that had dominated phylogenetics for the previous 20 years. Although the problem of gene tree discordance was well described and likelihoods for gene trees could be calculated on a species tree, no one had yet proposed statistical methods for inferring species trees, except in the

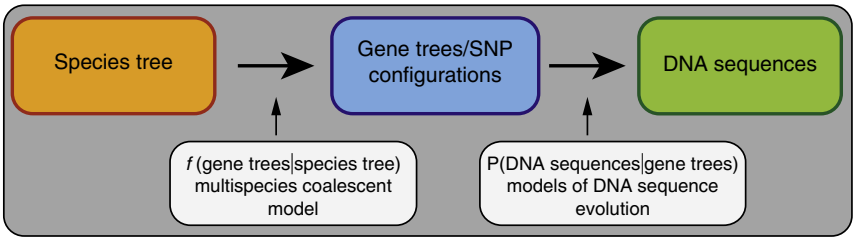

Figure 3 Processes generating parameters in species tree models and relationships among likelihoods in species tree models. The component of the overall species tree likelihood at the left is given by the probability density function (f) of the gene trees given the species tree and is based on the multispecies coalescent model. The more traditional likelihood in phylogenetics appears at right, the probability of the DNA sequence data given the gene tree. Many species tree methods combine these two likelihoods in order to estimate species trees from DNA sequence data. However, only Bayesian and SVD quartet methods estimate the species tree directly from DNA sequence data. Most other methods, such as STEM, MP-EST, STAR, and STELLS, employ a 'two-step' approach, in which the gene trees used to build the species tree are estimated separately and without reference to an overarching species tree. See **Table 1** for further details on each method.

simple case of three species (Pamilo and Nei, 1988; Takahata, 1989). Maddison and Knowles proposed minimizing deep coalescences (MDC) as a species tree method based on the principle of parsimony (Maddison and Knowles, 2006). Ané *et al.* (2007) proposed a method for summarizing Bayesian posterior distributions of gene trees and the concept of a concordance tree, a consensus tree with proportions on each branch indicating the proportion of the genome with a gene tree containing that branch. While the concordance tree idea was not based explicitly on the MSC and is strictly speaking not a species tree, it was still extremely useful for promoting the concept of and summarizing heterogeneity at the level of gene trees. Liu and Pearl (2007) proposed the first Bayesian method for estimating species trees, one that combined the likelihoods then available for calculating the probability of DNA sequence data given a gene tree and the probability of a set of gene trees given a species tree. Their software, Bayesian Estimation of Species Trees (BEST) (Liu, 2008) was the first widely used species tree method, one that employed a full Bayesian model. Other, simpler methods of species tree inference, such as GLASS, were also proposed (Mossel and Roch, 2010). Edwards *et al.* (2007) described using simulations and analysis of multilocus DNA sequence data some of the parameters for species tree estimation, showing that species trees could be estimated for a small or large number of loci depending on the shape of the species tree, in particular the length of the internodes. Kubatko and Degnan (2007) showed through extensive simulations that traditional maximum likelihood analysis of concatenated DNA sequence data could fail and was likely inconsistent if gene tree heterogeneity due to ILS was high. New insights into the behavior of alternative models of gene tree heterogeneity, such as that by Steel and Rodrigo (2008), quickly followed. The groundwork for species tree inference had been laid.

The anomaly zone: Another concept to emerge from this early work was the anomaly zone. The anomaly zone, first recognized by Degnan and Rosenberg (2006), is a region of species tree branch lengths that generate a distribution of gene trees in which the most common gene tree is actually different from the species tree. This paradoxical situation arises when essentially all gene lineages coalesce in the common ancestor of all species in the species tree, with the result that the topology of basal branches of the gene trees are dictated more by coalescent processes in the common ancestral lineage uniting all species rather than by the MCS. Although primarily a theoretical construct and apparently very rare in nature (Huang and Knowles, 2009), the anomaly zone has been proposed to apply in some empirical datasets (Linkem *et al.*, 2016). The anomaly zone is a useful analogy to the Felsenstein zone, a region of gene tree space leading to long-branch attraction and in which collecting more sequence data will positively mislead phylogeny inference with increasing certainty (Felsenstein, 1978). The anomaly zone is the gold standard for tests of the adequacy of a species tree method: if the method fails consistent inference in the anomaly zone, it cannot be said to be universally consistent. This criterion is often (Liu and Edwards, 2009; Liu *et al.*, 2009, 2010a) although not always (Mirarab *et al.*, 2014; Mirarab and Warnow, 2015) applied to newly proposed species tree methods.

Methods of Species Tree Inference

As of this writing, there are now numerous methods for estimating species trees (**Table 1**), reflecting a diversity of approaches and substantial research activity. The MSC has been exploited to take advantage of the diversity of DNA sequence data types as well as varying sophistication of statistical models employed. This diversity of approaches shows how the MSC can be used in all its complexity to infer species trees or can be distilled into simple rules that can guide species tree inference. The need for simpler models that do not employ the full likelihoods of the MSC quickly became evident as phylogenomic datasets grew in size (Liu *et al.*, 2015). For example, even the most sophisticated and popular Bayesian method for species tree inference in the PCR-era, *BEAST (Heled and Drummond, 2010), fails to converge with even moderate datasets consisting of a few tens of species and loci.

The various approaches highlighted in **Table 1** show both how flexible the MSC is and some of the practical challenges to implementation. For example, some methods, such as the MP-EST method, have thus far been developed to handle only one allele per species. Below is a brief summary of the logic behind some of the major pieces of software implementing species tree methods constraints and opportunities afforded.

Bayesian methods

Both *BEAST (Heled and Drummond, 2010) and BEST (Liu and Pearl, 2007; Liu, 2008) employ full and simultaneous likelihood estimation of gene trees and species trees. Both BEST and *BEAST take in DNA sequences as data and yield posterior distributions of gene trees, ancestral population sizes, and species tree branch lengths, as well as other parameters. The ability to study changes in effective population sizes over time is of general interest but has only rarely been used for testing various biological hypotheses, for example, in primates (Schrago, 2014a,b,c).

Maximum likelihood methods

Maximum likelihood species tree methods use estimated gene trees, rather than DNA sequence data, as input for species tree estimation. Thus they are sometimes called 'two-step' species tree methods (Liu *et al.*, 2015). MP-EST (Liu *et al.*, 2010a) is a popular pseudo-likelihood method that calculates the likelihood of a triplet of taxa in the species tree using gene tree triplets. It is a pseudo-likelihood method because the likelihood of the different triplets is not always independent, because they may share one or a pair of taxa (for example, triplet A, B, C is not independent of triplet A, B, D). Even the likelihood of two species tree triplets that do not share any taxa may not be independent if the genealogy of the two triplets shares branches internal to the species tree. Still, the pseudo-likelihood method has been shown to outperform concatenation methods in many situations, especially when gene trees are robustly estimated (Roch and Warnow, 2015). Other likelihood methods, including STEM (Kubatko *et al*, 2009) and STELLS (Wu, 2012), evaluate the likelihood of a set of gene trees. Whereas STEM uses branch lengths in the gene trees, STELLS focuses solely on gene tree topologies and a special peeling algorithm to estimate the species tree, which allows it to estimate large species trees rapidly from multiple

Table 1 Overview of methods of species tree inference. The table is not meant to be exhausted and highlights methods commonly used in the literature. Updated and expanded from Edwards (2009)

Method (References)	*Methodological basis*	*Basis in coalescent theory?*	*Data required*	*Accounts for stochastic variation or gene tree error?*	*Yields species tree branch lengths?*	*Yields effective population sizes?*	*Applicable to many loci?*	*Applicable to many taxa?*
Heuristic methods								
Species trees using average rank of coalescence time (STAR – Liu *et al.*, 2009)	Ranks of pairwise coalescence times	Yes	Coalescence times/Gene trees	Via bootstrapping	No	No	Yes	Yes
Specie trees using estimated average coalescence time (STEAC – Liu *et al.*, 2009)	Pairwise coalescence times	Yes	Coalescence ranks/gene trees	Via bootstrapping	No	No	Yes	Yes
Maximum tree (Liu *et al.*, 2010b); GLASS – Mossel and Roch, 2010	Divergence in gene trees/ coalescent	Yes	Rooted gene trees	No	Yes (assuming ultrametricity)	No	Yes (maximum and glass)	Yes
Neighbor-joining species tree (NJst – Liu and Yu, 2011)	Ranks of pairwise node distances	Yes	Unrooted gene trees	No	No	No	Yes	Yes
Gene tree quartets (ASTRAL–I and II – Mirarab *et al.*, 2014; Mirarab and Warnow, 2015)	Minimum quartet distances	No	Unrooted gene trees	No	No	No	Yes	Yes
SNP, haplotype or allele configuration methods								
Pruning algorithm (SNAPP – Bryant *et al.*, 2012)	Likelihood/ coalescent	Yes	SNPs	Yes	Yes	No	Yes	Moderate
Haplotype method (STELLSH – Wu, 2015)	Coalescent likelihood	Yes	Haplotypes with no recombination	No	Yes *	Yes *	Yes	Yes
Singular value decomposition (SVD Quartets – Chifman and Kubatko, 2014)	Site probabilities	Yes	SNPs	Via bootstrapping	no	no	Yes	Yes
Allele Frequencies (treemix – Pickrell and Pritchard, 2012)	Genetic Drift	No	Allele Frequencies	No	Yes *	Yes *	Yes	Populations within species
Likelihood Methods								
Maximum likelihood (STEM – Kubatko *et al.*, 2009)	Likelihood/ coalescent	Yes	Rooted gene trees	Via bootstrapping	Yes	No	Yes	Yes
Pseudo-likelihood (MP-EST – Liu *et al.*, 2010a)	Likelihood/ coalescent	Yes	Rooted gene trees	No	Yes *	Yes *	Yes	Yes
Likelihood for lineage sorting (STELLS – Wu, 2012)	Likelihood/ coalescent	Yes	Rooted gene trees	No	Yes *	Yes *	Yes	Yes
Joint inference of species and tree (JIST – O'Meara, 2010)	Likelihood/ coalescent	Yes	Gene trees	No	No	No	Yes	Moderate
Bayesian methods								
Bayesian estimation of species trees (BEST – Liu and Pearl, 2007; Liu, 2008)	Bayesian	Yes	DNA sequences	Yes	Yes	Yes	Moderate	Moderate
Bayesian concordance analysis (BCA – Ané *et al.*, 2007)	Bayesian	Yes	DNA sequences	Yes	No	No	Yes	Moderate
Bayesian inference (*Beast – Heled and Drummond, 2010)	Bayesian	Yes	DNA sequences	Yes	No	No	Yes	Moderate
Heuristic methods								
SINE method (discordance – Waddell *et al.*, 2001)	Likelihood	Yes	Binary characters	No	No	Yes	Yes	No

*Branch lengths in coalescent or drift units.

loci. These methods can handle larger datasets than Bayesian methods in part because they do not simultaneously estimate gene trees and species trees.

Maximum tree and related methods

Coalescent theory leads to the inference that the depth of a common ancestor of taxa A and B in a species tree can be no greater than the most recent common ancestor for A and B detected across the set of estimated gene trees. A consistent estimate of the species tree therefore will be that species tree whose branch lengths are as long as possible given the minimum divergences among pairs of species in the set of gene trees. Liu (2010b) showed that if the gene trees are estimated in a consistent manner, the MT tree will be a consistent estimate of the species tree. A similar method called GLASS was proposed by Mossel and Roch (2010), and STEM (Kubatko *et al.*, 2009) is a maximum likelihood implementation of the MT approach.

Parsimony methods

The main species tree method implementing the principle of parsiomony is the MDC method, first proposed by Maddison and Knowles (2006) and implemented in the software Phylonet (Than *et al.*, 2008). MDC finds the species tree that minimizes the number of deep coalescence events across gene trees. Although a powerful method backed by sophisticated dynamic programming algorithms and accurate under a wide range of species tree space, it has been shown to be inconsistent in the anomaly zone (Than and Rosenberg, 2011).

Fast nonparametric methods

A variety of fast, nonparametric methods have appeared in recent years, including STAR, STEAC (Liu *et al.*, 2009), ASTRAL (Mirarab *et al.*, 2014; Mirarab and Warnow, 2015), NJst (Liu and Yu, 2011), and other methods. These methods all share the property of using gene trees as inputs for species tree estimation, although methods differ as to whether they use rooted (STAR, STEAC) or unrooted (NJst, ASTRAL) gene trees. Most methods are based on distillations of coalescent theory whereas ASTRAL is a heuristic method that minimizes a score of the number of quartet trees required by a given species tree. STAR and STEAC both take advantage of the fact that the average rank and divergence time, respectively, of a pair of species across a set of gene trees is a consistent estimator of the rank and divergence time of that same pair in the underlying species tree. These methods show considerable power to estimate the topology of species trees but require many gene trees and, in the case of STEAC, the estimated branch lengths in the species tree are biased (Helmkamp *et al.*, 2012).

Methods based on SNPs

SNPs constitute an increasingly common form of genetic data, especially with new next-generation sequencing methods such as Rad-seq or Genotype-by-sequencing (GPS). Even when sequence reads consist of more than 100-bp, many such loci only have a single, often bi-allelic polymorphic site, hence the tendency to reduce the dataset to SNPs. SNAPP ('SNP and AFLP Phylogenies': Bryant *et al.*, 2012) was the first species tree method specifically tailored to SNP data. SNAPP assumes the classic Wright–Fisher MSC model and uses a novel pruning algorithm and dynamic programming to calculate the likelihood of particular site patterns in the data given a species tree. Another species tree method, singular value decomposition (SVD) on quartets (Chifman and Kubatko, 2014), is applicable to both SNP and multilocus DNA sequence data and uses algebraic statistics and a matrix of site pattern probabilities to determine the validity of quartets of species. After inferring all or a random subset of possible quartet relationships, a quartet assembly method is used to construct the estimated species tree. SVD quartets can be applied to very large datasets and estimates the species tree quickly.

Gene Duplication and the Multispecies Coalescent

Gene duplication is also an important source of genomic information for inference of species trees and genome history (**Figure1**; Rasmussen and Kellis, 2012; Boussau *et al.*, 2013; Szollsi *et al.*, 2014; Wu *et al.*, 2014). Gene duplication and loss can be considered a specific instance of more general birth–death models, which can account for births (speciation, gene duplication, and HGT) as well as losses (species extinction, gene loss) (Szollsi *et al.*, 2014). Powerful models now exist for estimating the history of multigene families given a species tree through coalescent processes as well as gene duplication and loss (Rasmussen and Kellis, 2012). Such methods are increasingly useful for more accurately defining orthologs, paralogs, and the history of gene duplication and loss in genomes. Although not covered in detail in this article, such models are a useful complement to methods for inferring the history of species themselves.

Networks, Reticulation, and Species Trees with Hybridization

Several species tree methods have begun to incorporate hybridization, gene flow, and reticulation as part of the model of diversification (Nakhleh, 2013). This represents a significant step in the evolution of species tree inference methods because, while ILS is likely to be ubiquitous in nature, it alone cannot explain all the patterns observed in gene trees and DNA sequence data. Networks have been a consistent component of systematics for decades, and it had been routine to depict variation within single genes as networks, where the reticulations often signaled homoplasy in the DNA sequence. However, the concept of networks is much more powerful when analyzed at the level of populations, lineages, and species, i.e., in a species tree framework (Pickrell and Pritchard, 2012). In this context, however, distinguishing processes such as reticulation, HGT and hybridization from the ever-present patterns of ILS can be challenging, since both processes induce gene tree discordance. Special models are required to accommodate multiple processes simultaneously.

Justification for Species Trees

Although the intellectual foundation for species trees goes back several decades, methods for inferring species trees

represent fundamentally new approaches to estimating phylogenetic and population history and combining information from multiple loci. Unlike concatenation methods, they acknowledge the ubiquity of independent gene histories mediated by recombination – a universal aspect of eukaryotic and prokaryotic genetics. Species tree inference methods are applicable not only to situations in which ILS is evident or on recent timescales, but really in all situations, whether or not ILS is occurring. Although empirically they often yield estimates of history that are not as highly supported as a similar concatenation analysis, this discrepancy is often attributable to the model misspecification inherent in supermatrix methods and the slower rate at which signal accumulates when gene trees are considered as conditionally independent from one another (Edwards, 2009; Song *et al.*, 2012). Although species tree inference methods are far from perfect and still apply to only a limited number of speciation models, they are superior to concatenation methods in so far as they acknowledge and accommodate basic aspects of the genetics of populations that concatenation methods do not. No doubt the ensuing decades will see a further proliferation and empirical application of new species tree inference methods for diverse types of molecular and other data.

See also: Coalescent and Models of Identity by Descent. Consensus Methods, Phylogenetic. Maximum Likelihood Phylogenetic Inference. Phylogenetic Networks. Supertree Methods, Phylogenetic

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Sperm Competition

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Glossary

Monogamy A mating system in which both males and females mate with only one individual during a reproductive episode, most commonly a socially paired partner.

Polyandry A mating system in which a female mates with multiple males during each reproductive period.

Polygyny A mating system in which a male mates with multiple females during each reproductive period.

Promiscuity A mating system in which both males and females mate with multiple partners during each reproductive period.

Sperm competition Competition to fertilize egg(s) between ejaculates of different males that overlap spatially and temporally in the fertilization environment.

Sperm quality A composite measure of the ability of a male's sperm to successfully fertilize ova relative to other males in the population, as determined by sperm morphology, number, and performance.

Introduction

Darwin (1871) recognized that males who are better able to attract mates or out-compete rival males would leave more offspring to future generations, and would therefore be favored by evolution. Perhaps not surprisingly then, the pursuit of reproductive opportunities has led to the evolution of some of the most striking features in the animal kingdom. Males produce conspicuous ornaments (e.g., the peacocks tail) that are used to attract more mates than rivals or arm themselves with costly weapons (e.g., a stag's antlers) that are used to outcompete rivals for access to mates. However, for Darwin and evolutionary biologists who followed for the next century, competition among males ended at mating. We now know that this is not the case. Molecular evidence have revealed that females frequently mate with multiple males during a single reproductive period and different males commonly sire eggs from a single clutch (Birkhead and Møller, 1998). Such female promiscuity means that competition between males continues after mating in the form of sperm competition, the contest between sperm from rival males to fertilize an egg(s). The evolutionary significance of sperm competition was first recognized by Parker (1970), sparking a paradigm shift that resulted in an explosion of interest in the importance of sperm competition in shaping the evolution of reproductive behaviors and phenotypes.

The pervasive influence of sperm competition has played a vital role in the evolution and extraordinary diversification of reproductive traits. Adaptation to the intense selective pressures imposed by sperm competition has generated an astonishing variety of reproductive phenotypes, from male genitals that resemble medieval weapons (Crudgington and Siva-Jothy, 2000), to testes so large that they account for more than 10% of adult male body mass (Montgomerie and Fitzpatrick, 2009), to gigantic sperm measuring a remarkable 6 cm in length (20 times longer than the male who produced them!) (Pitnick *et al.*, 1995). Here we explore how sperm competition generates the tremendous diversity in reproductive traits by describing the conditions under which sperm competition occurs and examining how sperm competition influences the evolution of animal behaviors, anatomy, and physiology.

Among species, female mating behavior varies widely and this has important implications for the risk of sperm competition experienced by males (**Figure 1**). In species with monogamous females, who only mate with one male, the risk of sperm competition is low (or absent) and in these species sperm competition is unlikely to represent an important selective force shaping reproductive traits. However, genetic monogamy is exceedingly rare in animals. More often, socially monogamous females mate with, and produce offspring by, males outside of their social pairing. Furthermore, in many species female promiscuity is common, as females either seek out multiple mating opportunities or have little control over the number of males attempting to fertilize their eggs (as is often the case in externally fertilizing species). Therefore, the prevalence of female multiple mating means that males of the vast majority of species will experience sperm competition, albeit to varying extents, and researchers have capitalized on this variation in female promiscuity and animal mating systems to gain a better understanding of how sperm competition shapes reproductive traits.

As with females, males too have evolved a suite of behaviors that influence the risk of sperm competition (**Figure 1**). Males can actively guard their mates to prevent subsequent matings by rival males and thus reduce their risk of sperm competition. In an effort to prevent sperm competition males can even take the extreme action of detaching and lodging his genitalia inside the female to physically block access to the female's reproductive tract (Fromhage, 2006). In contrast, male reproductive behaviors can also increase sperm competition risk. For example, socially subordinate males who are unable to successfully attract females often adopt 'sneaking' behaviors (**Figure 2**), attempting to surreptitiously fertilize eggs without the knowledge of socially dominant males (Gross, 1996). Therefore, despite the best efforts of males to reduce their risk of sperm competition, both male and female reproductive behaviors ensure that sperm competition persists.

For internally fertilizing species, male genitalia, which deliver sperm to the female's reproductive tract, are at the front

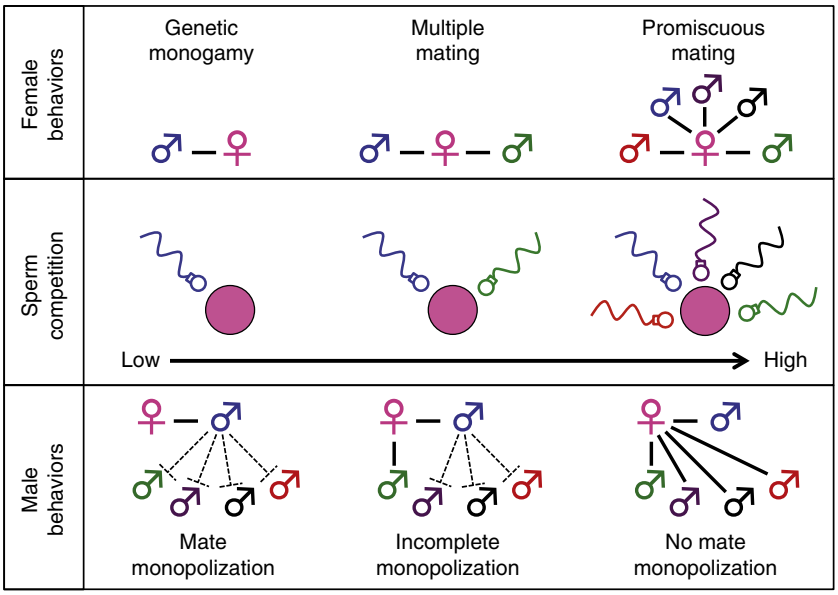

Figure 1 Summary of variation observed in male and female reproductive behaviors and how this variation influences the risk of sperm competition experienced by males. Male and female behaviors can work synergistically or antagonistically to influence sperm competition risk. Solid lines between males and females represent matings, while dashed lines represent thwarted mating attempts.

Figure 2 Sperm competition and sneaky matings. (a) Comparison of a large guarding male and a smaller sneaking male in plainfin midshipman (*Porichthys notatus*), a species with two alternative male reproductive tactics. Both males are reproductively mature. Guarding males court females, defend territories, and provide parental care for developing embryos, while sneaking males attempt to fertilize eggs surreptitiously and then leave the costly parental care to guarding males. Because sneaking males exclusively release sperm in the presence of a rival male they experience a higher sperm competition risk compared with guarding males. Testis size of a (b) guarding and (c) sneaking male demonstrating increased investment in testes in sneaking males, which represents a characteristic response to increased sperm competition risk. Photo credit: John Fitzpatrick.

line of competition among rival males. Male genital morphology is extraordinarily diverse (Figure 3), even among closely related species, and this diversity is attributable in large part to the selective pressures imposed by sperm competition (Hosken and Stockley, 2004). Male genitalia exhibit an astonishing variety of adaptations to maximize their chances of success in sperm competition. For example, male genitalia can displace sperm from previous matings, as is the case in the damselfly *Calopteryx maculata*, where male genitalia are covered in spines that remove almost all previously deposited sperm of rival males from the female reproductive tract, thereby virtually eliminating sperm competition (Waage, 1979). When looking across species, genitalia have more elaborate sexual 'weaponry,' including adaptations for sperm removal or displacement, in species where the risk of sperm competition is high compared with closely related monogamous species (Stockley, 2002). Similarly, populations of seed beetles (*Callosobruchus maculatus*) evolved under experimentally enforced monogamy

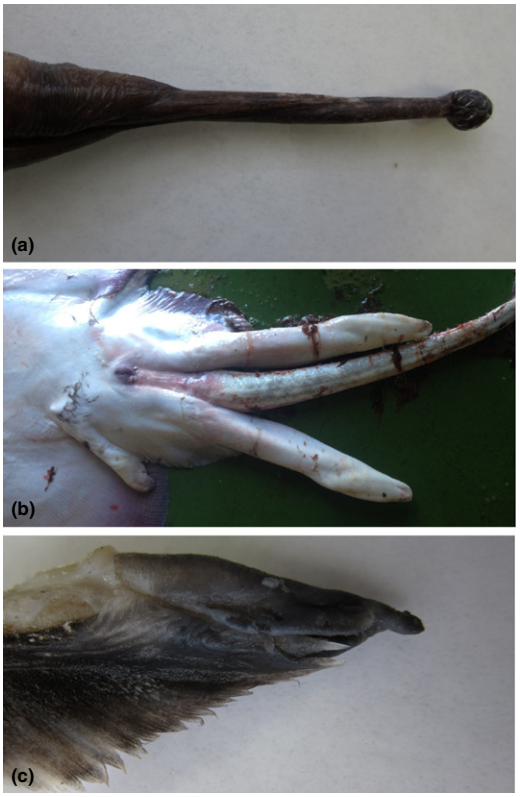

Figure 3 Diversity in genital morphology among cartilaginous fishes (sharks, skates, rays, sawfish, and chimaeras). Clasper (paired genitalia in cartilaginous fish) morphology differs dramatically in size and shape among (a) Australian ghostshark, *Callorhinchus milii* (Photo credit: Eduardo Garza Gisholt), (b) common skate, *Dipturus batis* (Photo credit: Amy Rowley), which shows paired claspers with the tail extending beyond the claspers and outside of the margins of the photo, and (c) New Zealand lanternshark, *Etmopterus baxteri* (Photo credit: Eduardo Garza Gisholt). Note the genital hooks and spurs observed at the terminal portion of claspers in (a) and (b).

show reduced genital spine length relative to polygamous populations (Cayetano *et al.*, 2011).

Sperm competition also influences genital size and shape. For example, in house mice (*Mus domesticus*), males maintained under breeding regimes where sperm competition occurred for 27 generations had thicker penis bones (baculum) than males maintained under enforced monogamy (Simmons and Firman, 2013). Across rodent and carnivores, the baculum is longer in species where sperm competition is prevalent (Ramm, 2007). There is good reason for genital morphology to respond to sperm competition risk, as several studies reveal that genital morphology predicts male reproductive success during sperm competition (Simmons *et al.*, 2009; Stockley *et al.*, 2013).

Following the release of sperm, either from male genitalia inside the female's reproductive tract or into the external environment in the case of internal and external fertilizing species, respectively, the sperm themselves are the primary combatants in male–male competition. Consequently, sperm are under intense selection, as those sperm traits that provide an advantage during sperm competition will be favored by selection. In particular, sperm competition influences the evolution of sperm number and sperm quality, both of which predict male fertilization success during sperm competition, albeit to varying degrees (Simmons and Fitzpatrick, 2012).

The number of sperm competing to fertilize an egg(s) can dramatically influence a male's competitive fertilization success. In many species, sperm competition is thought to follow a 'raffle principle,' where success in the raffle (in this case fertilizing eggs) is related to the number of 'tickets' (in this case sperm) a male holds (Parker, 1982). Under the raffle principle, all sperm have an equal chance of fertilizing eggs and therefore the probability of fertilization during sperm competition increases with the number of sperm transferred. Therefore, males are expected to invest more in sperm number when the risk of sperm competition is elevated (**Figure 2**; Parker, 1982).

The testes are the site of sperm production, and therefore an important target of selection for sperm number. Indeed, increases in testes size (in relation to body size) represent one of the most robust responses to sperm competition (**Figure 2**). The impact of sperm competition on testes size is especially well documented among primates (Harcourt *et al.*, 1981). For example, gorillas (*Gorilla gorilla*) experience a very low risk of sperm competition, as a single dominant male controls a harem of females and mates with them (almost) exclusively, and have remarkably small testes for their body size. In contrast, the closely related chimpanzee (*Pan troglodyte*) is highly promiscuous, facilitating very high levels of sperm competition, and has testes four times larger than those of a gorilla, despite weighing a quarter of a gorilla's body mass (Harcourt *et al.*, 1981). A similar pattern of increasing investment in testes in response to increase sperm competition risk is observed across a much broader range of primates (Harcourt *et al.*, 1981) and indeed across a wide range of other taxonomic groups, including birds, fish, amphibians, and reptiles (Simmons and Fitzpatrick, 2012).

Sperm competition not only selects for increases in sperm number but also influences the way males allocate their sperm during mating (Wedell *et al.*, 2002). While the costs of producing an individual sperm may be negligible, the ejaculate as a whole can be costly to produce (Dewsbury, 1982). Consequently, to maximize their reproductive success males are expected to strategically allocate their sperm during mating in response to cues of sperm competition (Wedell *et al.*, 2002; Parker and Pizzari, 2010). Males in many species indeed show strategic patterns of sperm allocation, but only under specific conditions. There is now clear evidence that males allocate more sperm when mating in the presence of a single rival male compared with matings where no rival males were present (Delbarco-Trillo, 2011; Kelly and Jennions, 2011).

However, sperm number is not the sole determinant of reproductive success during sperm competition, and under a broad range of conditions sperm quality (i.e., sperm morphology and performance) plays an important role in determining male fertility. Unique among cells, sperm must survive and travel outside the body in order to fulfill their function of fertilizing ova. Thus, as sperm moves toward the egg, various aspects of sperm quality, including sperm motility and swimming speed, will experience intense selection (Simmons and Fitzpatrick, 2012). For example, in domestic fowl (*Gallus domesticus*) and Atlantic salmon (*Salmo salar*), when females are artificially inseminated with an equal number of sperm

from two males, males with greater relative sperm velocity sire more offspring (Birkhead *et al.*, 1999; Gage *et al.*, 2004). Thus, in most species (but, for an exception see Dziminski *et al.*, 2009; Fitzpatrick *et al.*, 2012), sperm competition selects for increased sperm swimming speed (Simmons and Fitzpatrick, 2012).

Selection on sperm quality is predicted to impact on both sperm size and speed due to an assumed link between sperm swimming speed and sperm morphology, with longer flagella expected to generate greater propulsive force and allow sperm to swim faster (Gomendio and Roldan, 1991). While the underlying relationship between sperm morphology and swimming speed is far from clear (Humphries *et al.*, 2008; Fitzpatrick *et al.*, 2010; Simpson *et al.*, 2014), numerous studies have evaluated how sperm size responds to varying levels of sperm competition risk. In almost every taxonomic group studied to date (including mammals, birds, fish, reptiles, amphibians, and insects) there is evidence that sperm size increases with sperm competition risk (Simmons and Fitzpatrick, 2012). Moreover, a handful of recent studies have demonstrated that sperm competition also selects for faster swimming sperm across species, and this appears to be due to a positive relationship between sperm size and speed in these groups (Gomendio and Roldan, 1991; Fitzpatrick *et al.*, 2009; Lüpold *et al.*, 2009).

However, gaining a robust understanding of how sperm competition influences sperm size and speed has remained a contentious issue, as results contrary to the general pattern outlined above abound. Increasingly, efforts to understand how selection shapes sperm size and speed are focusing on better understanding the relationship and potential trade-off between sperm number and sperm size (Immler *et al.*, 2011). Although this work remains limited in scope, what is becoming increasingly clear is that sperm number, size, and speed are all important targets of selection by sperm competition, and which of these sperm traits are favored depends on the mechanism of sperm competition operating in a species (Immler *et al.*, 2011). For example, sperm are produced in greater numbers at the expense of size in large species with dilute female reproductive tracts where sperm competition adheres to the raffle principle. Conversely, where sperm displacement is the primary mechanism of sperm competition, as in many insects, selection acts to increase sperm size at the expense of producing greater sperm numbers (Immler *et al.*, 2011).

Increasingly, researchers are recognizing the importance of the non-sperm component of the ejaculate – seminal fluid – in mediating male reproductive success during sperm competition (Chapman, 2001). Sperm are released from males in the company of seminal fluid, a medium rich in proteins and other molecules produced by the accessory glands. These proteins exert a powerful influence on sperm competitive dynamics. First, seminal fluid proteins act on female physiology and behavior, which has knock-on effects for the risk of sperm competition faced by a male's ejaculate. For example, seminal proteins can reduce the risk of sperm competition experienced by an already inseminated ejaculate by reducing female receptivity to future matings (Chapman, 2001), aiding the displacement of rival males' sperm (Harshman and Prout, 1994), and contributing to the production of mating plugs that block access to the female's reproductive tract (Chapman, 2001). Second, seminal proteins can act to diminish the performance of sperm from rival males. In some social insect species, sperm perform better in the presence of the male's own seminal fluid than seminal fluid from a different, competing male (Den Boer *et al.*, 2010). However, the influence of seminal fluid on rival male sperm performance was not observed in monogamous social insect species, where sperm competition risk is low, indicating that seminal fluid function evolves in response to sperm competition risk (Den Boer *et al.*, 2010). Similarly, in the grass goby (*Zosterisessor ophiocephalus*), sperm of males using sneaky behaviors when attempting to fertilize eggs, and thus experiencing a higher risk of sperm competition, swim faster in the presence of the seminal fluid from a rival male, while sperm from males that use conventional courtship to woo females showed reduced fertilization rate when exposed to the seminal fluid of sneaky male (Locatello *et al.*, 2013). Thus seminal fluid has an important function during competitive matings and as such both the accessory gland and the proteins they produce evolve rapidly in response to sperm competition (Ramm *et al.*, 2005; Linklater *et al.*, 2007; Crudgington *et al.*, 2009).

In the four decades following Parker's (1970) recognition of the evolutionary significance of sperm competition, enormous advances have been made toward developing a comprehensive understanding of the evolutionary dynamics that govern sperm competition and its pervasive influence on male reproductive behavior, anatomy, and physiology. However, the study of sperm competition is still relatively young and many novel and exciting discoveries undoubtedly remain as advances in analytical tools and genomic approaches allow an ever widening set of questions to be addressed. Moreover, efforts to move the focus of sperm competition studies from the researcher's microscope to the female's reproductive tract (Manier *et al.*, 2010), the site of sperm competition in internal fertilizing species, promise to revolutionize our understanding of how sperm competition proceeds under 'real world' conditions. In this review, we focused our attention exclusively on evolutionary responses observed in males, paying particular attention to how sperm competition influences the evolution of male behaviors, genitalia, sperm, and seminal fluid. However, any discussion of sperm competition inevitably runs into the related topics of cryptic female choice, where females can influence the outcome of sperm competition by biasing fertility in favor of preferred males, and sexual conflict, where the evolutionary interests between the sexes differ. While these related topics are outside the immediate scope of this entry, we acknowledge their impact on shaping reproductive traits and encourage interested readers to follow up on these topics.

See also: Mating Systems in Flowering Plants. Polyandry and Female Postcopulatory Choice. Sexual Selection, Theory of

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Supertree Methods, Phylogenetic

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Supertrees

With the growth of evolutionary studies and consequently the availability of phylogenetic trees and data across many groups of taxa, there is increasing interest in combining insights from disparate sources to construct more comprehensive phylogenetic trees. Supertree methods and supermatrix methods are the two most common approaches to synthesize phylogenetic data. A supertree method combines a collection of phylogenetic trees with overlapping, but usually not identical, sets of taxa to create a single phylogenetic tree (i.e., the supertree) that, in most cases, contains all taxa from the input trees (**Figure 1**; e.g., Sanderson *et al.*, 1998; Bininda-Emonds *et al.*, 2002; Bininda-Emonds, 2004). In short, supertree methods generally combine smaller phylogenetic trees to build a larger phylogenetic tree (**Figure 1**), but any method to build a phylogenetic tree using other trees as input may be considered a supertree method. Consensus methods combine collections of trees with identical taxa (see Bryant, 2003). Thus, they may be considered a type of supertree method.

Although not designed specifically for phylogenetics, the BUILD algorithm (Aho *et al.*, 1981; but see Semple and Steel, 2003) is often described as the first supertree method. It builds a graph from a collection of input trees, and, if the trees are compatible, it outputs a tree that contains the topologies of all of the input trees. Numerous algorithms have enhanced or broadened the application of the original BUILD algorithm (e.g., Constantinescu and Sankoff, 1995; Ng and Wormland, 1996; Henzinger *et al.*, 1999; Daniel and Semple, 2004; Berry and Semple, 2006), but in practice, almost any collection of phylogenetic trees with taxonomic overlap will have topological conflict (i.e., no single tree can contain the topologies of all of the input trees). Algorithms such as MinCut (Semple and Steel, 2000), Modified MinCut (Page, 2002), and MultiLevelSupertree (Berry *et al.*, 2013) modified the BUILD or similar graph-based algorithms to combine incompatible trees. However, whether due to their complex, mathematical approach for resolving conflict among trees, questions about their performance on real datasets (e.g., Burleigh *et al.*, 2004; Eulenstein *et al.*, 2004; Chen *et al.*, 2006), or a lack of available, easy to use software implementations, these graph-based algorithmic approaches for supertree construction are seldom used in phylogenetics.

The matrix representation with parsimony (MRP) supertree problem provided the first pragmatic and intuitive method to construct supertrees (**Figure 2**; Baum, 1992; Ragan, 1992; see Baum and Ragan, 2004). For MRP, a collection of input trees is first transformed into a matrix of binary characters (**Figure 2**). Each column in the matrix represents a clade in an input tree. Usually taxa present in the clade are scored as '1,' and taxa not present in the clade, but otherwise present in the tree, are scored as '0'; the taxa that are not found in the input tree are scored as a '?,' representing missing data. After building the matrix, a phylogenetic tree (i.e., the supertree) is inferred from the matrix using maximum parsimony. With the availability of

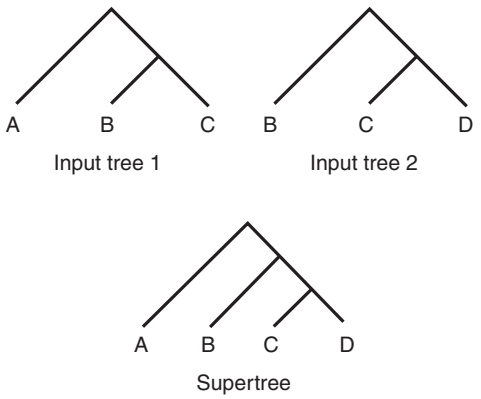

Figure 1 Supertree example. The two 3-taxon input trees on top can be combined to form a supertree that contains all four taxa found in input tree 1 and input tree 2. In this case, the supertree contains the topologies of input tree 1 and input tree 2, indicating that the two input trees are compatible. Interestingly, while the two input trees each contain information about one three-taxon tree (A|BC and B|CD, respectively), the supertree contains information about the relationships of four sets of three taxa (A|BC, A|BD, A|CD, and B|CD). Thus, the supertree induces two new sets of three-taxon relationships that are not present in the two input trees taken separately.

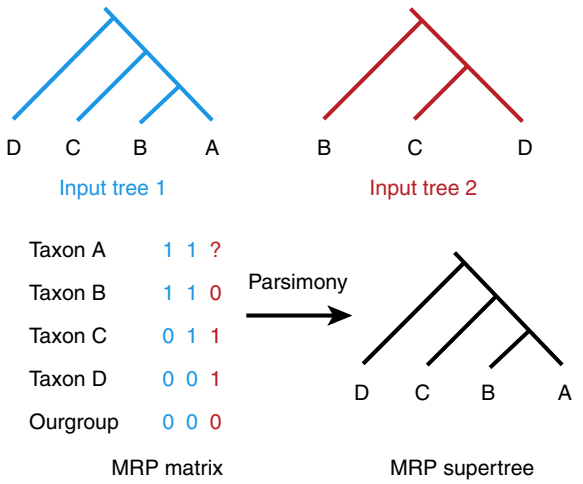

Figure 2 Matrix representation with parsimony (MRP). The two input trees can be coded as an MRP matrix. The first two columns of the MRP matrix describe clades in input tree 1 (a clade with A and B and a clade with A, B, and C), and the third column describes a clade in input tree 2 (a clade containing C and D, with taxon A missing). A maximum parsimony analysis of the MRP matrix will yield the supertree on the bottom right of the figure. Interestingly, even though input tree 1 and input tree 2 have conflicting topologies, the supertree is identical to input tree 1. This may be interpreted as a size bias for the MRP supertree method, in which the supertree more closely resembles larger input trees than smaller input trees.

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fast and effective software for parsimony analyses, MRP quickly became the most commonly used supertree method. It has been used in some of the largest and best known supertree studies, including those for mammals (Liu *et al.*, 2001; Bininda-Emonds *et al.*, 2007), angiosperms (Davies *et al.*, 2004), dinosaurs (Pisani *et al.*, 2002; Lloyd *et al.*, 2008), and birds (Davis and Page, 2014). However, MRP also is the most commonly criticized supertree method (e.g., Pisani and Wilkinson, 2002; Gatesy and Springer, 2004; Goloboff, 2005). For example, there are numerous ways to code the input trees a matrix of binary or discrete characters, and these different codings can result in different supertree topologies (e.g., Purvis, 1995). Furthermore, it is not clear why a parsimony criterion would be appropriate or desirable for the MRP matrix, as the columns in the matrix generally represent membership in a clade, not any character (e.g., Rodrigo, 1993, 1996; Slowinski and Page, 1999). MRP can be biased, with the MRP supertrees reflecting the topologies of input trees based on their shape or size (**Figure 2**; Purvis, 1995; Wilkinson *et al.*, 2005), and the resulting MRP supertrees can include clades that are unsupported by all input trees (Bininda-Emonds and Bryant, 1998; Pisani and Wilkinson, 2002; Wilkinson *et al.*, 2004; Goloboff, 2005). Furthermore, if the input trees represent gene trees evolving through a coalescent process, MRP can be inconsistent, converging toward the incorrect species tree topology with more gene tree data (Steel and Rodrigo, 2008). Much of the debate surrounding MRP may be summarized as a difference in perspective between those who dislike MRP because it can fail versus those like MRP because it often does not (e.g., Bininda-Emonds, 2003). This is the paradox of MRP: although it is unclear why the optimality criterion is appropriate and there are numerous ways MRP can fail, MRP remains a popular supertree approach that often produces credible supertrees.

Matrix representation with flipping (MRF) is an error correction method to construct supertrees from the same binary character matrix of the input trees used in MRP (e.g., Chen *et al.*, 2002, 2003; Burleigh *et al.*, 2004; Eulenstein *et al.*, 2004). MRF assumes that the conflicts among input trees are due to error. However, these errors can be fixed by changing the character states in the binary character matrix (i.e., changing 0's to 1's or 1's to 0's) until the input trees are compatible. MRF seeks the supertree that implies the fewest changes (i.e., the least error) the binary character matrix. MRF appears to perform well compared to MRP in simulation and empirical analyses (Burleigh *et al.*, 2004; Eulenstein *et al.*, 2004; Chen *et al.*, 2006), but it has not been used as often as MRP.

Although matrix-based supertree methods like MRP and MRF often perform well, their results are based on a matrix representation of the trees, not the trees themselves, and their optimality criteria can be difficult to understand. Another class of supertree methods uses optimality criteria that directly seek to maximize the similarity between the input trees and the resulting supertree. There are a number of different ways to measure the similarity (or distance) between the input trees and a supertree. For example, the Robinson–Foulds supertree methods seek to supertree that minimizes the Robinson–Foulds distance between that input trees and the supertree, maximizing the number of clades shared between the supertree and the input trees (Bansal *et al.*, 2010; Chaudhary *et al.*, 2012, 2013). Other approaches use the triplet distance (Lin *et al.*, 2009; Ranwez *et al.*, 2010) or the SPR distance (Whidden *et al.*, 2014) to find the supertree that is most similar to the input trees. Each of these approaches appears to perform well in some contexts (but see Goloboff and Szumik, 2015), but it is difficult to know which distance measure is most appropriate and ultimately how the conflict among trees should be resolved.

Other supertree methods take a more conservative approach and do not attempt to resolve conflict among the species trees (Goloboff and Pol, 2002; Berry and Nicolas, 2007; Ranwez *et al.*, 2007; Scornavacca *et al.*, 2008). For example, semi-strict supertrees (Goloboff and Pol, 2002) or matrix representation with compatibility (MRC; see Ross and Rodrigo, 2004) seek cliques (clusters) of pairwise compatible characters in the MRP matrix, which are then used to construct supertrees of the uncontradicted groups in the input trees. Similarly, PhySIC (Ranwez *et al.*, 2007), and later PhySIC_IST (Scornavacca *et al.*, 2008), are veto methods that infer supertrees that contain only relationships in the input trees or induced by input trees. While these methods can reveal areas of conflict among input trees and may avoid some potential biases and undesirable properties of more liberal supertree approaches (e.g., Goloboff, 2005), the resulting supertrees also generally contain less phylogenetic information. As we add more input trees, more conflict will arise, and simply avoiding the conflict ultimately may not yield a useful synthesis.

It is not always clear how to choose among the tremendous diversity of supertree methods. Generally, there is a dearth of studies that have carefully characterized properties of different supertree methods (but see Steel *et al.*, 2000; Wilkinson *et al.*, 2004, 2007; Dong and Fernández-Baca, 2009). Instead, there have been two general approaches to evaluate supertree methods that represent distinct perspectives on what a supertree method should be. First, simulation experiments have been used to evaluate how well a supertree method can infer the species phylogeny (e.g., Bininda-Emonds and Sanderson, 2001; Eulenstein *et al.*, 2004; Piaggio-Talice *et al.*, 2004; Swenson *et al.*, 2010; Chaudhary *et al.*, 2013). These approaches generally simulate alignments from a 'known' species tree, construct input trees from these alignments, and then construct supertrees from the inferred input trees. The performance of the supertree method is based on how well the resulting supertrees reflect the original species tree. In the second approach, the supertree method is judged by how well the resulting supertree reflects the relationships in the input trees (e.g., Chen *et al.*, 2006; Lin *et al.*, 2009; Bansal *et al.*, 2010). For example, one might measure the average Robinson–Foulds or triplet distance between the input trees and resulting supertrees. This approach values the similarity of the supertrees to the input trees and penalizes the differences between the supertrees and the input tree.

Thus, ultimately users must choose whether the supertree should reflect the true biological species tree or the relationships within the input trees. For example, imagine inferring a supertree from a dataset consisting of a single, erroneous species tree. Should the supertree method return the true species tree or should it return the erroneous input species tree (which best reflects the input)? This is the difference between a phylogenetic inference method and a tree synthesis method. Although many

biologists ultimately want the true species tree, the optimality criteria of most of the traditional supertree methods are based on tree synthesis, seeking to retain the relationships from the input trees. In practice, the results from a tree synthesis method and a tree inference method hopefully will not differ greatly. In fact, recently developed maximum likelihood supertrees, which use a model of phylogenetic error to infer a supertree from a collection of input trees, essentially provide a synthetic tree that should reflect the true species tree (Steel and Rodrigo, 2008; Akanni *et al.*, 2015). Maximum likelihood supertrees also can be a statistically consistent method to build species trees from gene trees (Steel and Rodrigo, 2008).

For an approach designed to unite disparate phylogenetic data, supertree methods have been particularly divisive area of phylogenetics. Some of the criticism has focused on the properties or performance of specific supertree methods (e.g., Gatesy and Springer, 2004; Gatesy *et al.*, 2002, 2004; Goloboff, 2005; Wilkinson *et al.*, 2005; Goloboff and Szumik, 2015), but there also are general limitations to supertree approaches. For example, by using trees as input, rather than then underlying character data used to build the tree, they fail to capture a great deal of information that could help to resolve the phylogeny. Also, the quality (i.e., accuracy) of the input trees can greatly affect the results (Bininda-Emonds *et al.*, 2004). While all phylogenetic methods depend on the quality of the input data, supertree methods may be especially susceptible to input data error because the data are usually estimates of inherently difficult phylogenetic analysis problems. Furthermore, simply obtaining and finding published phylogenetic trees, either from the literature or even tree databases, can be difficult (e.g., Chen *et al.*, 2008; Stoltzfus *et al.*, 2012). Finally, nearly all supertree methods output topologies without branch lengths, which are necessary for many evolutionary analyses. Methods exist to add branch lengths to supertrees (e.g., Gittleman *et al.*, 2004; Vos and Mooers, 2004; Webb and Donoghue, 2004; Torices, 2010; Eastman *et al.*, 2013), but the original supertrees may have limited use for evolutionary analyses.

In spite of the limitations of supertree methods, there are several ways in which supertrees are a part of cutting edge phylogenetics work. Recently, a supertree approach was used to combine a taxonomy of named species with published phylogenetic trees to create a supertree of ~2.3 million species, by far the most comprehensive representation of the tree of life to date (Hinchliff *et al.*, 2015). Interestingly, this approach constructs the supertree from a graph database, which can be modified to build supertrees using approaches similar to the BUILD algorithm and other related supertree algorithms that addressed the original supertree problems (see Chaudhary *et al.*, 2015). Perhaps the most relevant use of supertrees methodology is in the field of species tree methods, in which the species tree topology is inferred based on variation in phylogenetic trees, usually using a gene coalescence or duplication and loss model (see Liu *et al.*, 2009; Knowles, 2009; Anderson *et al.*, 2012). Although not covered in this article, most species tree approaches are supertree methods, in which a species tree is inferred from a collection of input (i.e., gene) trees based on a biological criterion. The first species tree methods, gene tree parsimony, are very similar to the distance based supertree methods described above (e.g., Goodman *et al.*, 1979; Guigó *et al.*, 1996; Page and Charleston, 1997). They take a collection of gene trees and infer the species tree that implies the fewest number of evolutionary events, such as duplications and losses, lateral transfer, or deep coalescence events.

Supermatrix Methods

Supermatrix methods combine phylogenetic character matrices from different sources with overlapping, but usually not identical taxonomic coverage (**Figure 3**; e.g., De Queiroz and Gatesy, 2007). To build a supermatrix, individual phylogenetic character matrices are concatenated into a single matrix (i.e., the supermatrix), which can be analyzed using conventional phylogenetic methods such as maximum parsimony, maximum likelihood, or Bayesian approaches. These matrices often represent molecular sequences (e.g., Driskell *et al.*, 2004; Thompson and Shaffer, 2010), but some supermatrices combine both molecules and morphology (Gatesy *et al.*, 2003; Goloboff *et al.*, 2009). In practice, not all taxa will be found in all input character matrices, and consequently, the supermatrices often have much missing data, which could represent either unsampled or missing characters. Although extensive missing data can be problematic (Lemmon *et al.*, 2009; Simmons and Goloboff, 2013), credible phylogenetic trees have been constructed from supermatrices with extremely high percentages of missing data (e.g., Driskell *et al.*, 2004; McMahon and Sanderson, 2006; Thompson and Shaffer, 2010; Pyron and Wiens, 2011; Hinchliff and Roalson, 2013; Pyron *et al.*, 2013; Soltis *et al.*, 2013; Burleigh *et al.*, 2012, 2015). Today, almost any phylogenetic analysis of large

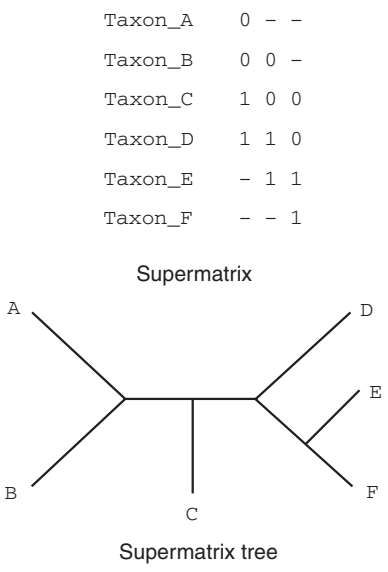

Figure 3 An example of a supermatrix analysis. The three columns in the supermatrix may come from different sources of data. Individually, each column can only describe the relationships among four taxa, but together the three columns can describe relationships among 6 taxa. Interestingly, if we remove data from Taxon_3 and Taxon_4, we cannot resolve the relationships among the remaining taxa. Only when we include data from Taxon_3 and Taxon_4 can we resolve the relationships among Taxon_1, Taxon_2, Taxon_5, and Taxon_6.

multi-locus or genomic data matrix may be considered a supermatrix, especially since, even with complete sampling, taxonomic sampling may not be even due to processes such as gene loss. Thus, there is often not an obvious distinction between a supermatrix and any combined data phylogenetic matrix.

Like supertree analyses, supermatrix analyses can induce relationships among taxa that are not apparent from analyses of any single data source (**Figure 3**). However, supermatrices have several seemingly intrinsic advantages over supertrees. First, they infer phylogenetic trees directly from the primary phylogenetic data, or characters. Thus, they can take advantage of sophisticated models of character evolution and phylogenetic inference software. Also, it is straightforward to quantify and interpret uncertainty for different tree nodes using either nonparametric bootstrapping or Bayesian posterior probabilities (e.g., Felsenstein, 1985; Huelsenbeck *et al.*, 2001). Finally, the output tree includes branch lengths, representing the number of substitutions, which can be easily transformed to represent time or make the tree ultrametric. This makes it easier to test rates and patterns of evolution and use the tree for phylogenetic comparative analyses.

Yet supermatrices are not without controversy. As supermatrices include more data, the data within the supermatrix will include more heterogeneity in rates and patterns of evolution. To encompass such heterogeneity within a single phylogenetic analysis, it may be necessary first to partition the supermatrices. Perhaps more worrisome is that the datasets may have different underlying trees due to processes such as hybridization/introgression or incomplete lineage sorting (e.g., Maddison, 1997), and combining datasets with different phylogenetic histories can lead to errors in the phylogenetic inference (e.g., Mossel and Vigoda, 2005; Kubatko and Degnan, 2007; Beiko *et al.*, 2008; Penny *et al.*, 2008). Thus, in some case, a species tree approach may be more appropriate than a supermatrix method.

While supertree and supermatrix methods may be viewed as competing methods for constructing large phylogenetic trees, they often have different objectives. Most applications of supertree methods seek a synthetic tree that best represents the relationships represented in the input trees. Thus, they are useful for representing the combined phylogenetic insights for a group of organisms. In contrast, supermatrix methods combine organismal character data to infer the species tree. Both approaches result in large trees, but the trees should be viewed or interpreted differently. However, some innovative new approaches for phylogenetics combine insights from both supertree and supermatrix methods. For example, with the growth of genomic data and a better understanding of the complexities of genomic evolution, it is clear that resolving a phylogeny often involves understanding not only patterns of variation among individual nucleotides, like a supermatrix method, but also patterns of variation among gene trees, like a supertree method. Thus, approaches that simultaneously infer gene tree from nucleotides and species trees from gene trees (e.g., Heled and Drummond, 2010; Bossau *et al.*, 2012) demonstrate that the solution to difficult phylogenetic problems may not be come from supertree or supermatrix methods alone, but by combining together the different perspectives from both approaches.

See also: Parsimony Methods in Phylogenetics. Species Trees, Inference of

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Support Measures, Phylogenetic Tree

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Glossary

Aligned sequence data A collection of amino acid, codon, or nucleotide sequences for organisms under study. Computer algorithms are used to align the sequence so that positions or sites are likely to be ancestrally related.

Alternative hypothesis A hypothesis of interest about the process or properties of the population that gave rise to data. In statistical tests, the probability of falsely rejecting a null hypothesis is controlled and small. Thus rejection of a null hypothesis suggests the alternative hypothesis is correct.

Bootstrap proportion The percentage of times a tree or split arose among trees estimated from bootstrapped alignments. Alternatively referred to as bootstrap support.

Bootstrapping Data-based simulation methods that are used to calculate *p*-values or confidence measures.

Conservative test A test is conservative if its actual false positive rate is smaller than its stated false positive rate.

False positive rate The probability of false rejection under the null hypothesis.

Likelihood The probability of observed data, treated as a function of the parameters in a model. Maximum likelihood estimation estimates parameters as those that maximize the likelihood.

Markov model A model for changes in discrete characters (e.g., nucloetides) over time where the probability of changes in the future depend upon past character states only through the current character state.

Nonparametric bootstrapping Bootstrapping where data sets are generated by sampling observational units at random and with replacement from the original data.

Null hypothesis A hypothesis of interest about the process or properties of the population that gave rise to data. In statistical tests, the probability of falsely rejecting a null hypothesis is controlled whereas the probability of falsely rejecting an alternative hypothesis is not. Thus failure to reject a null hypothesis is not usually considered evidence that it is correct.

Parametric bootstrapping Bootstrapping where data sets are generated using a parametric model, using parameters estimated from the original data.

Phylogenetic tree A graph showing evolutionary relationships between biological species.

Power The probability of correctly rejecting a null hypothesis when it is incorrect.

***p*-Value** The probability, calculated assuming a null hypothesis of interest, that a random test statistic is larger than the one observed in a study.

Split A partition of the taxa under study into two groups that are separated by an edge in a tree.

Test statistic A transformation of the data usually chosen so that values tend to be larger when the alternative hypothesis is correct than when the null hypothesis is correct.

Introduction

Modern methods of phylogenetic analysis use aligned sequence data to estimate evolutionary trees. A wide variety of methods are available for estimation. However, the information contained in aligned sequences varies substantially from study to study. Small sequence lengths or large evolutionary distances between sequences can lead to a great deal of uncertainty as to whether an estimated tree is correct. In this article methods for assessing uncertainty and their statistical properties will be discussed. One measure of support that will be considered is *p*-values coming from tests for significant evidence in favor of one tree by comparison with another. Another measure is bootstrap support for the claim that a set of taxa group together in the evolutionary tree. Due to its good statistical properties, maximum likelihood (ML) estimation is the most widely used method for estimating trees and some emphasis will be placed on measures of uncertainty associated with it. However, the bootstrap methods discussed apply more broadly to most methods for estimation.

Trees and Data

Figure 1 gives trees (Tree 1 and Tree 2A–2C) that might be considered possible for the $n=3414$-site mammalian mitochondrial data considered in Goldman *et al.* (2000):

		1	2	3	4	5	6	7	8	9		3414
0:	Mouse	I	N	I	L	T	L	L	V	P	…	L
1:	Opposum	I	N	L	L	M	Y	I	I	P	…	P
2:	Human	A	N	L	L	L	L	I	V	P	…	W
3:	Rabbit	I	N	T	L	L	L	I	L	P	…	W
4:	Harbor seal	I	N	I	I	S	L	I	I	P	…	W
5:	Cow	I	N	I	L	M	L	I	I	P	…	W

We will generally let x_i denote the data at site i; x_{ij} is the character state (nucleotide or amino acid, depending on the nature of the alignment). For instance $x_3=$ILLTII for the mammalian mitochondrial data.

Measures of support considered here include: (1) Two-tree tests: whether there is significant evidence against one specific tree in favor of another specific tree; for instance, against

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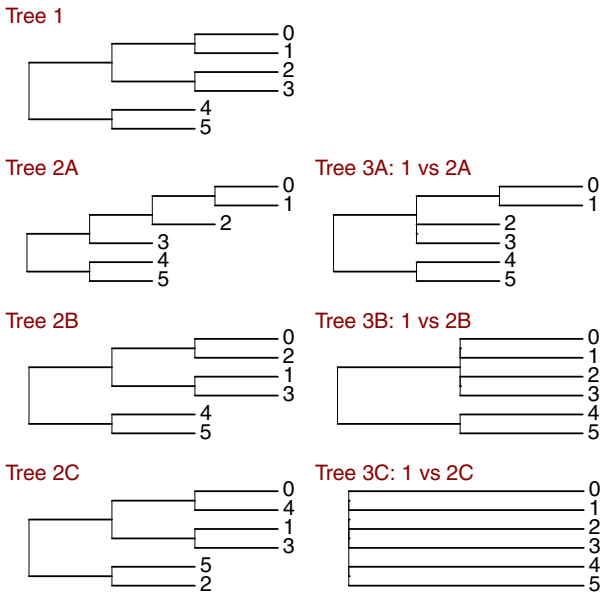

Figure 1 Tree 1 gives an example of a tree that significant evidence is being sought for. The trees 2A–2C give examples of trees that Tree 1 might be tested against with the KH test. The trees 3A–3C give the corresponding null trees obtained by collapsing edges in Tree 1 to make it equivalent to trees 2A–2C, respectively.

Tree 2A and in favor of Tree 1 in Figure 1. (2) Edge or split measures: whether there is significant evidence that a split in an estimated tree is truly present. For instance, one of the edges in Tree 1 can be described as splitting taxa 0 and 1 from the rest: 01|2345. The split 01|2345 is also present in Tree 2A, so one might be interested in whether that split is present without committing to either Tree 1 or Tree 2A as the correct tree.

Models and Methods

It is usually assumed that the observed character states at a site have been aligned in such a way that they are ancestrally related: the data that is observed at a position arose through a set of substitutions along the tree. Since algorithms like MAFFT (Katoh *et al.*, 2005) or PRANK (Löytynoja and Goldman, 2008) are used to convert raw sequence data into an alignment like the one for the mammalian mitochondrial data, there is some uncertainty about whether character states are correctly aligned to be ancestrally related, but this is a source of uncertainty that is usually ignored.

Treating the alignment as correct, and considering evolution at a particular site, most models make the reasonable assumption that given the ancestral character state for the immediate ancestor of an edge, evolution along that edge is independent of evolution along other edges. With this assumption, to model evolution at a site, it suffices to model evolution along an edge, which is usually assumed according to a time-homogeneous, continuous-time Markov chain (Felsenstein, 2004). Markov models require stationary frequencies as parameters and, in the case of nucleotide data, additional rate parameters are required; for amino acid models, empirical rate matrices derived from large databases are utilized to reduce the number of Markov model parameters requiring estimation. In most applications, additional parameters are required to allow different evolutionary rates over sites (Yang, 1994). A wide range of less commonly used, more complicated models and software are available allowing, for instance, variation of rates or substitution parameters across lineages (cf. Galtier, 2001; Jayaswal *et al.*, 2014). For the purposes of discussing tree support measures, the key issue is that there are a number of 'substitution model parameters' requiring estimation and providing additional sources of uncertainty: stationary frequencies of character states, rates-across-sites parameters, rate matrix parameters in the case of nucleotide data and, sometimes, additional parameters for more complex models.

Finally, most models assume that evolution across sites is independent. Because of interactions between sites in protein folding and other biological processes, this assumption is most likely not satisfied but, if the models of evolution at sites are reasonable, composite likelihood theory (Varin *et al.*, 2011) suggests treating sites as independent should still give reasonable tree estimates; the effect of ignoring site dependence on tree support measures is less clear and likely depends on the strength and nature of the dependence. In any case, the independence-across-sites assumption is convenient in that it yields a simple sum for the log likelihood or log probability of data:

$$l(\theta,\tau) = \sum_{i=1}^{n} l(x_i,\theta,\tau)$$

where $l(x_i,\theta,\tau)$ denotes the log of the probability of the aligned data at site i, τ is the tree that the probability is being calculated under and θ represents edge-lengths and substitution model parameters; θ_τ will be used to emphasize dependence on τ when it is important to do so. While most tree support measures adjust for uncertainty about edge-lengths, because of the wide variety of models, they sometimes do not adjust for uncertainty of all other parameters.

Tree support measures depend on the methods of estimating trees. ML estimation is perhaps the most frequently used method of estimation. It seeks to find the tree τ and substitution parameter estimates $\hat{\theta}$ that maximize $l(\theta,\tau)$. Packages available for ML estimation include PhyML (Guindon *et al.*, 2010) and RAxML (Stamatakis, 2014). Popular alternatives include parsimony and distance methods. Parsimony methods have a number of support measures like the consistency and retention indices that are specific to it. Distance methods start by considering the simplest ML problem: ML estimation for a pair of taxa. Because there is only one possible tree for a pair of taxa, ML estimation amounts to obtaining the evolutionary distance for the pair. Distance methods repeat this process for all pairs of taxa and then search for a tree for which the ML distances are well approximated by sums of edge-lengths over paths between taxa; one frequently used search strategy is the neighbour-joining algorithm (Saitou and Nei, 1987). Most of the measures of support considered here arise in an ML context. Bootstrapping methods apply generally to any method of estimation, including parsimony and distance methods.

Statistical Testing Concepts

Formal applications of hypothesis testing have a null hypothesis H_0 and an alternative hypothesis H_A. Given a suitable test statistic, T, chosen so that it tends to be large when H_A is true, an a-level test rejects the null hypothesis when $T > c_\alpha$ where c_α is chosen so that $P_{H_0}(\text{reject } H_0) = \alpha$. By definition, the test has probability of false rejection under H_0, alternatively referred to as the 'false positive rate,' that is controlled at α and is small. Since no control is provided over the probability of rejection when H_A is true, alternatively referred to as the 'power' of the test, H_0 and H_A are treated asymmetrically: if $T > c_\alpha$, it is unlikely H_0 is true but if $T \leq c_\alpha$, all that can be concluded is that it is not unlikely that T would have arisen under H_0; it remains possible that it was also not unlikely under H_A.

A specific example, whose relevance to tree tests will become clear later, is the z-test that a mean difference, μ, is 0 ($H_0{:}\mu = 0$) tested against $H_A{:}\mu > 0$. Such tests arise, for instance, when considering whether, say, the difference d in cholesterol levels of patients before and after some type treatment tends to be positive. Given a relatively large sample of differences, $d_1, \ldots, d_n$, under H_0, $z = \sqrt{n}\bar{d}/s_d$ should have an approximate standard normal distribution; here $s_d^2 = (n-1)^{-1}\sum_i (d_i - \bar{d})^2$. An α-level test rejects when $z > z_\alpha$ where z_α is the upper $(1-\alpha) \times 100$th percentile of the standard normal distribution. To have an $\alpha = 0.05$ false positive rate, the test must reject when $z > 1.64$. If $n = 100$ and the standard deviation of the d_i is 1, the power of the test is approximately 0.06, 0.64, and 0.99 when $\mu = 0.1$, 0.3, and 0.5.

In some cases, H_0 does not provide enough information to determine the false positive rate. For instance, a more natural H_0 for the cholesterol z-test example is $H_0{:}\mu \leq 0$ but different $\mu \leq 0$ give different false positive rates; in the example with $n = 100$, the false positive rate is 0.05 when $\mu = 0$ but 0.04 when $\mu = -0.1$. The convention in such cases is to require that the false positive rate be at most α for any parameters satisfying H_0, which usually amounts to requiring that the false positive rate be α at the boundary between H_0 and H_α. For the z-test, this criteria is satisfied if the false positive rate is α when $\mu = 0$; consequently the z-test is the same whether $H_0{:}\mu = 0$ or $H_0{:}\mu \leq 0$.

In theory, tests are designed to have known false positive rates, α. In practice, $P_{H_0}(\text{reject } H_0)$ often can only be calculated approximately, assuming a relatively large sample size. In some cases, while the false positive rate cannot, even approximately, be explicitly calculated, it can be said to be at most α. Such a test is referred to as 'conservative.' It will have less power than if the threshold, c_α, for rejection were determined so that the false positive rate is α. Nevertheless, knowing that a test is conservative is useful because it gives an upper bound on the false positive rate.

Finally, while it is natural to describe testing in terms of test statistics, T, it is convenient to calculate a p-value as $P_{H_0}(T > t_o)$, where t_o represents the observed test statistic. For instance, for the z-test, if the data gave $z = 1.3$, the p-value, $P = P(Z > 1.3) = 0.10$ where Z is standard normal. Note that since the test statistic T is a random quantity, the p-value, which is a transformation of the observed test statistic, is also a random quantity. Because $P_{H_0}(T > t)$ gets smaller as t increases, $t_o > c_\alpha$ if and only if $P = P(T > t_o) < P_{H_0}(T > c_\alpha) = \alpha$. Thus the α-level test that rejects when $T > c_\alpha$ is the same as the test that rejects when $P < \alpha$. Putting aside formal testing, it is useful to note that P is the probability of seeing a test statistic at least as large as the observed. If this is very small, either a rare event has been observed or H_0 is false.

Bootstrap Support

The 'bootstrap' broadly refers to data-based simulation methods that are most commonly used to obtain measures of uncertainty. Bootstrapping was originally described in Efron (1979) and introduced to phylogenetics in Felsenstein (1985). 'Nonparametric bootstrapping' is the most frequently used form of bootstrapping. The procedure generates a random sample of integers $i_1, \ldots, i_n$ with replacement from $1, \ldots, n$ which gives rise to a bootstrapped data set, $x_{i_1}, \ldots, x_{i_n}$. For instance, for the mammalian mitochondrial data, one might generate $i_1 = 3$, $i_2 = 6$, $i_3 = 3, \ldots, i_n = 9$ giving the alignment

		1	2	3		3414
0:	Mouse	I	L	I	…	P
1:	Opposum	L	Y	L	…	P
2:	Human	L	L	L	…	P
3:	Rabbit	T	L	T	…	P
4:	Harbor seal	I	L	I	…	P
5:	Cow	I	L	I	…	P

The term nonparametric is used because the procedure does not directly utilize the parametric model in simulations but rather the observed data. Bootstrapping is a class of 'resampling methods': methods that resample data from the original data.

For a given bootstrapped or resampled data set, parameter estimation can be done just as for the original data set. Estimates are denoted $\hat{\theta}^*$ and $\hat{\tau}^*$ to distinguish them from estimates for the original data. In applications of bootstrapping this is repeated many times, giving many data sets each in turn resulting in $\hat{\theta}^*$ and $\hat{\tau}^*$. The result is $\left(\hat{\theta}^*_{(1)}, \hat{\tau}^*_{(1)}\right), \ldots, \left(\hat{\theta}^*_{(B)}, \hat{\tau}^*_{(B)}\right)$ where the number of bootstraps B should, in principle, be large.

The results of bootstraping are commonly used to obtain a measure of support for tree τ as the percentage of $\hat{\tau}^*_{(b)} = \tau$, which is referred to as bootstrap support or 'bootstrap proportion' (BP) for the tree. The most common measure of uncertainty in phylogenetics is BP for splits, which is defined as the percentage of $\hat{\tau}^*_{(b)}$ that contained the split. Note that splits might arise in different trees. For instance, suppose that for mammalian mitochondrial data, only the trees 1, 2A–2B of Figure 1 were estimated among $B = 100$ bootstrapped data sets, and that Tree 1 arose 50 times while Tree 2 arose 10 times. Since these are the two trees with the split 01|2345, the BP for 01|2345 would be 60%.

If the value of B is small, BP will vary depending upon what particular bootstrapped data sets happened to arise through the random bootstrap generation, which is undesirable. For large B, it can be shown that BP will converge upon a value; denote it BP_c. The theory for bootstrapping is developed treating BP as if it were BP_c, so in principle, B should be as

large as possible. In practice, because phylogenetic estimation is computationally intensive, many applications take $B=100$. Binomial calculations can be used to obtain the chance that BP differs from BP_c by more than d% and are tabulated below

		BP_c(%)						
		50	60	70	80	90	95	99
$B=100$	$d=2$	0.62	0.61	0.59	0.53	0.40	0.25	0.02
$B=100$	$d=5$	0.27	0.26	0.23	0.17	0.06	0.01	0.00
$B=100$	$d=10$	0.04	0.03	0.02	0.01	0.00	0.00	0.00
$B=1000$	$d=2$	0.19	0.19	0.16	0.10	0.03	0.00	0.00

The table is symmetric around $BP_c=50\%$. For instance, if $BP_c=40\%$ and $B=100$, there is a 61% chance BP will differ from 40% by more than 2%. Since it is the more extreme BP_c values that are of particular interest, the relatively large chance of being more than 5% away from a correct BP of 50 or 60% need not be of concern. Nevertheless, one can see that using $B=1000$ leaves much less uncertainty as to whether results may be due to insufficient bootstrapping.

Adjusted Bootstrap Support

Bootstrap support is appealing because it can be applied to any estimation method whereas other tree support measures are tailored to the methodology used. Intuitively it is clear that large BP suggests strong support for a tree or split. If after deleting some sites and repeating others several times over, the same tree almost always gets estimated, it is likely the correct tree. Nevertheless, it would be nice to have a conventional statistical interpretation as a p-value, which would help to decide what constitutes large BP. For some time this was believed to be the case. Specifically, Efron *et al.* (1996) argued that 1-BP for a split could be considered a p-value for the test of H_0: the split is not present against H_a: the split is present. Under this interpretation, $BP>95\%$ should arise approximately 5% of the time when the split is not present. Susko (2009) showed that for ML estimation, due to the unusual nature of tree space, BP is actually conservative: $BP>95\%$ should arise less than 5% of the time. Through simulation it was found that the threshold required to have a 5% false positive rate depended upon the edge-lengths in the true tree and varied between 70–85%.

Susko (2010) provides an 'adjusted' BP, denoted aBP which is an approximation to $P(BP>BP_o)$ where BP_o is the observed BP and probability calculation is under H_0. As discussed in the statistical concepts subsection, $1-P(BP>BP_o)=P(BP\leq BP_o)$ can be interpreted as a p-value for the test of H_0 that uses BP as a test statistic; thus $1-aBP$ is approximately a p-value. Susko (2010) showed that $P(BP>t)$ can be approximated using simulations of normal random variables. The approach is a form of 'parametric bootstrapping.' It is parametric because the model for the data is implicitly used in generating normal random variables and bootstrapping because parameters in the simulation are estimated from the data.

An issue that needs to be borne in mind with most measures of support, including BP, is selection bias or the bias when splits for which support is obtained are selected post data collection; usually as splits in an estimated tree. BP for a split in an estimated tree can be expected to be larger than BP for a split specified prior to data collection. This makes sense, since for a fixed split, BP occasionally can be as low as 10% but this usually only occurs when that split was not present in the estimated tree. Distinctions can get blurry here as a split can be both a priori of interest and estimated. Considering the trees in Figure 1, imagine estimating separate trees from a large number of different alignments. The conservativeness of BP implies that BP for the split 01|2345, assuming it is not present, will tend to be larger than 95% less than 5% of the time. By contrast, the BP values for the splits in the estimated trees, which would change from alignment to alignment, might be larger than 95% far more frequently than 5% of the time.

RELL Resampling

An alternative type of bootstrapping that arises in phylogenetics is referred to as 'resampling estimated log likelihoods' or 'RELL resampling' (Kishino *et al.*, 1990). It is used in a number of statistical tests. We will illustrate its use for the Kishino–Hasegawa (KH) test developed in Hasegawa and Kishino (1989) and Kishino and Hasegawa (1989), which is the most widely used test for whether one tree is better supported than another. One tree (Tree 2) is fixed as true under H_0 and the other (Tree 1), the one for which significant evidence is sought, is the true tree under H_A; in principle, both trees should be specified prior to the collection of data. The KH test statistic is the average site log likelihood difference:

$$n^{-1}\{l(\hat{\theta}_1,1)-l(\hat{\theta}_2,2)\}=n^{-1}\sum_{i=1}^{n}\{l(x_i,\hat{\theta}_1,1)-l(x_i,\hat{\theta}_2,2)\}$$

$$=\sum_{i=1}^{n}d_i/n=\bar{d}$$

Expressed in terms of d_i, H_0:$E[d_i]=0$ and H_A:$E[d_i]>0$ and the test rejects when $\bar{d}$ is large.

As with the nonparametric bootstrap, in RELL resampling, a random sample of integers $i_1,\ldots,i_n$ is generated with replacement from $1,\ldots,n$. However, instead of selecting site patterns, $x_{i_1},\ldots,x_{i_n}$, to determine a new data set, the estimated log likelihoods, $l(x_{i_1},\hat{\theta}_j,j),\ldots,l(x_{i_n},\hat{\theta}_j,j)$ are resampled for the two trees, $j=1$, 2. A bootstrapped KH test statistic is then computed as $\bar{d}^*$, the average of the $d_k^*=l(x_{i_k},\hat{\theta}_1,1)-l(x_{i_k},\hat{\theta}_2,2)$. Because sites are being selected from the original data, the $\bar{d}^*_{(1)},\ldots,\bar{d}^*_{(B)}$ give the distribution for $\bar{d}$ that one would expect when data are generated from whatever the original process is, whether H_0 or H_A is correct. Because calculating a p-value requires the distribution for d when H_0 is true, the RELL version of the KH test replaces $\bar{d}^*_{(b)}$ with a centered version: $\bar{d}^*_{(b)}-B^{-1}\sum_b\bar{d}^*_{(b)}$. This ensures that mean of the distribution of $\bar{d}^*$ is 0 as required under H_0. A p-value is calculated as the proportion of centered $\bar{d}^*_{(b)}$ that are larger than the observed $\bar{d}$.

The original version of the KH test was effectively a z-test that the mean of a population is 0. As discussed earlier, if the d_i are independent and sequence length is relatively large, the z-test of H_0:$E[d_i]=0$ against H_A:$E[d_i]>0$, has p-value $P(Z>z)$ calculated with standard normal Z and $z=\sqrt{n}\bar{d}/s_d^2$ where

$s_d^2 = (n-1)^{-1}\sum_i (d_i - \bar{d})^2$. It can be shown that, as long as n is relatively large, the p-values using the original KH test or the RELL version will be almost identical, assuming B is large. For the types of sequence lengths encountered in practice, the RELL version is rarely worth the additional computation for the KH test.

Susko (2014) shows that the KH test is conservative (the false positive rate of an α-level test is usually much smaller than α) and provides a version that has an approximately correct false positive rate. Karin *et al.* (2014) provide a version of the KH that adjusts for alignment uncertainty.

A heuristic argument for the conservativeness of the KH test is that it is a consequence of using RELL in place of usual bootstrapping. In usual bootstrapping, the KH test statistic would be calculated as $\{l^*(\hat{\theta}_1^*,1) - l^*(\hat{\theta}_2^*,2)\}/n$ rather than the $\{l^*(\hat{\theta}_1,1) - l^*(\hat{\theta}_2,2)\}/n$ used in RELL resampling; here l^* denotes the log likelihood for a bootstrap sample. Because $\hat{\theta}_j^*$ is the ML estimate for the bootstrap sample, it makes $l^*(\theta,j)$ as large as possible; neither $l^*(\hat{\theta}_1^*,1)$ nor $l^*(\hat{\theta}_2^*,2)$ are likely to be very small. By contrast, $\hat{\theta}_1$ or $\hat{\theta}_2$ can occasionally be poor estimates for the given bootstrap sample, causing one of the $l^*(\theta_j^*,j)$ to be small, in which case a large or small KH test statistic will result. In short, RELL resampling is expected to occasionally produce more extreme bootstrapped KH test statistic, giving rise to more variability in the centered $\bar{d}_{(b)}^*$ and making it more frequent that the proportion of centered $\bar{d}_{(b)}^*$ will be larger than the observed $\bar{d}$.

Parametric Bootstrapping

For two-tree tests, the two trees can always be made equal by collapsing internal edges resulting in a tree referred to as the consensus tree. Figure 1 gives examples where the trees 3A–3C are the consensus trees obtained by collapsing edges in Tree 1 to make it equivalent to trees 2A–2C respectively. Recall that in testing the mean hypothesis, $H_0{:}\mu \leq 0$ against $H_A{:}\mu > 0$, p-values or critical values are determined by calculation at $\mu = 0$, the value on the boundary of the null hypothesis or the closest μ satisfying H_0 to values of μ under H_A. Consensus trees play this role in phylogenetics. Tree 3A, for instance, is on the boundary of tree space between the H_0 Tree 2A and the H_A Tree 1. Thus p-values or critical values should be determined by calculation under the consensus tree.

An alternative way of calculating p-values for the KH test statistic is through parametric bootstrapping. The idea is a general one applicable to any statistical test. Suppose the test rejects when test statistic T is large; for the KH test, T is the KH test statistic or difference in log likelihoods between Tree 1 and Tree 2. Parametric bootstrapping is similar to usual bootstrapping but instead of repeatedly generating bootstrap data sets from the original data, data sets with the same sequence length as the original data are generated from a model and assuming H_0 is true. For the KH test, data is generated from the H_0 tree, the consensus tree of trees 1 and 2, under whatever model was used to calculate likelihoods. Given estimated substitution model parameters and edge-lengths, software implementations like the seq-gen program of Rambaut and Grassly (1997) can generate simulated data on a tree. Let $T_{(1)}^*, \ldots, T_{(B)}^*$ denote the test statistic for each of the B bootstrap data sets. Then an approximate p-value is the proportion of $T_{(b)}^* > t_o$ where t_o is the observed test statistic. Similar comments as for usual bootstrapping apply as to how large B should be taken. Indeed, calculations are the same with BP_c and BP there replaced by 1 minus the correct and approximate p-value here.

See also: Consensus Methods, Phylogenetic. Molecular Evolution, Models of. Parsimony Methods in Phylogenetics. Phylogenetic Tree Comparison

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Symbiogenesis, History of

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Glossary

Archaea Oldest Domain of Life, previously designated as Archaebacteria.

Bacteria Second Domain of Life, previously designated as Eubacteria.

Domains of life/ three-domain classification Based upon results obtained from comparative molecular phylogenetics, Woese *et al.* (1990) undid the prokaryote/ eukaryote and five-kingdom classification and instead distinguished three major domains of life: Archaea, Bacteria, and Eukaryota.

Eukaryota/Eukaryotes Third domain of life, containing the Protist, Fungi, Plant and Animal Kingdoms. Eukaryotic cells have a membrane-bounded nucleus that contains the genome, and the cytoplasm often contains various cellular bodies called organelles.

Flagellum/Flagella Whip-like extensions of prokaryotic cells that enable motility. Contrary to eukaryotic undulipodia, flagella are made up of flagelin proteins.

Hereditary symbiosis Symbiotic association that becomes permanent and irreversible, foundational for symbiogenesis.

Horizontal transmission Any type of exchange between distinct individuals that happens during their lifetime and outside of the germ line.

Kingdoms of Life/ five-kingdom classification According to Whittaker and Margulis (1978), life can be classified into five kingdoms: Prokaryotic Monera (containing the Archaebacteria and Eubacteria), Eukaryotic Protoctista (Protists), Fungi, Plants, and Animals.

Lateral gene transfer Horizontal gene exchange between distinct organisms or distinct genomes within the same organism.

Metamorphosis Pre-evolutionary idea that living organisms can transform, or change in form. Transformation and metamorphosis are precursors to transmutation and evolutionary theory.

Microbiome The complete community of microorganisms that inhabit or live on the surface of an organism. The term is sometimes used to specifically refer to the genomes of the microbiota.

Microtubules Tubulin protein structures.

Prokaryotes All Archaea and Bacteria, typified by a free-floating instead of nucleated genome.

Defining Symbiogenesis

Symbiosis occurs when distinct organisms live in close association with one another, while symbiogenesis is both a phenomenon and an evolutionary mechanism (Merezhkowsky, 1905, 1910; Famintsyn, 1907; Kozo-Polyansky, 1924/2010) that results from permanent and hereditary symbiosis (von Faber, 1912; Buchner, 1921, 1939; Wallin, 1927; Lederberg, 1952; Sagan, 1967). Margulis and Dolan (2000, p. 157) define symbiogenesis as the "origin of a new organ, metabolic pathway, behavior, tissue, or other feature as a result of long-term hereditary symbiosis and includes the process by which organelles of the eukaryotic cell evolved from ancient bacterial symbionts."

Symbiogenesis today is well recognized to underlie the origin of mitochondria and chloroplasts. Both are cellular organelles (organ-like bodies), found exclusively in eukaryotic life forms (prokaryotes lack them, **Figure 1**), where they reside inside the cytoplasm of the cell (**Figure 2**). Mitochondria are present in aerobe protist, plant, and animal cells, while algal and plant cells also contain chloroplasts. These organelles once used to be free-living bacteria that, around 2 billion years ago, entered some of the first eukaryotic life forms through phagocytosis (eating or engulfment). The organisms engaged in symbiosis, this became permanent and hereditary, and the once free-living organisms evolved into the organelles (**Figure 3**). The bacterial lineages wherefrom mitochondria and chloroplasts evolved still exist as free-living bacteria today.

That mitochondria and chloroplasts evolved by symbiogenesis has for the most part of history been suggested based upon morphological comparisons between the organelles and bacteria, as well as the fact that these organelles contain their own DNA and have a double membrane, suggestive of bacterial engulfment (Ris and Plaut, 1962; Nass and Nass, 1963). Genetic comparisons between the organellar DNA and the DNA of bacterial lineages has now confirmed their bacterial origin. Chloroplasts evolved from cyanobacteria, and mitochondria evolved from proteobacteria (Bonen and Doolittle, 1975; Bonen and Doolittle, 1976; Bonen *et al.*, 1977; Gray and Doolittle, 1982). Molecular gene-sequencing techniques have furthermore shown instances of lateral gene transfer between the organelles and the nucleus, in both directions. After symbiogenetic acquisition, mitochondrial and chloroplast DNA underwent considerable gene loss (Archibald, 2014; Martin and Herrmann, 1998).

Eukaryotic cells often have many more organelles, and their evolutionary origin remains uncertain. A bacterial and symbiogenetic origin for the lysosomes has been suggested by its discoverer, the Belgian cytologist De Duve *et al.* (1974).

According to Margulis, the nucleated cell also evolved by means of symbiogenesis. As such, it was symbiogenesis that enabled both the evolutionary transition from prokaryotes to eukaryotes, and the subsequent evolution of the four eukaryotic kingdoms.

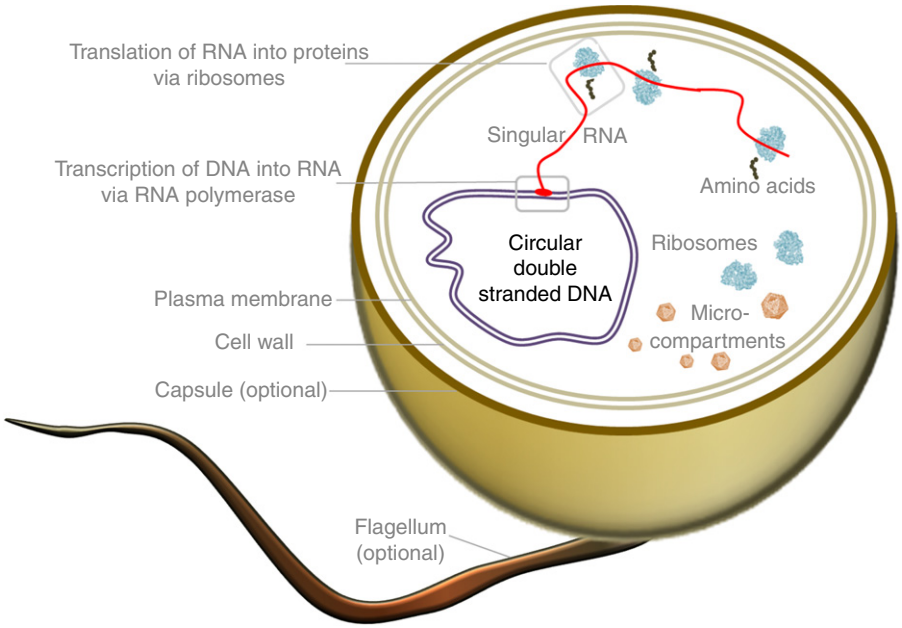

Figure 1 Schematic of a prokaryote. Prokaryotes neither have a membrane-bounded nucleus nor cellular organelles though they often contain micro-compartments that package enzymes and proteins.

From Hereditary Symbiosis to Symbiogenesis: The Origin of Mitochondria and Chloroplasts

Symbiosis research was often conducted in the margins of Darwinian evolutionary biology. Boveri (1904), for example, although a founder of chromosome theory, merely stated that Mendelian factors are transmitted via chromosomes. He also conjectured that the 'protoplasm' (cytoplasm) and chromosomes of the cell originated through symbiosis, an idea that was already introduced in 1893 by Shôsaburô Watasé (Sapp, 1994; Carrapiço, 2010, 2015).

Earlier in time, Schimper (1883, p. 112) suggested a single origin for "*Chlorophyllkörner*" (chlorophyll grains) and "*Farbkörper*" (pigment corpuscles) present in the plastids of plant and algal cells ("*plastiden*," subdivided into colorless "*leukoplastiden*," green "*chloroplastiden*" and yellow, orange and red "*chromoplastiden*"). He noted that they never arise *de novo* but develop from pre-existing 'protoplasmic' structures, and he thought they could divide and 'metamorphose' into one another. Because their protein structures ("*Proteïnkrystalle*") resemble those of 'living plasma' (bacteria), Schimper (1885, p. 202) later conjectured that chloroplasts might have resulted from a symbiosis between a colorless and chlorophyll-containing organism.

Merezhkowsky (1905, 1910, 1920a) identified these chlorophyll-containing bacteria as cyanobacteria ("*cyanophyceae*") and hypothesized that they underlie the evolution of all plant chromatophores ("*chromatophoren*," pigment-containing cells). Most importantly, Merezhkowsky argued that chloroplasts were different from their bacterial ancestors because they had evolved into new structures by a process he dubbed Symbiogenesis.

For Merezhkowsky (1910, p. 280, my translation), symbiogenesis is an evolutionary mechanism that forms part of a larger theory he introduced on the 'double' origin of life:

> All assumed and still assume today, that one plasma underlies all organisms, in other words, that out of non-being, life came forth from one root, from where one tree of organisms developed, first as a common trunk of protists, and then the tree split into two main axes – the plant axis and the animal axis. Until now, there was the general conviction, that the tree of life was a single one. The task set forth in this work, is to demonstrate that there are two trees of life, and that each tree originated on its own and independently from the other one, and this probably happened in different periods of earth's history. These trees partly developed on their own and independently from one another and partly stringed together and closely grew and developed together. Both trees are responsible for the diversity of organic beings. The idea of a unity of organic nature has to be abandoned in favor of the idea of nature's duality. (Merezhkowsky, 1910, my translation)

Life evolved from two distinct organismal types, each consisting of different 'protoplasms' ("*plasma-arten*"), "*Mykoplasma*" (Mycoplasma) and "*Amöboides Plasma*" (amoeba-like plasma). Mykoplasma is anaerobe, autotrophic, rich in phosphor and nucleic acid ("*nuclein*"), and experiments demonstrated a heat tolerance of up to 90 °C and a high resistance to poisons. Amöboplasma is aerobe and heterotrophic, low in phosphor and nucleic acids, it can only bear temperatures up until 50 °C, and it is easily poisoned. Based upon these features, Merezhkowsky (1910, p. 281) argued that "*Mykoplasma*" originated first because such organisms were better able to survive in the early earth's atmosphere and environment. Subsequently, and during different periods in time, these distinct life forms merged symbiogenetically. The first merger occurred between primitive bacteria and Monera and gave way to the evolution of amoebas. The second occurred when these amoebas merged with cyanobacteria (**Figure 4**).

Merezhkowsky thus pioneered in recognizing symbiogenesis' crucial role in the origin of complex life forms, and his

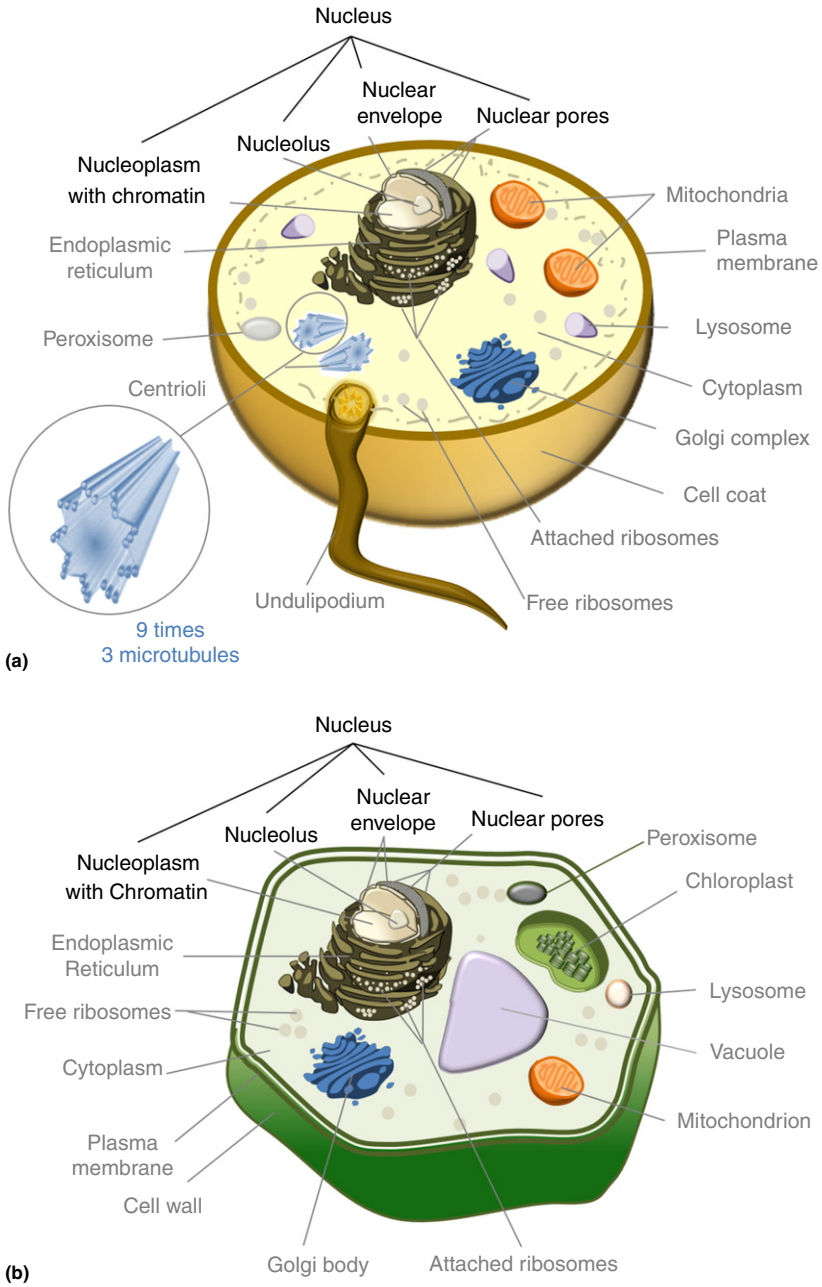

Figure 2 Schematic of an eukaryotic animal and plant cell. All eukaryotic cells have a membrane-bounded nucleus and many also possess numerous organelles.

double origin ideas made him recognize that a 'tree of life' needs to depict these symbiogenetic events.

Also Famintsyn (1907) and Kozo-Polyansky (1924/2010) developed ideas on symbiogenesis. Kozo-Polyansky understood evolution as the outcome of three phenomena: biotic potential or the ability to reproduce, symbiogenesis that generates variation and heritable novelty, and natural selection (Margulis, 1998, p. 1527).

The bacterial and symbiogenetic origin of mitochondria was first put forward by Kozo-Polyansky (1924/2010), Portier (1918) and Wallin (1923). Portier (1918) lived in France and when he suggested a symbiogenetic origin for mitochondria, he battled the successful Pasteur institute where bacteria became associated with disease (Sapp, 1994, 2003). In America, Wallin (1923) proposed that mitochondria evolved from oxygen-respiring bacteria, and he fought a similar battle against understanding bacteria merely as pathogens. For Wallin (1927), bacteria were the "building stones" or "primordial stuff from which all higher organisms have been constructed and modified," through *symbionticism*. Symbionticism is "the fundamental factor in the origin of species" because "microsymbiosis" can lead to new tissues and organs of such significance that they induce speciation.

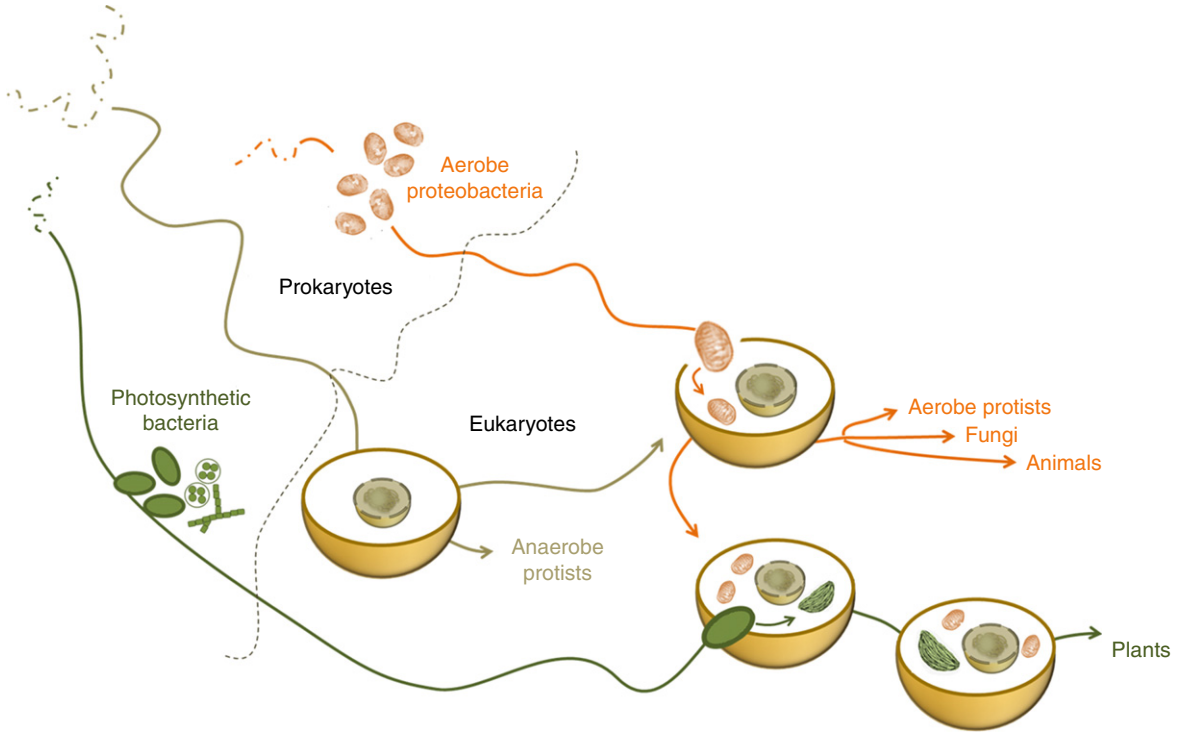

Figure 3 The origin of mitochondria and chloroplasts by symbiogenesis. Mitochondria evolved from aerobe proteobacteria, and chloroplasts from photosynthesizing cyanobacteria.

Earlier, von Faber (1912) had introduced the notion of "*erbliches Zusammenleben*," a concept Cowles (1915) translated as 'hereditary' and adopted by Buchner (1965). Also Wallin (1927) understood symbionticism as a 'hereditary mechanism.' Natural selection explained how species differentiate and evolve, but Darwin lacked clear theories on heredity and the origin of novel variation. For Wallin (1927, p. 121), symbionticism could complement Mendelian hereditary laws and theoretical genetics, because it introduces new genes and thus new hereditary variation: "on the basis of the new point of view that is associated with Symbionticism, we are forced to the conclusion that new genes must be acquired in organic evolution."

When Wallin suggested that mitochondria carry 'hereditary material' that can become part of the 'germ line,' 'genes' were still theoretical concepts presumably located on 'chromosomes.' Scholars that we now call adherents of 'cytoplasmic heredity' suggested that also the cytoplasm and the various organelles that house inside eukaryotic cells carry hereditary information.

One of them was Lederberg (1952), who in the 1940s reported on bacterial conjugation and transduction (**Figure 5**), two processes now characterized as mechanisms of horizontal gene transfer. For Lederberg (1952), however, both were instances of 'hereditary symbiosis' and 'infective heredity.'

Wallin and Lederberg's ideas that extrachromosomal, cytoplasmic structures such as plasmids or mitochondria carry hereditary material, and that these genes can become transferred to the nucleus were proven correct. Working at the Rockefeller Institute of Medical Sciences in New York, the Belgian cytoplasmic cell biologist Albert Claude, made the first electron-microscopy images of eukaryotic cells in 1945 (Palade, 1971). These enabled better visualizations of the cellular organelles and later, Ris and Plaut (1962) and Margrit and Silvan Nass (1963) respectively found DNA in chloroplasts and mitochondria, and linked it to symbiogenetic theories. Nonetheless, these ideas were overshadowed by the Modern Synthesis that advanced selectionist views of evolution. It was only through Margulis' work (Sagan, 1967) that data on symbiogenesis became widely recognized.

Lynn Margulis' Serial Endosymbiotic Theory

Our modern notions of symbiogenesis come from Lynn Margulis (Sagan, 1967), who from the 1960s onward, has introduced the Serial Endosymbiotic Theory (SET) (Sagan, 1967; Margulis, 1970, 1991, 1998; Margulis and Fester, 1991; Margulis and Dolan, 2001; Margulis and Sagan, 2002). Besides advancing a symbiogenetic origin for mitochondria and chloroplasts, according to SET also the eukaryotic nucleus evolved by symbiogenesis. In fact, according to Margulis, who endorsed a five-kingdom classification of life (Whittaker and Margulis, 1978; Margulis and Schwartz, 1997), all four eukaryotic kingdoms evolved as a result of three distinct symbiogenetic events (**Figure 6**).

SET gives the following chronological sequence of events (Margulis *et al.*, 2000; Margulis, 2010). In a first merger, fermenting thermoplasma-like archaebacteria (*Thermoplasma acidophilum)* merged with motile spirochete-like eubacteria, and evolved into the first anaerobe proto-eukaryotic cells (cells with a beginning nucleus). This first symbiosis is called motility symbiosis (**Figure 7**), because it presumably led to the

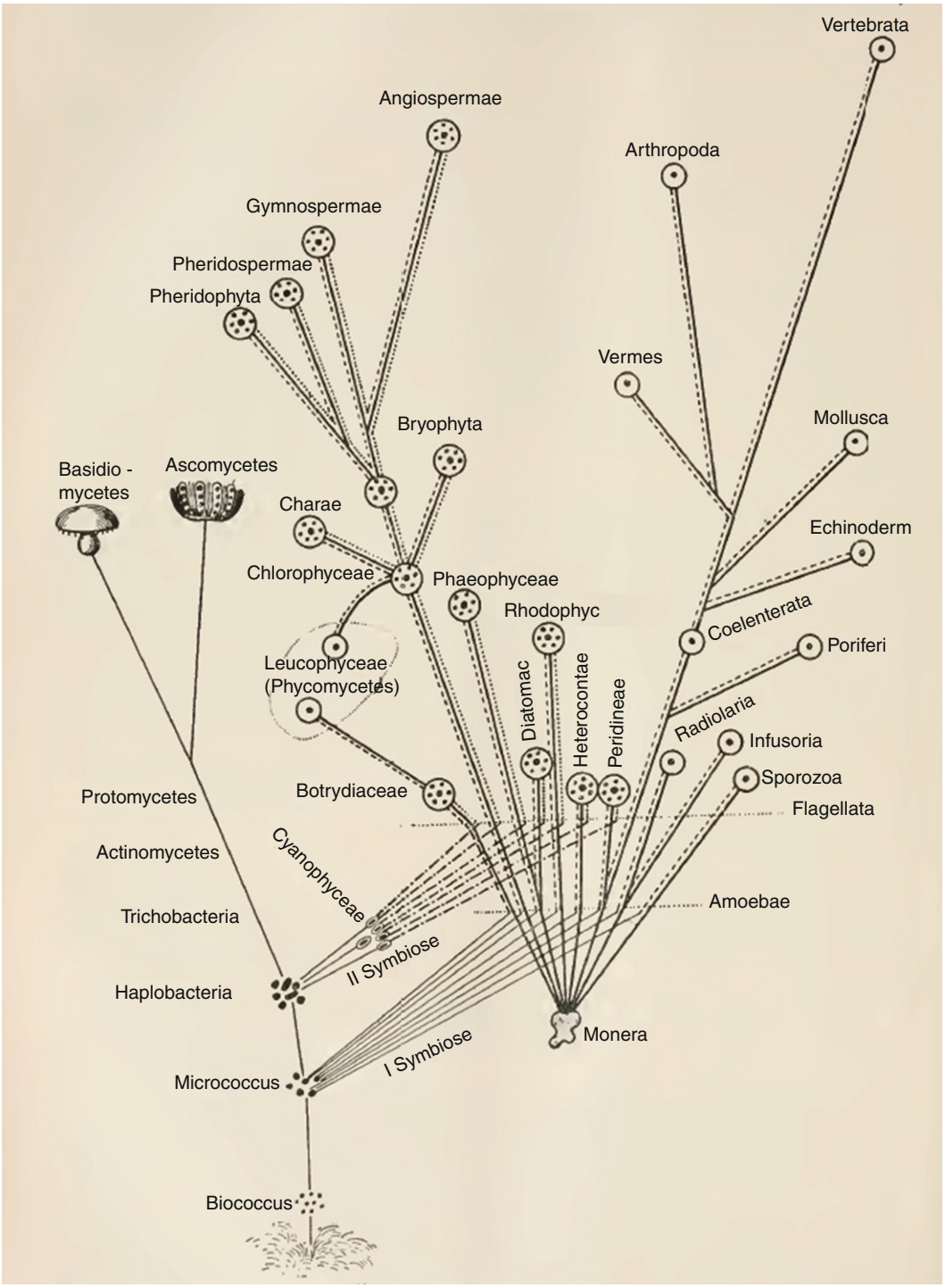

Figure 4 Merezhkowsky's polyphyletic tree of life (Merezhkowsky, 1910, p. 356). "*Mykoplasma*" is depicted by thin lines, "*Amöboplasma*" by thick lines, and the Cyanobacteria by interrupted lines. Mykoplasma evolved out of spontaneously generated biococci ("*Urbakterien*" or primitive bacteria), and this lineage evolved bacteria and fungi. Amöboplasma evolved out of spontaneously generated Monera. In a first symbiosis, Micrococcus on the Mykoplasma line merged with Monera resulting in the first Amoebae. A second symbiosis occurred between amoebae and Cyanobacteria (from the Mykoplasma line) resulting in flagellates. Consequently, he separated between three kingdoms: the kingdom of Mykoiden that never engaged in symbiosis (the thin line on the left); the animal kingdom resulting from a single symbiosis (the thick and interrupted lines); and the plant kingdom resulting from a second symbiosis event (thick, interrupted, and dotted lines). Leucophyceae were considered a side branch of plants.

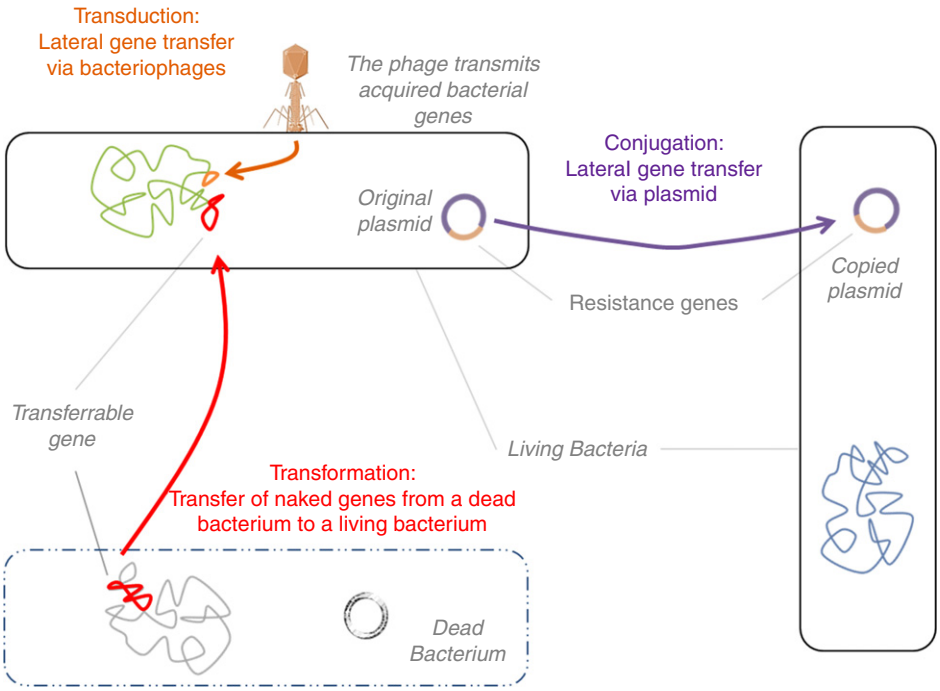

Figure 5 The three basic modes of lateral gene transfer amongst prokaryotes. In transduction, bacteriophages serve as vectors for the horizontal transmission of bacterial genes. In transformation, naked genes are picked up from their surroundings; and during bacterial conjugation, a single strand of a double-stranded plasmid (extrachromosomal DNA) is horizontally transferred from a donor to a recipient.

evolution of undulipodia and cilia (eukaryotic motility organelles that resemble tails and hairs) as well as centrioli (that form the centrosome which is the microtubule-organizing center that enables mitosis).

Evidence for motility symbiosis is found in the structure of undulipodia and centrioli. In cross section, centrioli are made up of microtubules organized according to a $[9(3)+0]$ pattern (**Figure 2(a)**). The same pattern is found in the cross section of the basal bodies (kinetosomes) of undulipodia and cilia (**Figure 8**). In their shaft (the axoneme), undulipodia and cilia have a $[9(2)+2]$ microtubular pattern. The structure of eukaryotic undulipodia is universal, and its morphological resemblance to the microtubular organization of centrioli makes Margulis assume that they share an evolutionary homologous origin, which she attributes to come from spirochete-like bacterial ancestors (Margulis *et al.*, 2000, 2006).

In a *second* merger, oxygen-respiring proteobacteria entered the cell's cytoplasm and engaged in permanent and hereditary symbiosis. The endosymbiotic bacteria evolved into mitochondria. Aerobic protoctists evolved, that, amongst others, includes amoebozoa and tailed (mastigote) cells, and from here all fungi and animals evolved.

In a *third* merger, early aerobic protoctists additionally engulfed photosynthesizing cyanobacteria that evolved into chloroplasts and gave way to the plant kingdom.

Wider Applications and Implementations of Symbiogenesis

Today, evidence is accumulating that endosymbiotic mergings occurred repeatedly. Sometimes the original merging bacteria as well as the organelles they evolved into were lost, and eukaryotes engulfed eukaryotes that already possessed organelles of bacterial origin. In this regard, scholars distinguish between primary, secondary, and tertiary endosymbiosis (Archibald, 2014; Raven, 1970; Stanier, 1974; Zook, 2015). In primary endosymbiosis, a prokaryote is engulfed by an eukaryote (a cyanobacterium enters a protist, leading to a photosynthetic eukaryote: a green algae). In secondary endosymbiosis, the product of primary endosymbiosis (the algae) is engulfed into another eukaryote where it wholly functions as an organelle (the transition from green to red algae); and in tertiary endosymbiosis, a eukaryote engulfs the product of secondary endosymbiosis (what happened when dinoflagellates evolved).

Nonetheless, an endosymbiotic origin for the eukaryotic nucleus remains debated. Some scholars assume that the outer membrane of prokaryotes merely folded inward thereby forming the membrane-bounded nucleus as well as the endoplasmic reticulum (Archibald, 2014). But these theories leave aside speculations on the origin of pro- and eukaryotic transcription and translation machineries that enable information to flow from DNA to RNA to proteins (**Figure 1**), as well as the origin of the eukaryotic chromosomes and mitosis.

Other scholars suggest that the eukaryotic nucleus evolved from viral symbiosis. For Livingstone Bell (2001), eukaryogenesis resulted from a symbiosis between a double-stranded DNA virus and a methanogenic Archaea. Villareal and Defilipps (2000) suggest that eukaryotic replication evolved from symbiogenetically acquired DNA viruses. The genes of these viruses and their host underwent hypercyclic organization and DNA compartmentalization into the complex eukaryotic chromosomes. Besides including virolution into the

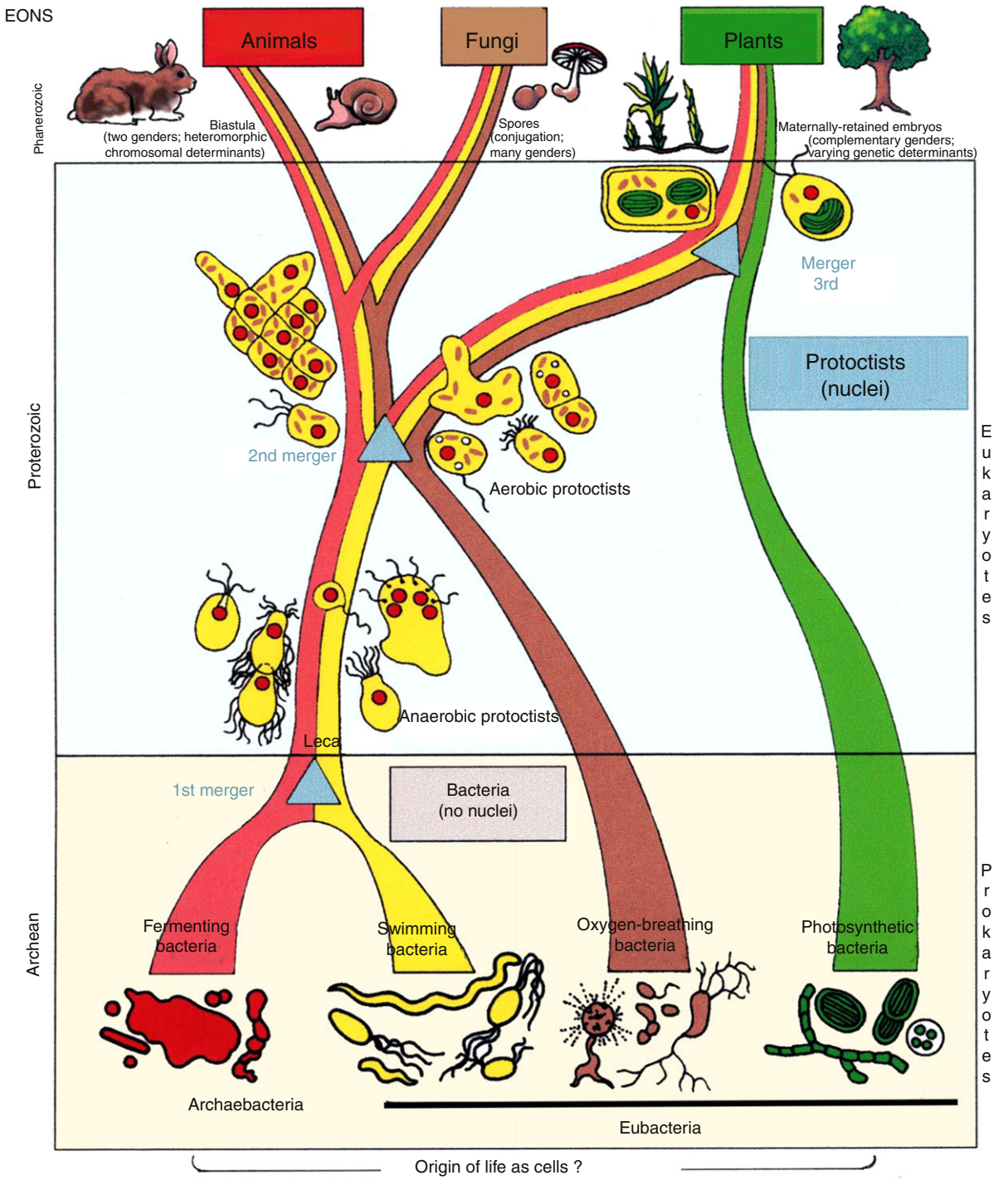

Figure 6 The origin of the four eukaryotic kingdoms according the Lynn Margulis' Serial Endosymbiogenetic Theory. The evolutionary transition from prokaryotes to eukaryotes happened because of symbiogenesis. Reprinted with permission and the courtesy of Ricardo Guerrero.

symbiogenetic framework, this research combines Eigen's (1996) theories on the origin of the genetic code with symbiogenesis theory, an idea already put forward in the 1980s by Dyson (1985).

Beyond eukaryogenesis, it is a fact that all eukaryotic life forms are prone to "viral colonization" (Villareal, 2000; Villarreal and Witzany, 2010, p. 699). Current microbiome and viriome projects are making scholars debunk the idea that viruses or microbial agents are mere pathogens or parasites. Rather, as mutual and commensal symbionts, these agents can introduce new genetic material that can become heredity and thus lead to symbiogenesis.

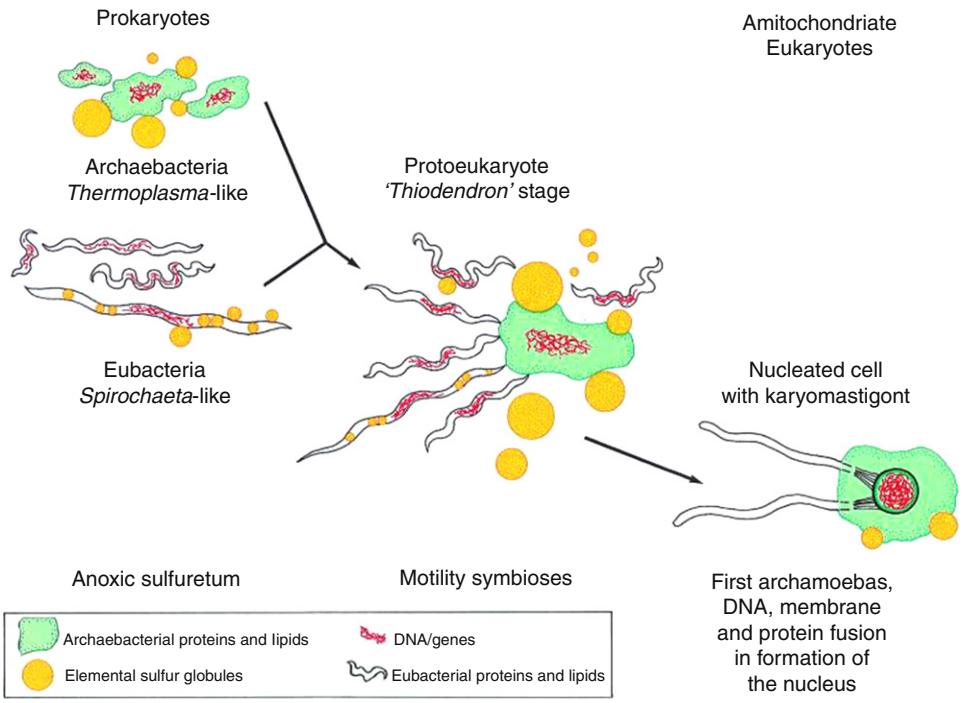

Figure 7 Schematic of motility symbiosis. The first symbiotic merger between spirochete and thermoplasma-like organisms enabled protoeukaryotic cells to acquire intra- and extracellular motility. Intracellular motility is necessary for the compartmentalization of genes into protein-rich chromosomes as well as for mitosis, the process whereby these chromosomes are doubled and pulled apart during division. Reprinted from Margulis, L., Dolan, M.F., Guerrero, R., 2000. The chimeric eukaryote: Origin of the nucleus from the karyomastigont in amitochondriate protists. PNAS 97 (13), 6954–6959, with permission and courtesy of Ricardo Guerrero.

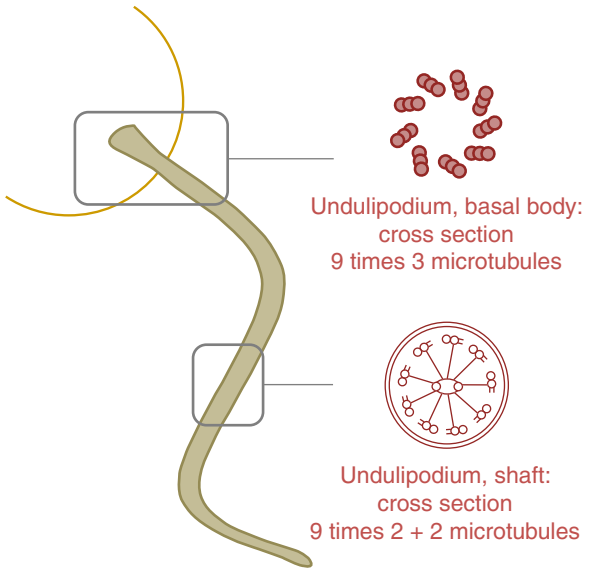

Figure 8 Cross section of a eukaryotic undulipodium. Its shaft has a 9 times 2 + 2 microtubular pattern while its basal body has a 9 times 3 microtubular pattern. The latter is identical to the icrotubular organization of centrioli, organelles that enable mitotic spindle formation (compare **Figure 2**).

Viruses mostly affect an organism's somatic cells during ontogeny. Upon infection, they either integrate into the host's DNA, or they use the host's metabolic apparatus to form new viruses, thereby destroying the cell upon release (Gontier, 2015a,b). Endogenous retroviruses (ERVs), however, are able to integrate into the germ line where they can influence phylogeny. Especially vertebrate genomes contain many remnants of ERVs, and ERVs in turn resemble mobile genetic elements (retrotransposons) that can become transferred laterally. This implies that ERVs "… have invaded the germ cell lines of every species of vertebrate. Here they replicate in Mendelian Fashion, as an integrated part of the sexual reproduction of the host, to inhabit the genome of all future generations" (Ryan, 2004, p. 560). Ryan (2002, 2004, 2009) considers such "… viral infection of host germ cells as a widespread but little-explored source of endosymbyotic creativity" (Ryan, 2006, p. 657), because the new symbiotic union can introduce new hereditary features.

Beyond influencing ontogeny and phylogeny, both symbiosis and symbiogenesis also impact evolution at the grand scale, by altering ecology from the environmental level all the way up to the biosphere. Winogradsky (1890, 1893) first discovered that the roots of legumes and the soil that surrounds these plants contain nitrogen-fixing bacteria. He later discovered the role these bacteria play in the earth's nitrogen-cycle. By launching 'microbial ecology' as a new research area, and by introducing the "*cycle of life*" concept (Ackert, 2007), Winogradsky helped bacterial research to transcend the medical disciplines.

Symbiogenesis also impacts the oxygen cycle. The Great Oxygenation Event marks the transition from an oxygen-low to an oxygen-rich atmosphere, estimated at 2.3 billion years

ago. The transition was induced by photosynthesizing cyanobacteria that originated 200 million years earlier. The new oxygen-rich atmosphere severely threatened the older obligate anaerobe Archaea and Bacteria. Oxygenation in turn caused methane to decrease which initiated the Huronian glaciation (Margulis and Fester, 1991). This environmental crisis triggered the first major extinction event (the oxygen catastrophe) as well as the evolution of aerobe bacteria. It also underlies the rise of symbiogenesis as an adaptive environmental response because some of the newly evolved aerobe bacteria became integrated as endosymbionts to subsequently evolve into cellular organelles.

As early as the twentieth century, such ecological and systems theoretical approaches led Reinheimer (1913) to provide a "*bio-economic*" view of life, wherein he introduced the concept of a "*web of life*" (Carrapiço, 2015). And many symbiologists today continue to link their theories to the idea that earth or 'Gaia' is a living superorganism (Lovelock, 1972; Lovelock and Margulis, 1974; Volk, 1998).

Reception of Symbiosis and Symbiogenesis in the Modern and Extended Synthesis

Ideas on symbiology first associated with sociopolitical ideologies and pre-evolutionary thought. After the introduction of natural selection theory, symbiology associated with vitalism, ecology, systems and hierarchy theory, cytoplasmic inheritance research, the biomedical sciences, and insight into the mechanisms of lateral gene transfer (understood as a form of hereditary symbiosis). These fields formed part of the 'eclipse of Darwinism' and developed in the margins of the Modern Synthesis that focused on selectionist, vertical-descent theories. From the onset, symbiologists have

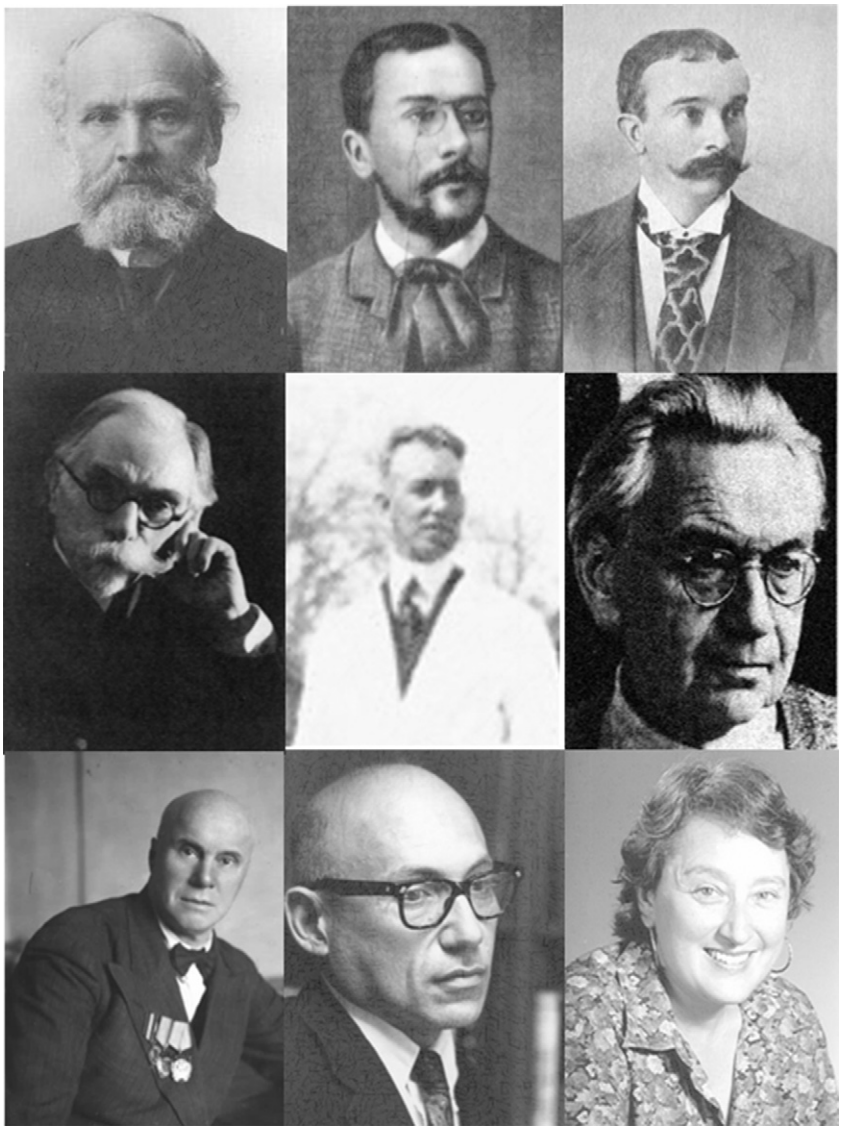

Figure 9 Pioneers in symbiogenesis research. From left to right and top to bottom: Andrey Sergeevich Famintsyn (1835–1918), Constantin Sergeevich Merezhkowsky (1855–1921), Andreas Franz Wilhelm Schimper (1856–1901), Paul Portier (1866–1962), Ivan Emmanuel Wallin (1883–1969), Paul Buchner (1886–1978), Boris Kozo-Polyansky (1890–1957), Joshua Lederberg (1925–2008), and Lynn Margulis (1938–2011).

in addition adhered to holistic, inter- and transdisciplinary stances, that counter the mechanical and reductionist approaches that characterized the division of the sciences at the turn of the twentieth century.

Its early associations with Western socialist thought (including Marxism) is not to be underestimated as a 'red flag' for neoliberal sociopolitical and Darwinian thought. In biology, symbiosis and symbiogenesis have often been typified as 'laws' of nature that either complement or contradict the 'laws' or 'mechanisms' of natural selection. Both presumed 'laws of nature' have been interpreted either in terms of struggle and competition, or cooperation and socialism, leading to both laws being understood as mutually exclusive. Nonetheless, by emphasizing cooperation and 'favoring' symbiosis over competition, symbiology too has, like competitive natural selection theory, been used to justify false believes on eugenetics, racism, hegemony, and national-socialism in order to obtain a 'higher good.' Early symbiologists and especially their critics often defined symbiosis in terms of parasitism, or as 'master–slave' relations (Sapp, 1994). Scholars such as Kropotkin, Reinheimer, Merezhkowsky, and Wallin understood symbiosis as a natural law necessary for progress, and especially Reinheimer and Merezhkowsky also saw symbiosis as a means for acquiring a 'higher good,' a 'better' and 'more cooperative' society that could be obtained by eugenetics. Merezhkowsky (1920b), for example, saw in symbiogenesis a justification for ethnic cleansing in order to develop a 'higher' society where mutualism would only arise amongst a select and chosen group (Sapp *et al.*, 2002).

Though both natural selection theory as well as theories on symbiosis and symbiogenesis find their historical roots in secular, Western sociocultural ideologies, both theories today are decoupled from such sociopolitical references. Nonetheless, the Serial Endosymbiogenetic Theory only became recognized post-synthetically, when molecular (phylo)genetics evidenced its basic morphologically obtained tenets.

Research on both symbiosis and symbiogenesis furthermore introduces new units and levels of evolution, including the *superorganism* (Spencer, 1876; Wheeler, 1928; Carrapiço, 2015), the *holobiont* (Margulis and Fester, 1991; Guerrero *et al.*, 2013), *symbiome* (Sapp, 2003), *symbiont* (Gontier, 2007), and *hologenome* (Rosenberg et al., 2007), as well as new means to draw evolutionary phylogenies (Brucker and Bordenstein, 2012), which today designates the rising field of *symbiomics* (after Sapp, 2003).

Currently, scholars associated with these disciplines are either pleading for an extension of the Modern Synthesis that incorporates the findings of symbiology with those of the Neo-Darwinian paradigm, while others are arguing for, or, a rupture with the latter in favor of a new evolutionary biology. The debates remain unsettled, but it is certain that increased genetic evidence for the symbiogenetic origin of life is causing for symbiosis and symbiogenesis to have finally received the scientific attention they deserve (**Figure 9**).

Acknowledgments

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See also: Complexity, the Role of Oxygen in Evolution of. Endogenous Retroviruses and Coevolution. Endosymbiotic Theory. Microbiome. Origin of Life, RNA World and. Origins of Life, History of. Plasmid Driven Evolution of Bacteria

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Symbiosis, History of

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Glossary

Deism The idea that the universe follows natural, mechanical laws even though it was created by a deity. The deity is presumed not to intervene after its original creation act. Deism was introduced by Natural Philosophers and is foundational for nineteenth century Natural History schools.
Ecology The study of the 'household' of organisms, broadly conceived as including biotic and abiotic environmental interactions, often depicted in hierarchy theories ranging from populations and species to ecosystems, biomes, and biospheres.
Metamorphosis Pre-evolutionary idea that living organisms can transform, or change in form. Transformation and metamorphosis are precursors to transmutation and evolution theory.
Symbiogenesis Evolutionary mechanism that results from hereditary symbiosis.
Symbiont Any organism that engages in a symbiotic relation with one or more organisms – often used to designate the smaller partner in the symbiosis.
Theism The idea that a supernatural being has created the universe and intervenes in creation at will. This belief is foundational for the Semitic religions as well as early Natural Theology schools.

Defining Symbiosis

Symbiosis refers to the phenomenon whereby two or more organisms with distinct genealogical, evolutionary histories live in close association with one another (de Bary, 1878, 1879). Together, the host and its symbionts form a new biological entity that is sometimes called a *superorganism* (Spencer, 1876; Carrapiço, 2015), *holobiont* (Margulis, 1991; Guerrero *et al.*, 2013), or *symbiome* (Sapp, 2003: 33); and this newly formed entity is considered to be a single unit, either of *natural selection* (through its hologenome, Rosenberg *et al.*, 2007; Zilber-Rosenberg and Rosenberg, 2008), or of other evolutionary mechanisms. Symbionts can be acquired both vertically (during phylogenesis) and horizontally (during ontogenesis). When symbiosis becomes obligate and hereditary, it can lead to evolution by symbiogenesis (Margulis and Dolan, 2000: 157). But even without causing symbiogenesis, symbiotic associations can affect an organism's adaptation, reproduction and fitness, species extinction or speciation, and symbiosis can influence ecological systems (Brucker and Bordenstein, 2012; Margulis, 1991; Zook, 2015).

Is Nature 'Red in Tooth and Claw' or 'Social' – Origins of Symbiosis Research in Natural History Research and Its Relation to the Sociopolitical Sciences

Research on symbiosis originated in nineteenth century European Natural History Research. Natural History Research marked the beginning of the modern sciences and encompassed the physical, biological, and cultural sciences (Gontier, 2011). Rather than adhering to 'divine laws' specified in religious writings, utopian scholars such as Thomas More or Bernard Mandeville, and moral philosophers such as Thomas Hobbes, Adam Smith, David Hume, and Jean-Jacques Rousseau would search for 'natural laws,' and transition from a theist to a deist worldview. These moral philosophers are called Social-Contract theoreticians because they founded sociopolitical, secular thought by debating the fair and just distribution of 'public goods' and 'natural resources' across nations, the 'natural' 'division of labor,' and the 'common goods' (shared belief states) that socially bond individual citizens into a 'common wealth' (a nation) (Gontier, 2009).

Their theorizing on the 'naturalness' by which humans 'live together,' and what the most righteous sociopolitical societal structure is, would come to define liberal and social thought and lead to the rise of the major secular sociopolitical doctrines of the nineteenth and twentieth centuries (libertarianism, liberalism, socialism, Marxism (communism), communitarianism, national socialism, and democracy). These sociopolitical ideologies were formulated by drawing idealized analogies with the animal world (often in the form of fables), which in turn founded preliminary researches on 'animal societies' that developed into naturalistic, and eventually evolutionary research on biological species.

It is well-known that Darwin (Burrow, 1972; Barrett, 1977; Bowler, 1983; Smocovitis, 1996) was inspired by:

1. Adam Smith's liberal idea of free-market economy and his metaphor of the 'invisible hand';
2. Thomas Malthus's notions of 'scarcity of resources' and 'struggle for existence';
3. Herbert Spencer and Thomas Huxley's liberal ideas on sociocultural 'progress';
4. Jean Baptiste Lamarck's ideas of 'adaptation' and teleological progress in biology;
5. Francis Bacon's 'induction theory';
6. Charles Lyell's 'uniformitarianism'; and
7. Wilhelm von Humboldt and Auguste Schleicher's 'natural genealogies' of the Indo-European languages.

The early Darwinists did not distinguish the natural from the sociocultural sciences, and their evolutionary theories were a natural extension of sociopolitical, liberal Hobbesian thought – how the latter ideology defined the 'natural'

condition as well as the sociocultural and political life of humans and animals. For Hobbes (1651/2010), humans were like wolves, who in a 'natural state' found themselves at 'war' with other humans because they wanted to defend their individual freedom. Early Darwinists extended these ideas to the whole of nature and, in the words of Alfred Lord Tennyson, they saw nature as 'red in tooth and claw.' A struggle for existence results from a scarcity of resources, leading to a natural selection of the fit, at the expense of the maladaptive (Huxley, 1888; Bouglé, 1909).

Etymological analyses of symbiosis jargon demonstrate that, prior to the introduction into biology, many of the foundational concepts were also used to define sociocultural human relations and sociopolitical ideologies (**Table 1**). Early symbiologists, however, applied less-liberal or non-liberal, often socialist and communitarian language to characterize the living arrangements present in the natural world – ideas that were inspired by Rousseau's social-contract theory. Rousseau (1762/2001) understood humans as 'good natured' and inherently social beings that became corrupted by artificial societies. The human family, one of the basic units of law, is typified by its sharing of resources, and societal living is ideally characterized by reciprocal exchange and a fair (re)distribution of natural resources. Reciprocal and altruistic social rule-following enables the formation of a 'social-contract' that in turn allows for the establishment of a 'higher, common good' that founds the resurrection of the welfare state. As members of the welfare state, persons become citizens that create a community where, for the sake of the whole, individuals give up part of their freedom to live together. These social ideas were further developed by Pierre-Joseph Proudhon (1840, 1849) under the form of 'mutualism' (**Table 1**), and by Pyotr Kropotkin's (1902) work on 'Mutual Aid.'

The Sociopolitical Life of Animals: Commensalism, Mutualism and Parasitism in the Economy of Nature

Research on symbiosis in the animal world followed as an extension of these sociopolitical theories. Linnaeus introduced the concept of an 'economy of nature' in the eighteenth century (Egerton, 2015), and Haeckel (1866) developed these ideas further when founding the field of ecology. The 'division of labor' concept was introduced by the French naturalist Milne-Edwards (1827), in the context of orthogenetic developmental laws, in order to describe the origin of complex anatomical forms in a hierarchical (systems theoretical) perspective (D'Hombres, 2012).

As a forerunner of ecological thought, Pieter Harting, working at Utrecht University, wrote a Dutch work in 1862, 'On the industry (economy) of animals: for all those who love nature,' wherein he discussed the numerous crafts found in the animal kingdom, ranging from 'carpenters' to 'architectural builders' and 'cleaners.' By applying sociopolitical terminology, Harting (1862) conceptualized the 'economy of animals' in terms of 'distributions' of 'common and public goods,' as well as 'divisions of labor' that underlie a hierarchically structured animal society.

Harting's work inspired the Belgian Zoologist and Paleontologist Pierre Joseph van Beneden, a student of Cuvier, which in turn specify the nature of the *social* lives of animals, in particular how they establish communal living and how they share their food resources. In this context, he would introduce the terms *commensalism* (*commensaux*), *mutualism and parasitism* (**Table 1**).

For van Beneden,

> When taking a closer look at the animal world, it does not take long to find more than one analogy with human society. If I'm allowed to say so, there is not one social position found in human society that is not also found in the animal kingdom. Most of the animals live peacefully from the fruits of their labors, and exercise a profession that gives them life. But, at the sideline of these honest industrials, we also find miserable ones, who cannot do without the assistance of their neighbors, some of whom establish themselves as parasites in their organs, others as commensals (*commensaux*) that take profit from the gains (labors) of the honest. (Van Beneden, 1875: 2–3, my translation)

Commensals or *messmates* are merely companions at the table, they are allowed to dine with the host and feed on their neighbor's catch. They can live inside or onside their host (what we today call *endo- or ectosymbiosis*), and they can either live independently or forever remain fixed (today called *facultative and obligate symbiosis*). *Parasites* are those animals who live at the expense of their neighbor, they take advantage of their host and can endanger its life. *Mutualists* are animals that live onto one another without being either *parasites* or *messmates*. They receive 'asylum,' and either return 'mutual services' or develop 'sympathetic bonds' which attracts them to one another (van Beneden, 1873, 1875). van Beneden described such associations to exist throughout the animal kingdom, and he dedicated a chapter on the nature of *parasites* as causative agents of disease (**Figure 1**).

The different social living arrangements of animals, for van Beneden, demonstrated the existence of a Great Chain of Being, which in turn evidenced divine providence. He did not use evolutionary vocabulary, but he did assume that especially the 'fixed commensals' undergo 'metamorphosis.'

With the dawn of evolutionary thought, two distinct paradigms, one of competition and one of socialism would emerge to characterize societal living of human and other animals. Both paradigms were well-recognized in both the biological and sociocultural sciences as valid means by which to describe the natural world. In a review article written by Bouglé (1909), for example, in a volume commemorating the 50th anniversary of Darwin's *Origin*, competition and socialism were presented as distinct and complementary 'laws' by which biological and sociological phenomena evolve.

Herbert Spencer (1876), famous for interpreting natural selection theory as leading to the 'survival of the fittest,' would write on the social living arrangements amongst distinct animals from within the competitive paradigm. Spencer introduced the concept of the 'superorganic,' and investigated how different 'life forms' (biological species but also sociopolitical, cultural, and linguistic systems), brought forth a division of labor and a hierarchical organization of the natural world that enables a unilinear and evolutionary progressive way of living or being in the natural world. For him, the superorganic structure comprises a higher, societal whole, of which he sought out the social laws.

Table 1 Etymology of symbiosis jargon

Term	*Etymology*	*First usage in biology*
Consortia	Plural for *consortium,* from Latin *consors* (partner, wife, companion) and *consortio* (having the same destiny), first introduced in French as *consorte* in the fourteenth century to designate a husband's wife, and later in England, where, from the fifteenth century onward, it first became a legal term for 'the right of a husband to access his wife,' and later a term to designate larger associations and societies that are bounded by duties and rights (e.g., in the form of divisions of labor)	Introduced in botany to characterize symbiosis by Reinke (1873) and Famintsyn (1907)
Commensalism	From the French word *commencaux*, derived from fifteenth century, Middle Latin *commensalis* (coming around a table), and *cum mensa* (eating at the same table)	Introduced in zoology by Van Beneden (1873, 1875) in that meaning, as *commensaux*. In a first English translation of his major 1975 work in 1976, the *commensaux* were translated as *messmates*, which either derives from Middle English *mes* (for table, dinner, food, and eating together at the same table), or from the old French word *mesme* or *même* (which means even, same or equal)
Mutualism	Introduced in French in the late fifteen century as *mutuel*, derived from the Latin *mutuus* (reciprocal exchange). Originally, the word was used to designate feelings of both love and hate between individuals. The term roots the French words *mutuel* and *mutuellisme*, first introduced by Pierre-Joseph Proudhon (1840, 1849), to designate societal socialism in human and animal societies; and a year later in Brittan as *communitarianism* by John Goodwyn Barmby, to designate societal, communal lifestyles (*consortia*) with social care (e.g., social justice such as health care)	First introduced in biology by van Beneden (1875) in that meaning, and today characterized as one type of symbiosis
Parasitism	From the Greek word *parásit*os (a person or organism who lives at the expense of another, who receives free nourishment and protection). Introduced in natural and medical sciences from the Greeks onward. First reintroduced in Medieval French as *parasite* in the sixteenth century	In biological and biomedical sciences fundamental to describe pathogens, though the first introduction in this sense remains obscure. de Bary (1878) in botany, and van Beneden (1875) in zoology, already identified several microorganisms as causal agents of disease, ideas that would become reintroduced as foundational for the germ theory of disease in the late nineteenth century
Symbiosis	From Greek *sumbio*sis (companionship) and *sumbioun* (to live together). First reintroduced in German and English languages in the early seventeenth century, to designate 'communal or social life,' including the union or living together of distinct individuals as companions, also in marriage (community) as husband and wife. From the seventeenth century onward used to describe societal, community life	First introduced in botany, by Frank (1877) as *symbiotismus* in 1877, and as *symbiosis* by de Bary (1878, 1879)
Symbiogenesis	*Symbio-* stems from the Greek *symbiosis* and *-genesis* from *genesis* (origin, birth, production, generation, creation). Symbiogenesis means generation or evolution by symbiosis	First introduced in biology by Merezhkowsky (1905) and later also by Kozo-Polyansky (1924/2010), by Wallin (1927) as *symbionticism*, and by Sagan (1967)
Hereditary symbiosis	A symbiotic association that becomes permanently transmitted to future generations, foundational for symbiogenesis	First introduced by von Faber (1912) in Germany as 'erbliche Zusammenleben' (hereditary living together) of bacteria inside tropical plants. Translated into English as 'hereditary symbiosis' by Cowles (1915), and later adopted by Buchner (1921, 1939), Wallin (1927), Lederberg (1952), and Sagan (1967)
Symbiont/Symbiote	Derived from symbiosis to designate an organism that entertains symbiotic associations, first known use in 1887 in Germanic languages, and from 1909 in French as *symbiote*	The term symbiote was first introduced by Paul Portier (1918) in France
Synergy	From the ancient Greek *Synergia* (working together, cooperation, joint work, assistance or help). First introduced in the middle of the seventeenth century. By the mid-nineteenth century, used to designate group cooperation and communal group living that advances effects unobtainable by the individuals, which is why the whole becomes more than the sum of its individual parts	First introduced in biology in bio-economic, systems theoretical and hierarchical approaches to life (see e.g., Corning, 2013, 2014)
	References: http://www.oed.com/; https://www.wiktionary.org/; http://www.etymonline.com/	(Carrapiço, 2015; Gontier, 2015; Sapp, 1994)

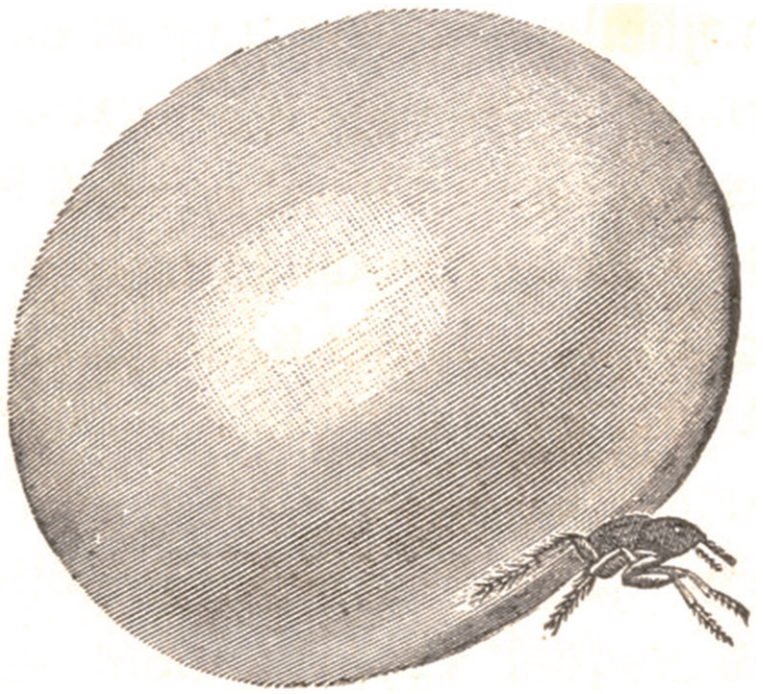

Figure 1 The swelling of a female chigoe flea (*Tunga penetrans*), a parasitic flea native to Latin America that partially penetrates the skin of mammals to breed, leaving an infectious blister. Used by Van Beneden (1875: 430) as an example of *temporary parasitism*.

Social Darwinians thus focused on competition, and many symbiologists understood these theories as direct extensions of liberal thought into biology. Symbiosis scholars critiqued by emphasizing that the sociopolitical and biological realms display many instances of social and mutualistic behavior, which they equally understood as 'lawful.'

Pyotr Kropotkin (1902) pioneered by understanding 'mutual aid' as a 'law of nature' that complements the principles or 'laws' of natural selection (Kropotkin, 1902). The first two chapters of his magnum opus focused exclusively on the 'mutual aid' found in animals. In the remaining chapters, from invertebrates to humans, he lists hunting and breeding associations as examples of socialism that contradict the sociopolitical ideas put forth by Hobbes, Malthus and Huxley. Battling, what Kropotkin (1902) called 'Huxley's (1888) gladiator show,' where organismal beings are characterized as 'naturally' amoral and asocial beings, Kropotkin instead makes the case that mutual aid and the division of labor is as 'instinctive,' and 'natural' as 'struggle for existence' is. For Kropotkin, the law of mutual aid helps eliminate competition and aids in the struggle for existence, enabling the establishment of social and political laws that bond organisms in communal lifestyles characterized by reciprocal altruism and cooperation (for a discussion see Dugatkin, 2011 or Sapp, 1994).

At the beginning of the twentieth century, parallels between the sociocultural and natural world appear to have come into disuse. An exception is Hermann Reinheimer (1913, 1915, 1920), who would continue to draw explicit parallels between the biological and social world. He developed a 'bio-economic' view of evolution and also identified symbiosis as a 'law of nature' (for a modern-day account on bio-economics and *synergy*, see Corning, 2013, 2014).

Today, and from within an extended synthesis, the similarities between reticulate biological and sociocultural evolution are being rediscovered (Gontier, 2007, 2012; Hird, 2010a,b; Kressing and Krischel, 2013).

Botanical Lichenology Studies and the Introduction of the Symbiosis Concept

Symbiosis research entered botanical studies through the study of lichens. Lichens are chimeric organisms composed of distinct symbionts (**Figure 2**). They grow on trees or rocks, and closer analyses of their foundational structures demonstrate thread-like, interwoven networks that resemble hair braids ('*Flechten*').

Lichens were first studied in pre-evolutionary times when German botanists including Georg Franz Hoffmann (1760–1826) and Georg Friedrich Wilhelm Meyer (1782–1856) attempted to provide morphological and taxonomic classifications of lichens. Hoffmann (1787, 1790–1801) classified lichens as plants and especially Meyer (1825) assumed that lichens arose 'spontaneously' while he interpreted the different morphological life-stages in terms of 'metamorphosis.' Both scholars' research perpetuated some of the basic terminology still used today to describe lichen morphology.

After the introduction of evolutionary thought, the works of Simon Schwendener (1828–1919), Christian Ernst Stahl (1848–1919), Johannes Reinke (1849–1931), Albert Bernhard Frank (1839–1900), and Heinrich Anton de Bary (1831–1888) and his students, would clarify the exact morphological nature of lichens (Kärnefelt *et al.*, 2012: 10).

Schwendener (1867, 1868a, 1868b) was the first to propose that lichens are 'new plants' with 'new characteristics' that

Figure 2 Lichens are chimeric organisms consisting of fungi that live in intimate symbiotic association with algae and/or cyanobacteria. These are some of Schwendener's (1868a) early drawings of lichens that demonstrate the dual nature of the organism.

originate from a reciprocal and intricate relation between two different organisms. For Schwendener (1868a: 3, my translation), lichens:

> enable insight into previously overlooked or completely misunderstood living relationships between two large plant groups, namely "algae" and "fungi" ("Pilze"). The algae are willing to serve as nutrition for the fungus that controls the algae. Despite these counterpropositions, however, the organisms are so intrinsically and reciprocally connected that through their penetration and merging, they constitute new plants with a clear individual character. For that reason, many authors classify them into an independent group as lichens ("Flechten").

The French scholar Jean-Baptiste Édouard Bornet (1828–1911), who first described the phylogeny of red algae, confirmed the dual nature of lichens experimentally. Bornet (1873, 1874) was able to separate the individual organisms, and noticed that when several of the fungal spores of the species he studies are disabled to establish associations with algae, they either die or are unable to reproduce because they germinate on the algae. He also noticed that such associations are formed rapidly during ontogeny (Fink, 1913), and thus outside the germ line.

Nonetheless, Schwendener's hypothesis and Bornet's experiments were fiercely criticized by their contemporaries (e.g., Kröber, 1874; and see Sapp, 1994), even though Famintsyn and Boranetski were also able to separate the individual organisms in 1876 (Khakhina, 1992), and experimental work by Stahl (1877) resulted in the laboratory formation of lichens from associating fungal spores with algae.

In the same year that Bornet conducted his experiments, Reinke (1873) had referred to the chimeric lichens in vitalist and sociopolitical terms, as a '*consortium*' ((**Table 1**), a concept that in turn relates to the *synergism* concept). This can be interpreted as a precursor of systems theoretical hierarchy theory and ecological thought in general as it would be developed by scholars such as Jacob von Uexküll and Ludwig von Bertalanffy in the beginning of the twentieth century. Reinke (1895, 1908), a scholar trained in both theology and philosophy, opposed both Darwin's selection theory as well as Haeckel's monism, and favored morphogenetic explanations for lichen development.

Two years after van Beneden had introduced his work on the social lives of animals, Frank (1877) characterized the association between the organisms that make up lichens as 'symbiotismus,' and defined it in terms of 'coexistence' (Sapp, 1994: 6). In the subsequent two years, de Bary (1878, 1879) reintroduced the ancient Greek concept of *symbiosis* (**Table 1**) to characterize the dual nature of lichens, and defined symbiosis as "the living together of unlike-named organisms." By basing himself upon the experimental work of his former students, Famintsyn and Stahl, de Bary developed the first theoretical framework on the appearance of symbiosis in the plant kingdom; a framework wherein he made direct reference to van Beneden's work. For de Bary, botanical symbiosis demonstrated the closest affinity to van Beneden's zoological mutualism concept (Seckback, 2002; **Figure 3**).

van Beneden and de Bary set the scene for all later theorizing on symbiosis because their work became available to a wide scholarly audience. The former's work was translated into English, and the latter's was summarized in the writings of his students and collaborators whom included Andrey S. Famintsyn (1835–1918), Sergei Winogradski (1856–1953), Martinus Beijerinck (1851–1931), and Ernst Stahl and Andreas Schimper (1856–1901). Scholars would opt for de Bary's symbiosis concept, and van Beneden's distinctions repeatedly became understood as types of symbiosis that specified the nature of the symbiotic relation.

Symbiosis in *All* Animals, Plants, and Protists and its Significance for Evolution

Microscopic advances steadily enabled better visualizations of pro- and eukaryotic cellular morphology, and botanists

Figure 3 Some of the pioneers in Symbiosis research, from left to right and top to bottom: Pierre-Joseph van Beneden (1809–1894), Simon Schwendener (1829–1919), Heinrich Anton de Bary (1831–1888), Andrey Sergeevich Famintsyn (1835–1918), Albert Bernhard Frank (1839–1900), Pyotr Kropotkin (1842–1921), Andreas Franz Wilhelm Schimper (1856–1901), and Paul Buchner (1886–1978).

identified the numerous cellular organelles found in plant cells. Between the 1860s and 1880s, Julius von Sachs (1859), Gottlieb Haberlandt (1876), and Schimper (1883) documented the role plastids (*'chlorophyllkörner'* or chlorophyll grains and *'Farbkörper'* or pigment corpuscles) play in the formation of starch and the coloring of the plant's leaves. Schimper (1885) furthermore noted that chlorophyll bodies (*'chloroplastiden'* or chloroplasts) divide in ways similar to bacterial division and suggested a symbiotic origin for the latter. As such, he first drew attention to symbiosis as an *intracellular* phenomenon, a theme that was later repeated and expanded by Paul Buchner (1921), who dedicated a full book on intracellular symbiosis (Sapp, 2002).

Andrey Famintsyn (1889a,b, 1892, 1907), the father of Russian plant physiology and a student of de Bary, studied both lichens and chloroplasts from a symbiotic point of view. Famintsyn is considered one of the pioneers of symbiogenesis theory, because he emphasized the adaptive role symbiosis plays in evolution by enabling the synthesis of new *consortia* (**Table 1**).

Symbiosis research also progressed from within the biomedical and bacteriological sciences where bacteria became understood as parasitic agents of disease. Ferdinand Cohn published a first systematic classification of bacteria in 1872; in 1876, Robert Koch associated the *anthrax* bacterium with the Anthrax disease ('Milzbrand-Krankheit') in cows; Pasteur's work on the germ theory of disease was read before the French Academy of Science in 1878; and Charles Louise Alphonse Laveran, the discoverer of the malaria parasite, was one of the first to, in 1880, recognize parasitic protozoa as causative agents of disease (Gontier, 2015b).

The positive effects of symbiosis also remained a topic of interest, especially in what regards its impact on ecology and the biosphere. Frank (1885) first described 'root symbiosis' (*'Wurzelsymbiose'*) that occurs between fungi and the roots of trees and plants, and he introduced the concept of *Mycorrhiza* (Carrapiço, 2015). At de Bary's lab, Winogradsky (1893, 1895) discovered nitrogen-fixing bacteria in the soil, which he called 'autotrophic' for their ability to synthesize chemical elements instead of devouring organic matter.

Beijerinck, another student of de Bary, also found *Rhizobia*, nitrogen-fixing, symbiotic bacteria present in the roots of legumes, and he was the first to point out their importance for agriculture (because rhizobia-rich roots and soil makes for fertile soil). Beijerinck is considered one of the founders of virology, and in nineteenth century academic circles, also de Bary (1861) was mostly known for his studies on plant diseases and for reporting on the life cycle of the fungus *Phytophthora infestans* that is parasitic on potatoes thereby causing potato blight (Gontier, 2015b).

Symbiosis found its way to America with the works of Roscoe Pound (1893) and Albert Schneider (1897) who integrated animal with plant studies in more general, and especially ecologically-oriented works on symbiosis (Sapp, 1994). Schneider (1897) first generalized de Bary's notion of symbiosis to *all* life forms, and he averred that symbiotic associations can occur between *more* than two individuals. He opens his work, 'On the phenomena of symbiosis' by saying that

> *All* living organisms manifest a more or less intimate biological interdependence and relationship. In fact, their very existence depends upon this condition, therefore no organism, no matter how simple or how complex its structure may be, is the result of a wholly independent phylogenetic development. (Schneider, 1897: 923, my emphasis)

Like Famintsyn, Schneider speculated about the evolutionary significance of symbiosis, when it first arose in

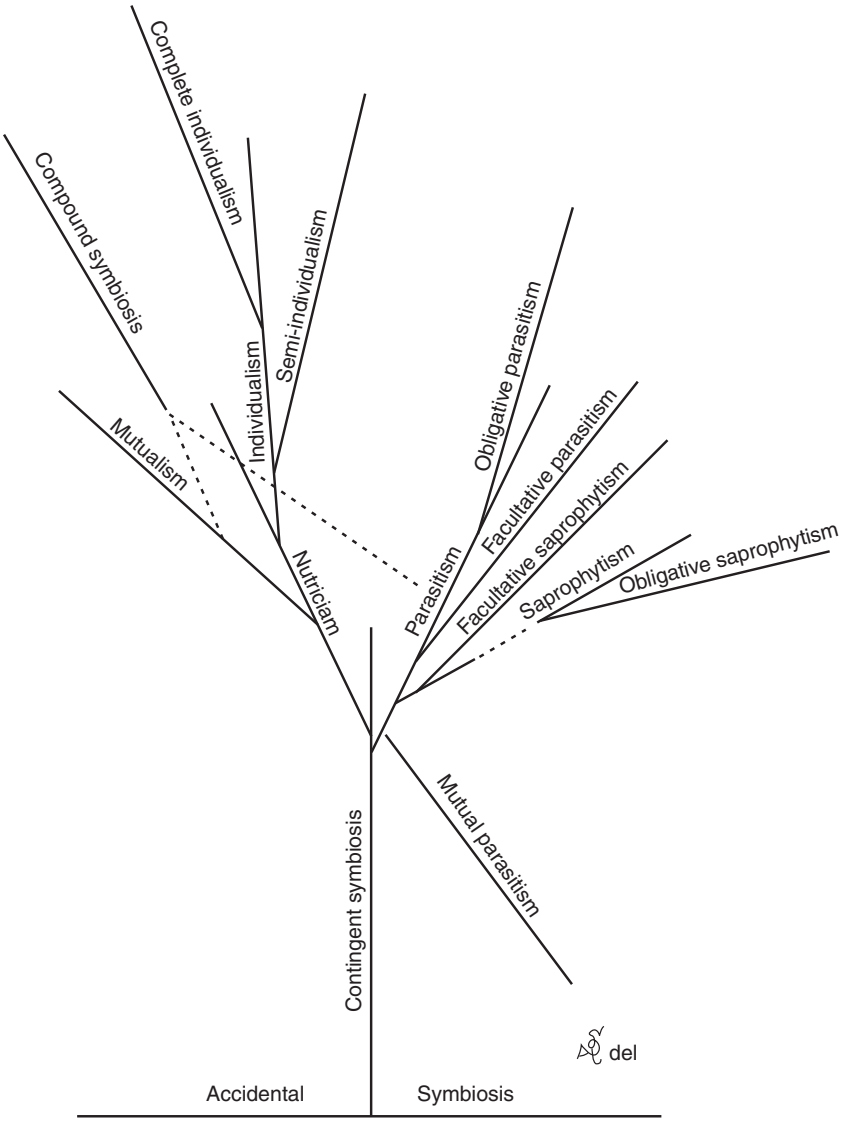

Figure 4 Schneider's phylogeny of the various types of symbiosis that evolved as an adaptive response to nutritional problems induced by the scarcity of resources and the struggle for existence.

time, and how it relates to natural selection. By associating symbiosis with a 'loss or acquisition of assimilated food-substances,' he understood symbiosis in nutritional terms, as an adaptive response to the struggle for existence that exists because of the scarcity of resources. In other words, for Schneider, it was hunger and a drive to reproduce ('food requiring and reproductive life-action of the organism'), that by necessity engaged organisms in forming symbiotic associations.

The emergence of symbiosis, for Schneider, was a process that required long periods of evolutionary time. He assumed that individual organisms evolved first, and because of their independent evolution, they were originally not equipped to form such relations, so they had to evolve them over the course of evolution. Schneider tried to synthesize natural selection theory with symbiology, in a 'coevolutionary,' 'ecological sense' *avant là lettre*. He described how symbiotic partners developed morphological adaptations to facilitate the symbiotic relationship, that went from parasitic and facultative to obligate and mutual.

Mimicry, for example, was a type of 'mutual adaptation,' a mutually evolved symbiosis. Other examples were the relation between the male and female reproductive cells, as well as the relation between mothers and their developing embryos. Schneider also brought symbiosis to entomology, by discussing numerous cases of symbiosis in insects and between insects and plants.

In his small but rich paper, he furthermore provided a layout for new terminology, by expanding the various types of symbiosis, and by pouring them into an evolutionary taxonomy (Figure 4). Schneider appears to have been engaged in reconstructing the evolutionary genealogy of the various types of symbiosis that evolved over the course of evolution.

Reduction of Symbiosis Studies to Ecology and Developmental Biology until the Advent of Symbiogenesis Theories

In 1885, Auguste Weismann developed his *Keimplasm* theory of descent that stated that hereditary traits are only transmitted through the germ line. It put a halt to neo-Lamarckian and evolutionary developmental studies, that because of their focus on ontogenetically acquired traits, became less and less understood as relevant for the study of evolutionary phylogenesis. Weismann's ideas were later synthesized with Theodor Boveri's and Walter Sutton's chromosome theory that identifies the chromosomes as bearers of hereditary traits. The rediscovery of Mendelian hereditary laws, and advances in theoretical and experimental population genetics as well as molecular genetics caused symbiosis to be studied from within fields such as ecology and developmental biology rather than evolutionary biology.

Symbiosis became understood as an ontogenetic or developmental, adaptive behavioral response to nutritional problems brought forth by the scarcity of resources and the struggle for existence. Nonetheless, in the margins of standard, Neo-Darwinian evolutionary theory, the evolutionary significance of symbiosis would remain studied by scholars who investigated cytoplasmic inheritance (for a discussion see Sapp, 2003; Gontier, 2015a) as well as ecological interactions (for a discussion see Egerton, 2015).

Some of the most important symbiologists at the beginning of the twentieth century were Frederick Keeble (1910), Paul Buchner (1921), Maurice Caullery (1922), George Nuttall who founded the *Journal of Hygiene and Parasitology* in 1901 and 1908, Lemuel R. Cleveland (1923).

Eventually, the recognition that 'hereditary symbiosis' can lead to symbiogenesis, and idea introduced by von Faber (1912) and brought to an English readership by Cowles (1915) and Buchner (1921), would reintroduce symbiosis studies into evolutionary biology.

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See also: Coevolution, Bacterial-Phage. Commensalism, Amensalism, and Synnecrosis. Cooperation and Public Goods, Bacterial. Lichen-Forming Fungi, Diversification of. Mutualism, the Evolutionary Ecology of. Plant–Pollinator Interactions and Flower Diversification. Predation and Parasitism. Sperm Competition. Symbiogenesis, History of. Symbiosis, Introduction to

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DNA Interactive, Cold Spring Harbor Laboratory (features animations and historical timelines on the discoveries of the biochemical nature of DNA).

http://iss-symbiosis.org/
International Symbiosis Society (International Society that groups symbiologists).

http://www.nature.com/nrmicro/index.html
Nature Reviews Microbiology (Journal that regularly features review articles on symbiosis and related topics such as the microbiome and viriome).

http://www.asm.org/index.php/choma3/71-membership/archives/7852-significant-events-in-microbiology-since-1861
Significant Events in Microbiology 1861–1999, American Society for Microbiology (features major events and timelines in symbiosis, lateral gene transfer, genetics, and biomedical sciences).

http://www.springer.com/life+sciences/evolutionary+%26+developmental+biology/journal/13199
Symbiosis Journal (Official Journal of the International Symbiosis Society).

Symbiosis, Introduction to

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Glossary

Aposymbiotic State in which a potential host lacks resident microbes.
Bacteriocyte Specialized host cells (sometimes called mycetocytes) for housing obligate bacterial symbionts.
Codiversification Corresponding lineage splitting between interacting partners, which signifies highly faithful interactions across evolutionary timescales and can be evidenced by matching host and symbiont phylogenies.
Facultative endosymbionts Maternally transmitted bacteria common in arthropods that are not required for host survival and reproduction. These have the potential for cross-species horizontal transfer, during which they move heritable traits to new hosts.
Metagenomics The study of the total genetic content associated with a particular environment, including the microbial communities associated with eukaryotic hosts.
Microbiome The assemblage of microbes associated with a particular host organism; the term has also been used to specifically refer to the collection of genes encoded by resident microbes.
Obligate symbiont A symbiont that is required by the host for survival and reproduction.

The term 'symbiosis' is derived from the Greek *σύν* 'together' and *βίωσις* 'living' and is generally defined as the persistent association of two or more dissimilar species. A large number of symbioses involve a single multicellular eukaryotic organism coupled with one or more microorganisms, including bacteria, viruses, eukaryotic microorganisms, or Archaea. By convention, the larger partner is usually called the 'host' and the smaller ones 'symbionts.'

'Symbiosis' has been notoriously difficult to define and hence there is often disagreement over which particular associations count as symbioses. In general, symbiotic interactions typically persist for most of the partners' lifespans, and many carry over to subsequent generations, exceeding tens of millions of years in age in the most ancient examples. This definition excludes transient interactions, such as most insect–plant pollination relationships, which are mutualisms but not symbioses. Further, many authors restrict the term symbiosis to refer only to persistent mutualisms, such as the interaction between diverse insect species and their obligate bacteriocyte-associated symbionts, which provide amino acids and other nutrients to their hosts. While this definition comports with the prevailing view of symbiosis outside of biology, it excludes many interactions that biologists typically consider symbioses, such as reproductive manipulators in arthropods, including the widespread bacterium *Wolbachia*. These maternally transferred bacteria can only survive and replicate in association with animal tissues, and they often provide no clear benefits to their hosts. Instead, they utilize a range of strategies that cause infected females to produce more female offspring than symbiont-free females, thereby favoring symbiont proliferation (Werren *et al.*, 2008; **Box 1**).

An additional complication with the 'narrow' definition of symbiosis is that particular symbionts, including rhizobia and mycorrhizal fungi (MF), confer net benefits to hosts under some conditions, but costs under others. Thus, a particular interaction could fluctuate temporally in 'symbiont' status. For this reason, symbiosis is more often defined (as above) to include interactions that span the continuum from mutualism to parasitism. While the broader definition resolves the aforementioned issues, new problems arise in its inclusion of interactions that are not generally considered symbioses by biologists, including persistent infections with specialized pathogens and parasites (e.g., malaria in humans). In accord with current practice, we take the broader definition, but exclude host–parasite/pathogen interactions.

Symbioses are Diverse and Ubiquitous in Nature

The first descriptions of symbioses in the nineteenth century provoked curiosity, but also a general perception that these interactions comprised interesting exceptions to the norm (Sapp, 2004). Today we recognize that virtually all multicellular eukaryotes are composites made up of multiple species and that microorganisms are often key players in the physiology, development, ecology, and evolution of eukaryotes (Douglas, 2014). Taking an even broader view, we also recognize that the success of eukaryotic life is itself largely due to the ancient acquisition of the symbiotic α-proteobacteria that evolved into today's mitochondria. This major symbiotic event brought to eukaryotes the capacity to execute aerobic respiration, enabling eukaryotic existence and expansion in an oxygen-rich world. Plants, algae, and a number of other unicellular eukaryotes similarly owe their success to ancient acquisitions of cyanobacteria, or more recent secondary or tertiary acquisitions of photosynthetic eukaryotes, enabling the conversion of sunlight, water, and carbon dioxide into sugars. While some groups appear to engage in symbiotic interactions more readily than others (e.g., Bacteria more than Archaea), symbioses are widespread in nature and occur within and among diverse groups. Here we focus on symbioses between plants or animals and their many microbial associates, using these to discuss patterns and processes common to nearly all life forms.

Box 1 Reproductive manipulation as a means of symbiont spread

While partner fidelity typically promotes the origin of beneficial symbionts, high fidelity of maternal transmission of symbionts in arthropods has opened the door to alternative symbiont transmission strategies collectively dubbed reproductive manipulation. Several bacterial lineages have evolved to employ one or more of these strategies. Most impressive among these are *Wolbachia* symbionts (α-Proteobacteria), which can execute all four types of manipulation (see below) (Werren *et al.*, 2008). *Wolbachia* are estimated to occur in 40% of terrestrial arthropods, thus possibly infecting millions of animal species (Zug and Hammerstein, 2012). While some *Wolbachia* strains benefit hosts, others manipulate reproduction, and both strategies are keys to success for one of the world's most prolific symbiont genus. Other common reproductive manipulators include, *Spiroplasma* (Mollicutes), *Rickettsia* (α-Proteobacteria), *Cardinium* (Bacteroidites), and *Arsenophonus* (γ-Proteobacteria). Bacterial invasion and maintenance in all four cases is achieved by increasing the number or fitness of infected females.

Male killing occurs when symbiont-infected males die during larval or embryonic development. Males are 'dead-ends' for maternally transmitted symbionts. If male deaths increase the fitness of infected females by increasing resource availability, then male killing symbionts may spread in the population.

Feminization occurs when maternally inherited symbionts block the production or action of masculinizing hormones. By causing genetic males to become female, symbionts create a transmitting host as opposed to a dead-end male.

Parthenogenesis induction takes place when a symbiont causes its host to reproduce asexually, such that females are produced from unfertilized eggs (thelytoky). This phenotype occurs primarily in hymenopteran insects, which have haplodiploid sex determination systems – the chromosomal complement of unfertilized, male-destined haploid eggs is doubled by activity of the symbiont.

Cytoplasmic incompatibility occurs when matings between infected males and uninfected females fail to produce viable offspring. As all other crosses are viable, infected females are favored over uninfected females as they can mate with any male. *Wolbachia* modify sperm to cause defects in early zygote development when a 'rescue' modification is not present in infected female mates carrying the same strain.

Plants and animals evolved, and still live today, in a microbial world. The result has been an astonishing diversity of eukaryote–microbe interactions. Microbial symbionts have evolved from many different lineages, playing key roles in eukaryote biology, as described in **Table 1**. In general, ectosymbionts colonize surfaces exposed to the environment, such as the root epidermis in plants, mammalian skin, insect exoskeletons, or animal guts. External surfaces are most often colonized by extracellular microbes that are acquired from the environment each generation. But social transmission also occurs, and the duration and specificity of the association varies greatly depending on the interaction. Ectosymbionts often have roles in defense, especially colonization resistance, and digestion. The other broad category of microbial associates, endosymbionts, tends to be much more specialized and typically restricted to internal tissues or cells. Many are vertically transmitted through seeds or eggs with high fidelity, and can persist within host lineages for timespans of thousands or even millions of years. Such parent-to-offspring transfer tightly links the fitness of interacting partners, allowing beneficial microbes to preferentially invade and persist in host populations. Benefits conferred by heritable symbionts include the provisioning of nutrients or secondary metabolites used in defense. An alternative strategy for the spread of heritable microbes, commonly employed by symbionts of arthropods, involves manipulation of host reproduction or development in ways that favor symbiont transmission (**Box 1**). Heritable endosymbionts occur in many plants and invertebrate animals, but not vertebrates, possibly due to differences in their immune systems.

Many symbioses involve the mutual exchange of benefits between host and symbiont. Microbes as a whole have a greater biosynthetic potential relative to plants and animals, and the microbial partner often provides a function or service that is lacking in the eukaryotic host. Through acquiring an entire organism, the eukaryotic host can expand its metabolic potential by obtaining not only complete functional pathways but also the accompanying regulatory machinery. In other cases, infection with microbial symbionts may modulate host regulatory pathways important for development and immunity, or those involved in resource acquisition (e.g., herbivores' symbionts may regulate plant defense pathways to better exploit plants as a food source). In general, resource acquisition and protection are the two most common services provided by microbial residents of plants and animals. These have had substantial impacts on hosts and, in some cases, on surrounding communities and ecosystems.

Symbiosis as a Source of Evolutionary Innovation

Mutation has long been seen as providing the raw material for evolution, yet studies in prokaryotes and viruses have revealed the importance of lateral gene transfer in microbial innovation (Ochman *et al.*, 2000). While the lateral acquisition of genes also affects the ecology and evolution of eukaryotes (Schoenknecht *et al.*, 2014), the acquisition of novel capabilities through symbiosis has played a larger innovative role in many eukaryotic lineages, including plants and animals (Moran, 2007). Like lateral gene transfer, the acquisition of a novel symbiont contrasts with the Fisherian model of gradual evolution through the accumulation of small changes. And while mutualistic symbionts may take some time to evolve fine-tuned relationships with novel hosts, symbiosis can be viewed as a type of macromutation that can rapidly alter host characteristics.

Symbioses Involved in Resource Acquisition

A diverse assemblage of organisms can photosynthetically fix atmospheric carbon into storable fuel supplies (Venn *et al.*, 2008). Interestingly, this incredibly important process, which underlies the bulk of primary production in both terrestrial habits (via plants) and oceans (via 'algae'), evolved only once in the ancestor to extant cyanobacteria, but has been shared

Table 1 Major animal and plant symbioses

Association	*Common eukaryotic hosts*	*Symbiont diversity*	*Major roles*	*References*
Plants – mycorrhizal fungi (MF)	a. Widespread: found in all major plant groups b. Angiosperms/ gymnosperms	a. Arbuscular MF (Glomeromycota) b. Ectomycorrhizal F (mostly Basidiomycota and Ascomycota)	Plants get phosphorus and other nutrients and pathogen protection; fungi get organic carbon; likely facilitated colonization of land by plants	Smith and Read (2008)
Plants – nitrogen-fixing bacteria	a. Legumes (Fabaceae) b. Various dicotyledon angiosperms c. Cycads and *Gunnera* (angiosperms)	a. Rhizobia (α and β-proteobacteria) b. *Frankia* (actinomycete) c. *Cyanobacteria*	Plants get usable nitrogen, bacteria carbon. Can expand host range to nitrogen-poor soils	van der Heijden *et al.* (2008)
Animals – photosynthetic bacteria or algae	a. Corals (Cnidaria) b. Sponges (Porifera) c. Mollusca d. Tunicates (Ascidia)	*Symbiodinium* (dinoflagellate) (a, b, and c) *Chlorella* (chlorophyta) (a, b, and c) *Cyanobacteria* (a and d)	Diverse animals derive photosynthetically fixed carbon from algae or bacteria. Corals symbioses support communities of marine life	Venn *et al.* (2008)
Animals – chemosynthetic bacteria	a. Annelid worms b. Bivalve mollusks c. Decapod crustaceans (arthropods)	Sulfur-oxidizing symbionts usually γ-proteobacteria (evolved many times), but some ε-proteobacteria Methane oxidizing γ-proteobacteria	Conversion of chemicals rather than sunlight into carbon-based energy. Allows for primary production and formation of ecosystems in deep sea where sunlight does not reach	Dubilier *et al.* (2008)
Nutrient-provisioning, heritable symbionts in insects	a. Sap-feeding Hemiptera b. Blood-feeding hemipterans, lice and flies c. Some ants, roaches, and beetles	Mostly Proteobacteria, but evolved many times independently; rarely fungi	Provides amino acids and cofactors for sap-feeders, B vitamins for blood feeders; symbionts get stable, nutritional environment. Allowed for niche specialization and subsequent diversification	Baumann (2005); Moran *et al.* (2008)
Gut symbionts in animals	Most bilaterian animals harbor gut microbiota	Mostly bacteria (diverse taxa), but also fungi and other eukaryotic microbes	Break down cellulose and other plant polymers, nutrient-provisioning, nitrogen recycling and fixation, detoxify plant defenses and pesticides, protect against ingested pathogens; can also affect development and immune function	Engel and Moran (2013); Ley *et al.* (2008b)
Facultative, heritable symbionts	Widespread in insects and other arthropods although infections are often sporadic within and among host lineages	Mostly bacteria	Protection against natural enemies, mediate plant–animal interactions, and reproductive manipulations; symbionts get stable, nutritional environment	Moran *et al.* (2008)
Plants – fungal endophytes	a. Grasses b. Most plant species	a. Clavicipitaceous fungal endophytes b. Diverse non-clavicipitaceous endophytes	Fungal produced alkaloids protect plants against herbivores and confer drought tolerance; fungi get carbon Pathogen protection, growth enhancement	Rodriguez *et al.* (2009)

(*Continued*)

Table 1 Continued

Association	*Common eukaryotic hosts*	*Symbiont diversity*	*Major roles*	*References*
Wasp – polydnarvirus symbioses	Ichneumonid and braconid parasitic wasps (Insecta: Hymenoptera)	Ichnoviruses and Bracoviruses	Protect wasps from insect host's immune system	Strand and Burke (2013)
Nematode – bacteria symbioses	Entomopathogenic nematodes (a) *Steinernema* and (b) *Heterorhabditis*	a. *Xenorhabdus* (γ-proteobacteria) b. *Photorhabdus* (γ-proteobacteria)	Help subdue and liquefy insect hosts to aid in resource acquisition	Stock and Goodrich-Blair (2008)
Animals – luminescent bacteria	Most common in marine animals, including fish, squid, and crustaceans	Mostly bacteria including *Vibrio* and *Photobacterium* (both γ-proteobacteria)	Used as a light source or lure in resource acquisition, defense, and interspecific communication	Widder (2010)
Agricultural symbioses	Leaf-cutter ants, some termites, and xylophagous beetles	Fungi	Special fungal cultivars gardened for consumption as a food source	Mueller *et al.* (2005)

among diverse eukaryotes through symbiosis. While plants photosynthesize through chloroplast organelles derived from cyanobacterial ancestors, many animals, including sponges and corals (**Table 1**), engage in photosynthetic symbioses with cyanobacteria or 'algae.' The latter is a polyphyletic group of unicellular eukaryotes that acquired this capability through an initial symbiosis with cyanobacteria. Of course, sunlight cannot reach deep-sea depths and drive primary production, yet life still thrives in locations such as hydrothermal vents through chemosynthesis – a process by which carbohydrates are derived from chemical energy via the oxidation of hydrogen sulfide or other inorganic molecules. Chemosynthesis has arisen numerous times throughout bacterial history, and bacteria from at least nine distinct chemosynthetic lineages are known to form symbioses with marine animals from at least seven phyla (Dubilier *et al.*, 2008; **Table 1**). Although first discovered near deep-sea hydrothermal vents, chemosynthetic symbioses are also found at deep-sea whale and wood falls, and even in shallow areas with ample sunlight. Symbiont-mediated fixation of inorganic carbon therefore fuels many of the world's ecosystems, with carbon-fixing plants and animals forming the base of many food chains. Thus, symbioses contribute greatly to primary productivity both locally and globally.

In addition to sugars, many macro- and micronutrients are essential for the growth, development, and reproduction of plants and animals. Due to the infrequent 'invention' of metabolic pathways required for nutrient biosynthesis, a more common solution has involved the acquisition of microbial symbionts that fulfill requisite nutritional needs. Animals, for example, ancestrally lack the ability to synthesize a subset of necessary amino acids (i.e., essential AAs) and many cofactors (e.g., vitamins) required for enzyme activity. While most animals acquire these by consuming a varied diet, others have acquired microbial partners that allow for niche specialization on previously unusable resources that are lacking in essential nutrients (Moran *et al.*, 2008). For example, some hemipteran insects have specialized on plant sap and harbor ancient, highly specialized bacteriocyte-associated symbionts that provision them with amino acids and other nutrients. As seen for some other eukaryotes, these nutritional symbionts are drivers of sap-feeding hemipteran diversity, having enabled novel niche occupation and subsequent species radiation. Nitrogen-fixing and nitrogen-recycling symbionts of plants and invertebrates also increase the availability of usable nitrogen to their hosts and facilitate exploitation of nitrogen-poor substrates, like wood by termites and shipworms, or the colonization of previously unusable habitats, such as desert soils by plants (van der Heijden *et al.*, 2008). In addition to nitrogen, phosphorus is also frequently a limiting macronutrient for plants, and most species engage in nutritional symbioses with phosphorus-providing MF (**Table 1**). Through their contributions to water and nutrient uptake, arbuscular MF likely facilitated the colonization of land by plants (Smith and Read, 2008).

In terrestrial habitats, plants comprise the bulk of biomass and thus represent an enormous, but challenging resource for heterotrophs (Hansen and Moran, 2014). In addition to low-nitrogen content, plants contain recalcitrant plant polymers and an array of chemical defenses that must be subverted for successful resource acquisition. Herbivorous and detritivorous animals can harbor digestive gut symbionts, which greatly aid in the breakdown of lignocellulose and other complex carbohydrates (**Table 1**). To counter the toxic and repellant secondary metabolites produced by their food plants, herbivores may also employ symbiotic microbes in addition to their own intrinsic mechanisms. For example, leaf-cutter ants, which are dominant herbivores in the tropics, engage in an agricultural symbiosis where they cultivate a fungus on plant material in the nest as a food source. In at least one interaction, the fungus produces enzymes that break down plant phenolics (Licht *et al.*, 2013). Leaf-cutter ants also harbor bacterial symbionts on their cuticles that produce antibiotics used to protect the cultivated fungus (Mueller *et al.*, 2005), demonstrating that symbioses often involve multiple parties. In addition to digestion, detoxification, and agricultural management, there is also emerging evidence that herbivores employ symbionts to modulate plant signaling pathways to

their benefit. Examples include orally secreted bacteria, which trigger plant responses to a microbial threat, constraining the abilities to defend against the herbivore (Chung *et al.*, 2013). Through these activities, animal–microbe symbioses can play important roles in terrestrial carbon and nitrogen cycling.

Symbiotic microbes are also known to aid parasitic animals in resource acquisition, including such hyper-diverse groups as insect parasitoids and entomopathogenic nematodes. For example, some hymenopteran parasitoids carry ancient, highly specialized polydnaviruses, which aid internally developing wasp larvae by compromising their host's immunity and creating an environment more suitable for development (Strand and Burke, 2013). Similarly, nematodes of the genera *Heterohabditis* and *Steinernema* harbor bacterial symbionts that help to subdue the insect host and break down tissues to increase resources to nematode juveniles (Stock and Goodrich-Blair, 2008).

Symbioses Involved in Host Protection

Virtually all plants and animals are attacked by natural enemies, and there is a growing appreciation that protective symbioses are widespread across eukaryotes (Clay, 2014). In some cases, the symbionts provide toxins, antimicrobials, or other bioactive compounds, defending hosts directly. For example, the larvae of the marine bryozoan, *Begula neritina*, are protected against predators by symbiont-produced bryostatins. Similarly, terrestrial beewolves (Hymenoptera) have bacterial symbionts that produce an antibiotic cocktail that protects brood cells from microbial pathogens. In other cases, symbionts can prime the host immune system, heightening vigilance against subsequent enemy attack. Defensive symbioses have been best studied in insects, grasses, and sessile marine invertebrates (**Table 1**).

In insects, facultative heritable endosymbionts are increasingly shown to protect hosts against natural enemies. Some are especially widespread and confer a wide range of phenotypes. For instance, in addition to executing all four types of reproductive manipulation (**Box 1**), *Wolbachia* symbionts confer protection against a wide range of natural enemies in dipteran insects and likely beyond (Hamilton and Perlman, 2013). In other cases, some insects harbor a diverse assemblage of more specialized heritable bacteria that mediate diverse ecological interactions. Pea aphids, for instance, are associated with seven common facultative endosymbionts that provide defense against high heat, parasites, and pathogens (**Figure 1**). Through defensive benefits, heritable symbionts may spread quickly through host populations, illustrating rapid adaptation through symbiosis (Jaenike *et al.*, 2010). Protective symbioses show much potential for coevolutionary interactions, with strain variation in the strength of protective phenotypes, symbiont strain specificity to particular enemy genotypes, and enemies that can evolve counter-responses to symbiont-based defenses (Clay, 2014). Protection against abiotic factors is an additional contribution of some symbioses, with microbes buffering hosts against the stressors of drought and heat (Gilbert *et al.*, 2010).

Origins of Symbiosis

Symbioses can originate from diverse interaction types, including antagonism, commensalism, or chance encounters. They can also be maintained and disrupted through a variety of

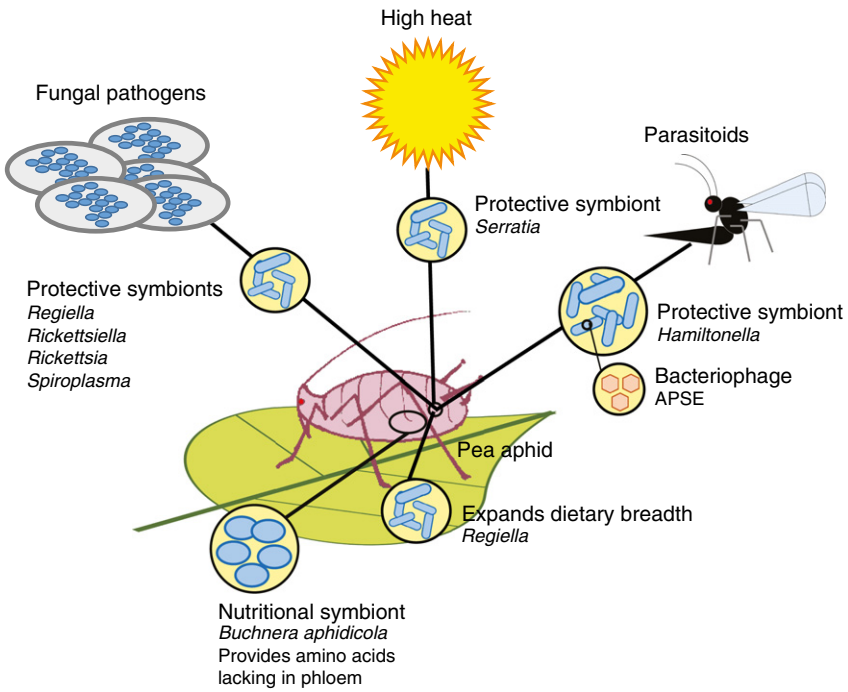

Figure 1 The pea aphid, *Acyrthosiphon pisum*, is a phloem-feeding insect that harbors at least eight heritable symbionts that have major effects on host ecology and evolution. All pea aphids carry an obligate nutritional symbiont allowing subsistence on a nitrogen-poor diet, and individual aphids may carry one or more facultative endosymbionts, with diverse roles in mediating important ecological interactions.

mechanisms. The result is substantial variation in the persistence and specialization of interactions. While germ theory was a major development for disease treatment it left the false impression that most microbes were pathogens. Given this, we expect that many symbioses, especially the microbial consortia inhabiting guts and external surfaces, originated from encounters with nonpathogenic organisms. Nevertheless, there is evidence that some symbiotic interactions are likely derived from antagonistic relationships. For example, phylogenetic evidence indicates that many plant and animal symbionts are closely related to pathogens, including the protective fungal endophytes of grasses, which likely evolved from entomopathogenic fungi (Sung *et al.*, 2007). In some extant symbioses, including *Photorhabdus* bacteria infecting entomopathogenic nematodes, a particular microbe can be a beneficial symbiont in one host and a pathogen or parasite in another (Stock and Goodrich-Blair, 2008). Transitions from pathogenicity to symbiosis can occur through several parallel processes including changes to the mode of transmission, the acquisition of symbiosis genes, and the amelioration of pathogenic effects of infection over time. In some cases, the eukaryotic host can even become dependent on a microbial partner, even when no novel benefit is conferred. The parasitic wasp *Asobara tabida*, for example, requires *Wolbachia* for oogenesis through its inhibitory effects on programmed cell death (Werren *et al.*, 2008). More broadly, although plants and animals can often develop and function in an aposymbiotic state, they often do so with impairments (Douglas, 2014). This highlights the integrated nature of symbiosis in eukaryotic biology that has come about through evolution in a microbial world.

Symbiont Maintenance and Stability

Symbiotic microbes must establish and persist in hosts with extensive means to recognize and remove microorganisms. Some symbionts borrow infective strategies employed by pathogens, such as evading recognition by the host, or if recognized as nonself, they may avoid elimination through the inhibition of mechanisms targeting their destruction. Conflict resolution allowing symbiont persistence can also be accomplished by restricting tissue tropism. Vertebrates, for example, tolerate high bacterial loads on external surfaces (including their gut lining), though their immune systems effectively eliminate internally circulating bacteria. In more specialized interactions, novel morphological structures have evolved to harbor specific symbionts, such as the light-emitting organs in some fish and squid, and the bacteriocytes of insects housing nutritional symbionts.

Many eukaryote–microbe symbioses have persisted for more than a hundred million years, including some obligate nutritional symbionts of insects. These ancient symbionts have undergone strict maternal transmission and show patterns of cospeciation between host and symbiont. Functional redundancy can lead to the loss of function in one or both partners. A striking feature of the obligate nutritional symbionts of insects is that their genomes are greatly reduced compared to free-living relatives, yet retain the specific metabolic pathways necessary for nutrient provisioning (Moran *et al.*, 2008). Genome reduction can lead to functional deterioration, potentially constraining suitable abiotic (temperature) or biotic (diet breadth) ranges. Potential solutions include the transfer of key symbiont genes to the host nucleus and symbiont replacement.

When symbionts are environmentally acquired there is considerable variation in the taxonomic specificity and persistence of the microbial partners, and the amount of specialization in the host. The gut microbiota of terrestrial animals, for example, are generally less specialized and partner specific, though there are exceptions (Engel and Moran, 2013). Mechanisms for partner recognition are also variable and can be sophisticated. Squid and their environmentally acquired *Vibrio* symbionts, for example, engage in a complex dialog that allows a 'winnowing' from thousands of bacterial species to one or two specific strains that occupy the light organ (McFall-Ngai, 2014). Policing of symbiont function leads to the removal of non-luminescing *Vibrio* strains. Mechanisms that ensure symbiont functionality appear critical for environmentally acquired symbioses to safeguard against cheaters. In contrast, policing in heritable symbioses occurs via host-level natural selection (**Figure 2**).

Correlations between particular environmentally acquired symbionts and host attributes have been hypothesized to reflect a role of microbes in host adaptation. For instance, most mammalian herbivores have specialized gut fermentation chambers to aid in the digestion of recalcitrant plant materials, and findings that unrelated herbivorous mammals harbor similar gut communities suggest symbiont roles in dietary evolution (Ley *et al.*, 2008a,b). Partner availability, host plasticity, and host or symbiont evolution, however, can all produce symbiotic variation in nature (**Figure 3**), and correlations between symbionts and host attributes should be carefully scrutinized when assessing symbiont roles in host adaptation. Common garden and microbial transplantation experiments can determine, for example, whether the host controls which symbionts colonize their bodies, and whether symbiont-correlated phenotypes are transported upon microbial transplantation.

Application of Symbiosis for Human Welfare

Understanding and manipulating symbioses may aid in solving many of the biological challenges facing our world today. Climate change is expected to affect symbioses more severely when partners have different environmental tolerances, a phenomenon exemplified by symbiont loss in bleached corals due to warming oceans and other anthropogenic impacts. Many symbionts, however, buffer environmental variability and these have the potential to confer tolerance to a rapidly changing climate. Thus, findings of such protective symbioses, including those that inhibit coral bleaching (Gilbert *et al.*, 2010), should be of growing interest in the coming years. Capacities of symbiotic microbes to degrade pesticides, influence the diet breadth of herbivores, affect host pest status, and to thwart biological control agents have important implications for the health and function of both natural and managed ecosystems. Alternatively, we may be able to leverage symbiont function to our own ends. For example, symbionts may represent an untapped source of antibiotics and other bioactive compounds with medical applications and provide a novel means to combat insect-vectored human pathogens either by changing vector characteristics or inhibiting pathogen

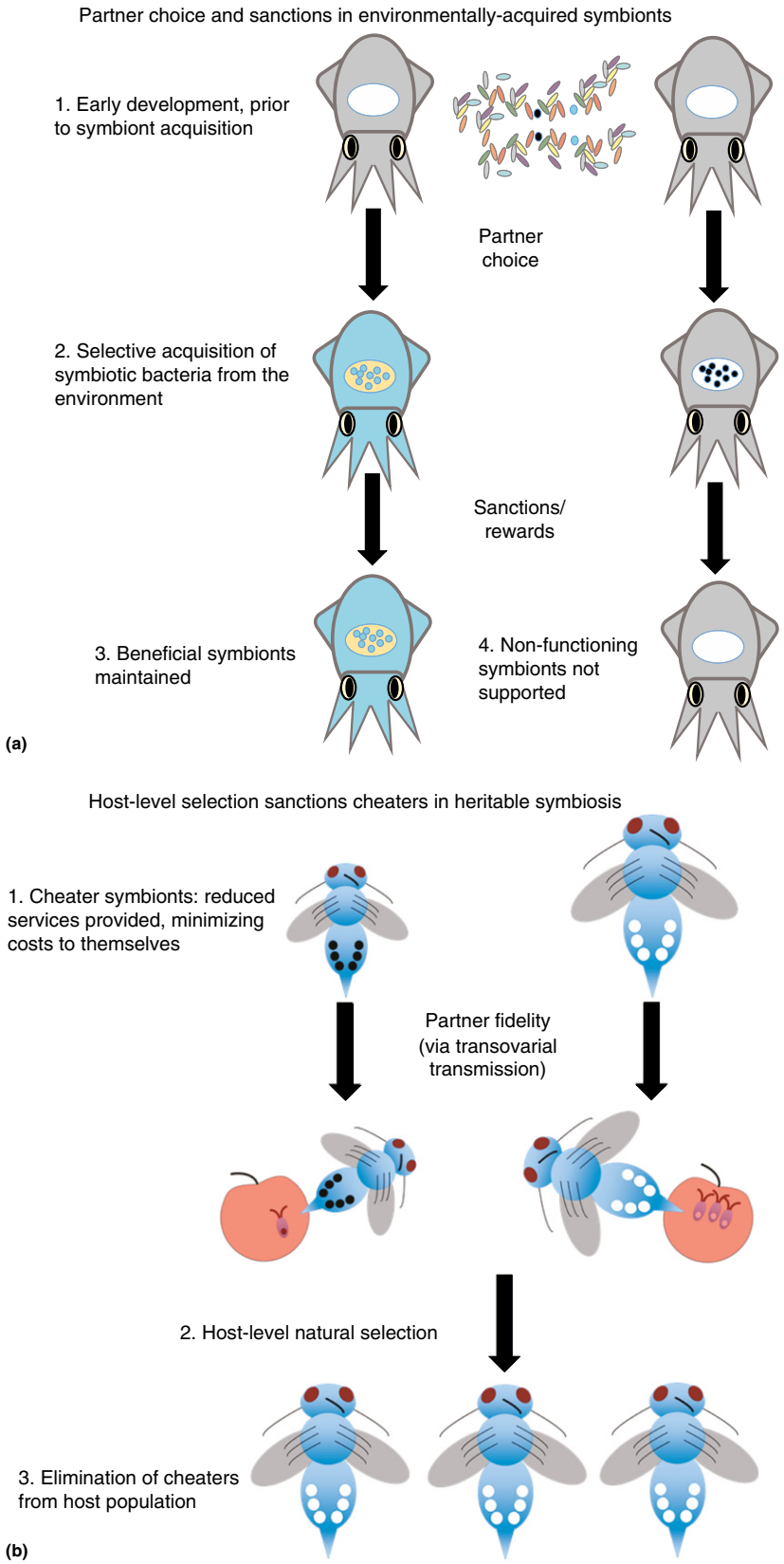

Figure 2 Forces stabilizing symbiotic mutualisms. For environmentally acquired associations, partner choice (i.e., selectivity/specificity of the hosts and microbes) and the capacity to sanction under-performing partners can stabilize beneficial symbioses (panel (a)). For associations with substantial vertical transfer (i.e., microbes move from parent to offspring), selection at the host level favors hosts with cooperative symbionts (panel (b)).

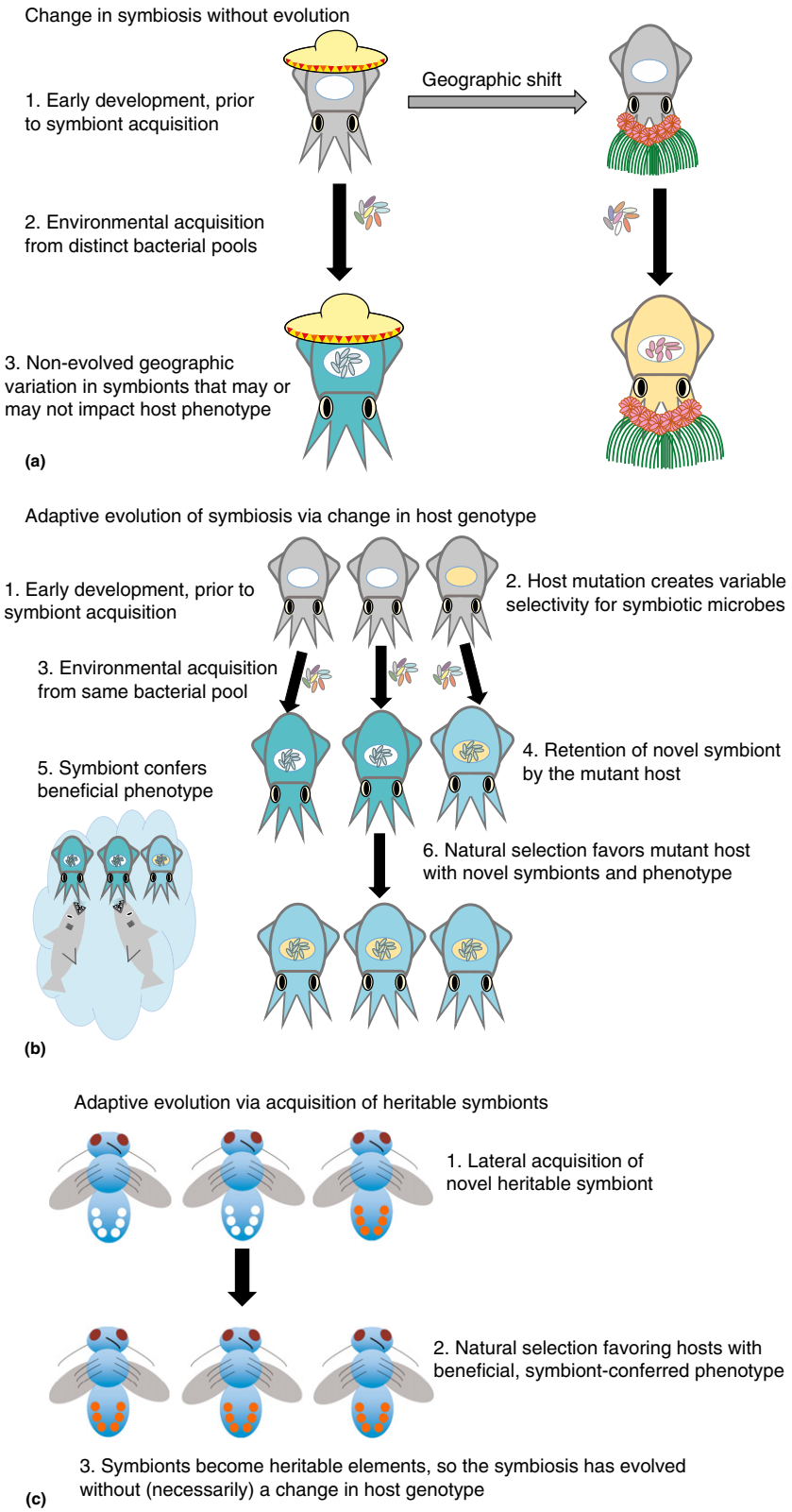

Figure 3 Change in symbiosis. Symbiotic associations vary over time and space and across ecological gradients. While this may be due to factors such as plasticity and shifting partner availability (panel (a)), it may alternatively reflect evolutionary change through symbiont-conferred adaptation (panels (b) and (c)). Panel (b) depicts this symbiotic evolution via host mutations that favor retention of beneficial microbes. Panel (c) depicts an acquisition of novel, beneficial symbionts; as the microbes are heritable elements of their hosts (like mitochondria), the hosts, and thus the symbiosis, have evolved without a change in host genotype.

Box 2 Symbiosis and human health

Discoveries on the diversity, abundance, and importance of microbes to human health have piqued public interest in symbiosis. Metagenomic research indicates that individual humans typically carry hundreds of bacterial species and trillions of cells and demonstrates that symbiont function varies across individuals with varying relatedness and diet. Moreover, our 'normal' microbiota may be altered in association with disease states. For example, obesity is associated with community-level microbiome shifts toward an increase in particular Firmicutes bacteria that drive an increased microbial capacity to extract calories from our diets (Ridaura *et al.*, 2013). The promise of probiotic therapies, fecal transplants, and other manipulations represent exciting possibilities for symbiosis research to improve human health (Spor *et al.*, 2011).

replication (Werren *et al.*, 2008). Manipulation of our natural gut microflora will also likely be important for the treatment of human diseases (see **Box 2**).

Conclusions

Modern molecular tools have led to an awareness that symbioses are widespread in nature and have contributed to major transitions in the evolution of life on Earth. Indeed, virtually all plants and animals, including humans, harbor microbial symbionts that can have large effects on host ecology and evolution. Symbionts can confer novel capabilities that influence ecological interactions, with effects that extend to other trophic levels. They may also impact the processes of speciation and diversification (Brucker and Bordenstein, 2012). Many particular symbioses are pertinent to medicine, agriculture, climate change, and frontiers in this field include exploiting symbiosis to improve the health of humans, our agricultural systems, and natural ecosystems. Among the many challenges include (1) understanding how genes and the environment interact to shape eukaryote–multipartite microbe associations, (2) how host immune systems regulate beneficial symbioses, and (3) how contributions from host and symbiont are coordinated between organisms from different domains.

See also: Coevolution, Introduction to. Endosymbiotic Theory. Microbiome. Mutualism, the Evolutionary Ecology of. Mycorrhizal Fungi, Evolution and Diversification of. Symbiosis, History of

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Further Reading

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Synthetic Theory of Evolution, History of

NA Johnson, University of Massachusetts-Amherst, Amherst, MA, USA

Glossary

Adaptive landscape A heuristic tool to visualize the relationship between genotype and fitness.

Blending inheritance The notion that traits are inherited like different colors of paint blend. Under blending inheritance, the value an individual has for a trait would be a blend of the values that the individual's parents have for that trait. This notion was largely discredited by Mendel's findings.

Particulate inheritance The notion that inherited traits are transmitted from one generation to the next by specific particles. Mendel's findings and subsequent work by early geneticists confirmed the generality of particulate inheritance.

What is the Synthetic Theory of Evolution?

In essence, the synthetic theory is a unification of Mendelian genetics and Darwinian evolution. However, exactly what is included in the synthetic theory of evolution is hard to definitively determine, and different biologists and historians of biology differ regarding what should or should not be included.

Julian Huxley, grandson of 'Darwin's bulldog' Thomas Huxley, synthesized the views of many biologists in his 1942 book *Evolution – The Modern Synthesis*. Like his grandfather had in the 1860s and 1870s, Julian defended Darwinian evolution. Unlike the evolution of his grandfather's time, the version that Julian Huxley promoted had been synthesized with a clear theory of heredity, namely that associated with Gregor Mendel and Mendelian inheritance and was more firmly rooted in mathematical theory. Moreover, the younger Huxley was battling with biologists at the time, who had accepted evolution, but not one based on natural selection. Although the terms 'the modern synthesis' and 'the synthetic theory of evolution' often are used interchangeably, they are not the same. Huxley's (1942) modern synthesis encompassed views about society that are not in the synthetic theory of evolution (see Mather, 1943; Smocovitis, 1996). The modern synthesis, but not the synthetic theory, includes the institutional framework that supported the science. Ernst Mayr, in particular, established the Society for the Study of Evolution, serving as its first President, and the journal *Evolution* (Smocovitis, 1996; Haffer, 2008). The institution building is not part of the synthetic theory but does accompany its establishment. Finally, the synthetic theory of evolution spans a more expansive span of time than did Huxley's modern synthesis.

In this article the author traces the development of the synthetic theory, beginning with why such a synthesis was needed and why it took so long. Then he discusses the first step of the synthesis, the establishment of theoretical population genetics that occurred during the quarter century before Huxley (1942) and then discusses the solidification of the synthetic theory that occurred during Huxley's time as various disciplines latched onto a common framework. He concludes with a discussion of the challenges the synthetic theory has faced since the 1960s.

Before the Synthetic Theory

Although Darwin's insurmountable evidence and forceful argument quickly convinced biologists and naturalists of the reality of evolution, Darwin's preferred mechanism for evolution – natural selection – was still controversial up until well into the twentieth century (Provine, 1971, 2001; Mayr, 1982; Gould, 2002). Most biologists of this time acknowledged that natural selection could operate, but were skeptical of its potency and scope (Provine, 1971; Gould, 2002). These biologists had advanced several alternatives to natural selection. For instance, orthogenesis referred to several postulated theories of the time wherein organisms evolved along certain directions dictated more by 'channels of internal constraint, rather than external pathways of natural selection' (Gould, 2002, p. 66).

One of the main reasons for the skepticism regarding selection involved the lack of a clear mechanism of heredity. For instance, in a review of Darwin's *Origin of Species*, the engineer Fleeming Jenkin (1867) posed a puzzle to Darwin that seemed to challenge the importance of evolution by natural selection. Jenkin argued that if inheritance is blending, new variants quickly would be lost. For instance, suppose a new mutation changed the flower color from white to red. If the red individual outcrosses, it would have to breed with a white individual, as the entire population (aside from it) is white. The resulting offspring would be pink. Nearly all of the rest of the population would be white, so these pink individuals would have still lighter pink offspring. Soon, any trace of red would be lost. Because evolution via natural selection requires variation in the population, blending inheritance would seem to preclude evolution via natural selection.

Transmission genetics as a science developed quickly after the re-discovery of Mendel's laws of heredity at the turn of the twentieth century (Sturtevant, 1965; Mayr, 1982). Mendelian genetics follows particulate inheritance: that is, the hereditary materials (genes) come in different varieties (alleles) that are passed on from one generation to the next, and that while the action of one allele may be masked, these alleles are not lost during transmission. In Mendelian genetics, new alleles arise from mutations in genes. Yet, despite this development, Mendelian genetics and Darwinian selection still were not unified.

During the first quarter of the twentieth century, evolutionists coalesced into two opposing groups, the Mendelians and the biometricians (Provine, 1971). The Mendelians rejected Darwinian gradualism and viewed evolutionary change as being driven by mutations of large effect. In contrast, the biometricians dismissed the mutations the Mendelians were observing as being irrelevant to the evolutionary process, and were skeptical of the value of Mendelian genetics (Provine, 1971). Instead, they viewed slight differences in continuous traits as being most important for evolutionary change, and generally took a statistical or biometrical approach in their assessment of this variation.

Some authors at the turn of the twentieth century, such as William Castle and Udny Yule, quickly saw that the views of the Mendelians and the biometricians were compatible. In addition the British mathematician Godfrey Hardy (1908) and the German physician Wilhem Weinberg (1908) independently developed an early null model demonstrating that allele frequencies would not change unless evolutionary processes were operating in the population (Provine, 1971; Crow, 1999). Despite these developments, most of the biologists of the time did not appreciate the compatibility between Darwinism and Mendelism and the synthesis had to wait another generation (Provine, 1971, 1986).

Early Development of the Synthetic Theory: Theoretical Population Genetics

The first phase of the development of the synthetic theory came when mathematical population genetic theory united Mendelian genetics and Darwinian evolutionary processes, including natural selection. Predominant among these theoreticians were the British geneticists Sir Ronald Fisher and J. B. S. Haldane, and the American geneticist Sewall Wright.

Early in his career, Fisher (1918) showed that Jenkin's objection regarding selection was groundless given Mendelian particulate inheritance; genetic variation can persist in a population under Mendelian inheritance, in contrast to blending inheritance, because the alleles are not lost in the process (Grafen, 2003). More generally, Fisher (1918) also showed that Mendelian rules of inheritance could apply to continuous traits being studied by the biometricians, accounting for the correlations of traits seen across different relatives. In his subsequent book *The Genetical Theory of Natural Selection*, Fisher (1930) began by emphasizing the particulate nature of heredity and its consequences. In particular, Fisher emphasized the role of additive genetic variation, the proportion of the variation that is owing to the additive effects of genes. The more additive genetic variation for fitness, the faster alleles will change in frequency due to selection.

Starting in 1924, Haldane published a series of papers that presented a quantitative theory of how natural selection would operate in Mendelian populations. For instance, Haldane (1924, Figure 2) demonstrated that an autosomal dominant allele with a selective advantage of 0.001 could increase in frequency from 10% to 50% in about 2500 generations. With stronger selection, the time to change frequency would be correspondingly reduced. Quantification of the effects

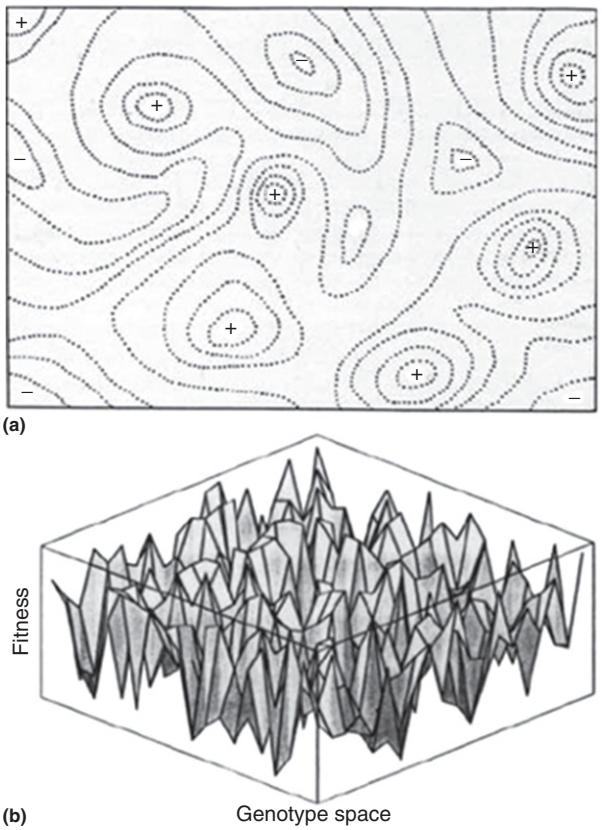

Figure 1 The adaptive landscape can be visualized in several ways. In (a), fitnesses are displayed in a contour map with the *x* and *y* axes representing different combinations of genotypes. In (b), high fitness is represented by peaks and low fitness is represented by valleys. Reproduced from Johnson, N., 2008. Sewall Wright and the development of the shifting balance theory. Nature Education 1(1), 52. Available at: http://www.nature.com/scitable/topicpage/sewall-wright-and-the-development-of-shifting-30508 (accessed 19.09.2015).

evolutionary processes had on changes in allele frequency, as done by Haldane (1924) and Wright (1931) among others, established theoretical population genetics as a science, and cemented the foundation for uniting Mendelian genetics and Darwinian evolution (Provine, 1971).

Wright (1931, 1932) emphasized that selection was not the only evolutionary process that could alter allele frequencies. Random sampling variation occurring every generation, which would become known as genetic drift, could also alter allele frequencies; the potency of this process is inversely related to population size (Hartl and Clark, 2007). Wright (1931, 1932, 1977) noted that drift could interact with selection even in large populations if those populations were subdivided.

Wright (1932) also introduced the metaphorical concept of the adaptive (or fitness) landscape, as a way to visualize the relationship between genotype and fitness (**Figure 1**). This heuristic enabled better understanding of how selection and other evolutionary processes could operate on populations and helped to open up theoretical population genetics to biologists with limited mathematical skill. Although Wright's own biographer, Will Provine (1986), criticized ambiguities in Wright's use of the adaptive landscape, it remains a key

concept in evolutionary biology (e.g., Arnold *et al.*, 2001; Gavrilets, 2004).

Population Genetics and the Synthesis

Although theoretical population genetics was established by the early 1930s, it did not actually reach most biologists interested in evolution for some time. The population genetics theory was largely in densely-written, equation-heavy papers that were inaccessible to many biologists. In addition, the theory needed to be applied to natural populations of organisms.

During the late 1930s and early 1940s, the publication of several books from a variety of authors advanced and solidified the synthesis (Mayr, 1982). These publications made the foundations of theoretical population genetics accessible to wider audiences of geneticists, zoologists, botanists, and paleontologists. Through these books and other works, these leading authors codified research agendas and influenced the next generation of evolutionary biologists (Smocovitis, 1996).

In his *Genetics and Origin of Species*, the Russian-born, American immigrant Theodosius Dobzhansky (1937) promoted and popularized the population genetic principles and theory established by Fisher, Haldane, and especially Wright. One key tenet emerging from this work was the promotion of selection as an evolutionary force: unless a population is very small, mutations will frequently appear in the population as a whole such that variation should be sufficiently abundant for natural selection to operate (Dobzhansky, 1937). To many, Dobzhansky's book functioned as the first textbook of evolution that integrated Darwinian theory with Mendelism. It also inspired other works, and influenced the German-born, American immigrant Ernst Mayr who had been studying the systematics of birds. Mayr (1942) folded systematics into the synthetic theory, viewing species through the lens of population thinking wherein the focus was on individual differences within a species and not an idealized type (see also Haffer, 2008).

Collaborations between the theoretical population biologists and empirical field researchers cemented the synthetic theory in empirical realism. The close collaboration with Sewall Wright helped Dobzhansky establish a research program to address the genetics of natural populations. Here, Dobzhansky's main organism of choice was *Drosophila pseudoobscura* (Dobzhansky, 1937; Lewontin *et al.*, 1981; Provine, 1986). In the United Kingdom, the Lepidopterist Edmund BriscoFord collaborated extensively with Fisher to develop what is now known as ecological genetics, the study of genetic variation and the evolutionary processes that influence it in natural populations (Connor and Hartl, 2004). Under Ford's supervision, Bernard Kettlewell (1955) tested and confirmed the hypothesis that bird predation was the selective agent behind changes in color morphs of the peppered moth, *Biston betularia*. This is one of the classic examples of evolution by natural selection taking place in a natural population (Grant, 1999).

The synthesis founders were not monolithic in their thinking, and indeed had frequent disagreements and debates. For instance, Wright (1931, 1932, 1977) emphasized the roles of population structure and gene interaction while Fisher was dismissive of these factors (Provine, 1986). This debate has persisted for several decades (cf Coyne *et al.*, 1997; Wade and Goodnight, 1998). Another major mid-century debate revolved around the nature of genetic variation within species and the processes that maintained such variation. As recounted by Lewontin (1974), the American geneticist Hermann Joseph Muller and the classical school thought that genetic variation was generally rare and was maintained mainly by a balance between mutation bringing in new variation and selection purging the variation, while Dobzhansky and the 'balance school' thought variation was much more common and was maintained by a balance between different forms of selection. Note that these disputes primarily revolved around model parameters and the relative frequencies of empirical biological phenomena, and were not about the types of questions to be addressed or the underlying mathematical theory.

Speciation and Macroevolution in the Synthetic Theory

In addition to examining evolutionary processes on variation within species, the modern synthesis also addressed speciation and evolution beyond the species level (macroevolution). In general, the proponents of the modern synthesis viewed evolution as a continuum with macroevolution as microevolution writ large (Dobzhansky, 1937; Huxley, 1942; Mayr, 1942; Simpson, 1944).

Both Dobzhansky (1937) and Mayr (1942) paid homage to Darwin in using 'the origin of species' in the titles of their books, and devoted much of those books to speciation. To Dobzhansky (1937), biological diversity came in more-or-less discrete entities (with species being the most basic level of biological diversity). He saw a central question being how can discrete packets of biodiversity emerge via evolutionary processes. Dobzhansky (1935, 1937) and Mayr (1940, 1942) argued for a biological species concept based on reproductive compatibility: species are entities of reproductively compatible populations that were reproductively isolated from other such groups. Influenced by Dobzhansky, Ledyard Stebbins (1950) applied the reproductive compatibility species concept to plants (Smocovitis, 2006). Although Haldane, among others, opposed this definition of species on the grounds that species were just human constructs, the Dobzhansky–Mayr species concept generally won out (Mayr, 1963; Coyne, 1994; Coyne and Orr, 2004).

Viewing species on the basis of reproductive compatibility enabled the founders of the synthesis to study speciation as the evolution of reproductive isolation.

Dobzhansky (1937) and Muller (1942) independently established a genetic framework for how the fitness of hybrids is reduced as nascent species diverge from one another. In the Dobzhansky–Muller framework, which is still commonly used today, hybrids have reduced viability and fertility due to changes at multiple interacting genes instead of single gene changes (Coyne and Orr, 2004; Johnson, 2002). Fisher (1930) and especially Dobzhansky (1940) also promoted the idea that reduction of hybrid fitness could lead to selection pressure against mating with other species. This idea, now known as

reinforcement, is generally accepted as a major factor in speciation (Coyne and Orr, 2004). Stebbins (1950) championed and provided evidence for the thesis that polyploidy – the acquisition of additional chromosome sets – could be an important mode of reproductive isolation, especially in plants (Smocovitis, 2006; Soltis *et al.*, 2014).

Beyond the species level, macroevolution was also brought into the synthetic theory. In his influential book *Tempo and Mode in Evolution*, the paleontologist George Gaylord Simpson (1944) argued that the paleontological patterns observed in the fossil record could be explained by the population genetics of the synthetic theory, and that no additional processes were needed to explain the patterns. Moreover, Simpson argued that the patterns observed (the tempo) could provide information about the types of evolutionary processes involved (the mode). Simpson (1944) adopted and expanded Wright's adaptive landscape to include phenotypes, with phenotypic measures represented locations on each of the axis and fitnesses depicted as contour intervals (see **Figure 1**). With this expanded adaptive landscape, Simpson could help unite population genetic and paleontological approaches to studying evolution (Arnold *et al.*, 2001).

The Synthesis as a Constriction

Provine (1989) contended that the synthesis was more of a constriction than a synthesis. Ideas that were dominant at the turn of the twentieth century, such as orthogenesis and Lamarckian inheritance of acquired characteristics, were removed from mainstream evolutionary biology thought during the synthesis of the 1930s and 1940s. It is true that many biologists working in the early part of the synthesis, including Ernst Mayr (Haffer, 2008; Mayr, 1982) started out accepting Lamarckian inheritance and later became convinced of the validity of evolutionary change occurring via Mendelian particulate inheritance as the synthetic theory developed. To describe the synthesis as strictly a constriction is unfounded given that it actually involved the fusion of Mendelian and Darwinian notions.

The views of the synthesis hardened and further constricted during the 1950s and 1960s (Gould, 2002). During this time, natural selection became the predominant explainer of biological phenomenon to the nearly complete exclusion of other evolutionary processes. For instance, successive editions of Dobzhansky's *Genetics and the Origin of Species* focused increasingly more on selection and de-emphasized the importance of genetic drift (Gould, 2002). In addition, approaches that attempted to link evolution with developmental processes such as those championed by Richard Goldschmidt and Conrad Waddington were de-emphasized during the evolution synthesis of the middle twentieth century (Gould, 1980, 2002; Jamniczky *et al.*, 2010).

Challenges to the Synthetic Theory

Beginning in the 1960s, new discoveries and ideas challenged the synthetic theory of evolution. Over the past half century, evolutionary biologists have debated whether the synthetic theory is still useful and whether we are in a new synthesis. There is no resolution to this debate (Laland *et al.*, 2014).

Advances in molecular biology led to new sources of data that ushered in the field of molecular evolution. Protein electrophoresis data revealed that natural populations of many organisms contain much more genetic variation than previously thought (Lewontin, 1974). Protein sequence data suggested that proteins evolve roughly linear to the time of divergence between species (a 'molecular clock') (Zuckerkandl and Pauling, 1965). Kimura (1968) noted that the observed rate of protein sequence evolution was higher than could be accounted for by selective deaths.

These and other observations led Motoo Kimura (1968, 1983) to argue against the primacy of selection as the driver of molecular evolution. Kimura's thesis, which became known as 'the neutral theory of molecular evolution,' posits that the vast majority of the variation at the protein and DNA level seen in natural populations is simply the result of mutational input, random genetic drift, and negative selection weeding out variants. Thus, most genetic variants in natural populations are effectively neutral. Deleterious mutations do occur but are weeded out.

Similarly, mutation and genetic drift– and not positive selection – drive divergence between species in molecular evolution (Kimura, 1983). Debates between advocates of the neutral theory (neutralists) and those who champion a larger role for selection driving molecular evolution continue to the twenty-first century (Lynch, 2007; Hahn, 2008). Even if the neutral theory is not a good representation of biological reality, evolutionary genetics of the twenty-first century generally think that mutation plays an important role in evolution, and especially molecular evolution (Stoltzfus and Cable, 2014).

Findings about the rates of phenotypic evolution also challenged the modern synthesis. Famously, Eldredge and Gould (1972) advanced the thesis that most phenotypic evolution involves long periods of relative stasis punctuated by rapid (on a geological scale) change. This observation, called punctuated equilibrium, is a pattern, but that pattern also generated much debate about the processes underlying evolution. For instance, Gould (1980) suggested that the observed pattern implied the need to examine additional mechanisms of evolution, including radical reorganizations of the genome. Population geneticists of the time strongly objected to this need, and proposed that stabilizing selection could be responsible for stasis (e.g., Charlesworth *et al.*, 1982). Why persistent stasis is commonplace remains the most enduring question from the punctuated equilibrium debate (Futuyma, 2010).

Although Darwin (1859) was well aware of correlated traits and had an intuitive grasp on how selection on one trait could constrain the evolution of other traits, the complications of correlated traits were generally not a focus in the mid-twentieth century synthesis. Starting in the early 1980s, quantitative evolutionary geneticists developed methods to deal with these complications (e.g., Lande and Arnold, 1983; Arnold, 2014). The expected evolutionary path of a population is dependent not just on patterns of selection, but also on internal genetic constraints (e.g., Futuyma, 2010). The extent to which internal genetic constraints channel evolutionary responses is still unresolved, but if such constraints are frequently strong, then

orthogenesis might bear a revisit as a cause of the observed stasis (Gould, 2002).

Developmental biology was generally not part of the synthetic theory of the middle twentieth century (Gould, 2002). Evolutionary developmental biology (evo-devo), the synthesis of evolutionary biology and developmental biology, came later. It posed challenges for the synthetic theory of evolution. One of these challenges is that of deep homology – very distantly related organisms (e.g., flies and mammals) share a common tool kit of important developmental genes (e.g., Palopoli and Patel, 1996). Deep homology was an unexpected surprise. In fact, Mayr (1963, p. 609) noted, "Much that has been learned about gene physiology makes it evident that the search for homologous genes is quite futile except in very close relatives" (see also Gould, 2002). While it was a challenge, the notion of deep homology is being weaved into the synthetic theory of evolution (Futuyma, 2013).

In recent years, the notion of inheritance has expanded beyond Mendelian genetics. Because the synthetic theory fuses Mendelism and Darwinism, epigenetics, defined in the broad sense as inheritance systems outside of DNA sequence, poses a challenge (Jablonka and Lamb, 1998; Goldberg *et al.*, 2007; Jamniczky *et al.*, 2010). At the time of writing this, the extent of the challenge remains unclear and a matter of contention (Laland *et al.*, 2014).

See also: Darwin–Wallace Theory of Evolution. Genetic Variation in Populations. Quantitative Genetic Variation

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Systems in Evolutionary Systems Biology

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Glossary

EvoSysBio A transdisciplinary framework for constructing reliable, testable, interactive overviews of nestable, dynamic, multi-dimensional fitness landscapes, which mechanistically predict: (1) changes in fitness of individual organisms when their states and environments change; (2) how populations evolve when organisms traverse fitness landscapes.

Extra-Organism Biology (EOB) Biology focusing on actions, reactions, processes, parts, and patterns in ecosystems that alter states of individual organisms as they interact with biotic or abiotic environments.

Fitness Causality Network (FCNet) A network of nodes (defined by IFTs) and links (defined by LIFTs) that describes causal influences (e.g., genotypes, environments, and initial states such as maternal methylation patterns of DNA) on consequential IFTs (e.g., survival, reproduction) in a given time period.

Fitness landscape An abstract 'landscape' defined by *causal* 'positions in a plane' and their *consequential* 'heights,' as defined by a corresponding Fitness Causality Network.

Incomplete Fitness Trait (IFT) A phenotypic trait that impacts the fitness of an organism, its offspring, or its genetic relatives and that, at least occasionally, affects rates of survival, reproduction, merging, etc., or modifies evolutionary factors like mutation rates in one or more environments.

Intra-Organism Biology (IOB) Biology focusing on actions, reactions, processes, parts, and patterns within a single, individual organism that enable it to live (grow, survive, reproduce, move, etc.) by changing its state in a given environment.

Landscape of Incomplete Fitness Traits (LIFT) An abstract representation of a fitness landscape that maps causal IFTs to consequential IFTs, thus providing a causality statement about how input governs output, comparable to probabilistic mathematical functions.

Nest Organism A single, individual organism that is the environment for one or more populations of different types of nested organisms, which may themselves be nest organisms.

Nested Organism A single, individual organism that is contained by an encapsulating nest organism, which is the nested organism's environment and may itself be nested in a larger organism of a different type.

Organism A single, individual system that consists of different parts that builds a whole, which – in biology – must be able to replicate and may be nestable.

Population-Genetics Biology (PGB) Biology focusing on heritable information (genotypes, alleles, traits, methylation patterns, etc.) that can directly or indirectly affect IFTs (survival, reproduction, etc.) when passed on by individual organisms in evolving populations.

Trans-Organism Biology (TOB) Biology focusing on integrating Extra-Organism Biology (EOB), Population-Genetics Biology (PGB), and all direct or indirect actions, processes, and patterns that otherwise fall through the disciplinary cracks of EOB or PGB (excluding the inside of organisms).

Systems approaches to biology and genome evolution are becoming increasingly important. Since the 1920s, the New Evolutionary Synthesis has been constructing an increasingly coherent view of evolution by synthesizing ideas from different parts of biology. Further progress of this relentless synthesis will increasingly depend on crossing disciplinary boundaries, complex simulations of biological systems, and reliable reproducibility. Evolutionary Systems Biology (EvoSysBio) is defined here as working towards integrated interactive overviews of dynamic multi-dimensional fitness landscapes that are testable in the real world and enable the prediction of evolution. EvoSysBio further formalizes the New Synthesis as illustrated here for cancer and antibiotics resistance evolution.

Systems Approaches to Genome Evolution

Biology has a long history with numerous independent efforts to integrate diverse aspects of biological systems in order to understand the whole. For example, R.A. Fisher (1918) resolved a big controversy about inheritance by creating an integrative systems model with a mechanism for combining the varying effects by which different genes could impact a given phenotypic trait. The rise of population genetics has since inspired mechanistic modeling of how systems with populations of organisms evolve in response to the five fundamental factors of evolution, which may vary between these biological extremes over space and time:

1. *mutation* (perfect heritability ⇆ fast change);
2. *selection* (harmful ⇆ neutral ⇆ helpful);
3. *genetic drift* (last survivor ⇆ largest finite population);
4. *recombination* (complete linkage ⇆ free segregation); and
5. *migration* (homogeneity of space ⇆ movement maximizing impact of heterogeneity).

These factors are fundamental, since they affect *all* real-world populations of organisms with a phenotype capable of reproducing heritable variations; the complexity of evolutionary

outcomes (see this Encyclopedia) is fueled by variations of these factors over space and time at nested levels of replication. Simple interactions are well understood, but complex, dynamic patterns in heterogeneous, nested, multi-locus systems pose many questions that will keep defining the cutting-edge of population genetics for a long time to come (Loewe and Hill, 2010a,b). There is no better theory of evolution than population genetics, the 'auto-mechanics of evolution' (Singh and Krimbas, 2000, p.1); yet it has much room for growth by integrating expertise from biochemical reaction networks to ecological interactions. For example, biochemistry was instrumental for generally understanding dominance in heterozygous genes (Kacser and Burns, 1981); now more detailed, dynamic models could explain patterns observed in specific genes. Population genetics can be abstract, ignoring many details (Haldane, 1964), yet its core abstractions are powerful enough for mechanistically connecting intra-organism and trans-organism biology and their environments with the phylogenetic patterns they govern together (see **Table 1**). The power of these abstractions places population genetics in a remarkably central role in biology and makes it very difficult to ignore whenever populations of replicating organisms are involved.

This article points to various systems approaches in biology and how they connect to population genetics, starting with Current Systems Biology (CSysBio) and increasingly important aspects of formal and computational modeling. Section 'Defining EvoSysBio' uses abstractions from population genetics to define EvoSysBio without loss of generality. Section 'Fitness Landscapes' discusses **L**andscapes of **I**ncomplete **F**itness **T**raits (LIFTs), which help to define EvoSysBio and represent the best albeit fractured glimpses of true fitness landscapes that will be available for a long time. The last section presents examples of five important milestones for EvoSysBio.

Current Systems Biology

Systems approaches in biology emerged from a confluence of several broad trends:

(i) Researchers have always known that cellular molecules had a complex context, but this complexity came into focus only after enough details accumulated from studying isolated molecules.
(ii) Increases in computing power inspired developers of quantitative and computational methods in biology, ushering in the era of bioinformatics and genomics.
(iii) As genome sequencing matured, some perceived its data-driven discoveries to lack inspiring transformational hypotheses; this fueled a desire for more hypothesis-driven quantitative models and opened biology up to many physicists and engineers excited about modeling.
(iv) Medical interest in understanding how varied diseases work in humans motivated the Human Genome Project ('*read* the blueprint'), but locating candidate disease genes rarely satisfied curiosity ('*understand* the blueprint'). As differences between genomes were revealed, they stimulated interest in personalizing medicine ('understand *my* blueprint' (Hood *et al.*, 2004)). A theoretical framework could guide the long journey from wishing to actually understanding biology.

These trends supported the rise of CSysBio, which can employ data from genomics and other -omics to construct a more integrated view of molecular interactions, ideally by simulating them in dynamic mechanistic models that yield predictions to be tested by perturbations in high-throughput experiments (Ideker *et al.*, 2001b). It was initially contrasted to (1) a deep-but-narrow focus on functions of isolated molecules, and (2) a broad-but-shallow, data-driven genomic search for candidate genes with functional annotations, but without corresponding mechanistic models (Ideker *et al.*, 2001a). CSysBio started in molecular biology (Westerhoff and Palsson, 2004), and since it has been expanding its scope to include cells, physiology, and more, it still lacks a generally agreed-upon definition. Some see it as a cycle that could characterize *any* system (Kitano, 2002a,b; Ideker *et al.*, 2001a):

(i) collect all details possibly relevant for a given system and research question;
(ii) define a quantitative model with all parts and interactions relevant to the question;
(iii) predict from the model properties of observable real-world perturbations;
(iv) test the model by comparing its predictions to relevant real-world data;
(v) refine the model if the distance between predictions and real-world data is too large (as usual), or expand it by including new questions.

This definition reassuringly echoes the Scientific Method; absence of system types studied, methods applied or questions asked, it is also too general for any field, which risks defining CSysBio only in 'the eye of the beholder.' Despite difficulties of definition and adoption of more thorough systems perspectives (Cornish-Bowden, 2006), CSysBio has been producing some impressive mechanistic models of systems ranging from the molecular to the physiological, including: circadian clocks (Dodd *et al.*, 2005; Huang *et al.*, 2012), metabolic fluxes in microbes (Edwards *et al.*, 2001; King *et al.*, 2015), viral replication (Endy *et al.*, 2000; Lim and Yin, 2009), full intracellular dynamics over a simple cell cycle (Karr *et al.*, 2012), human heart (Noble, 2011), rat physiology (Virtual Rat, see Section Relevant Websites), and more, occasionally even venturing beyond Intra-Organism Biology (IOB) into topics such as evolution.

Current Systems Biology Meets Evolution

Most CSysBio works toward empirically supported models of processes that help genotypes and environments shape phenotypes. However, if CSysBio models include replicating entities with traits that are both heritable and mutable, then these need to be treated as organisms in their own right. Heritable variation of such traits inevitably activates the five factors with all their complicated dynamics. If evolutionary processes occur within multicellular organisms, they are easily overlooked even if they are highly relevant for medicine, such as the evolution of tumor cell populations in cancer (Stearns and Koella, 2008). Similarly, fast evolution of bacteria

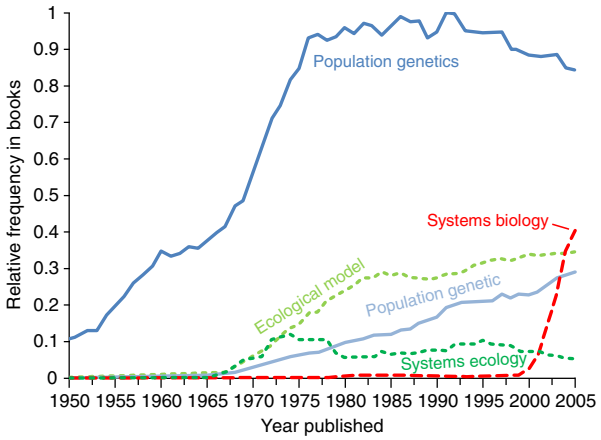

Figure 1 Rise of some terms related to systems approaches in biology as tracked by changes of frequency in books digitized by Google (terms are case insensitive; in relative units of $1 \approx 0.000\,037\%$; all big data cautions apply; Michel *et al.*, 2011). Data credit: https://books.google.com/ngrams.

(Edwards *et al.*, 2001) could be important to understand for industrial production plants.

Understanding population genetics mechanisms helps to recognize evolutionary processes and select the right types of tools and theorems for modeling them. Without some grasp of past population genetics work, researchers risk needlessly re-deriving past results instead of building on them. Trends in Figure 1 might provide rough indicators of this rich history (all caveats about 'big data' apply).

Ecological Systems Biology

CSysBio contrasts with an earlier era of 'systems theory in biology,' which inspired ecologists in the 1970s to build causal models of ecological systems (Wolkenhauer, 2001). As a discipline, ecology has learned important lessons about how to deal with extreme uncertainty and has accumulated statistical expertise in the art of developing knowledge by using empirical tests against real-world data to reduce uncertainty in models of complex systems. CSysBio can learn from Ecology's experience in quantifying uncertainty (Kirk *et al.*, 2015).

Advances in computing and the availability of 'big data' for *some specific* parts of systems provide opportunities for improving models from cells to ecosystems (e.g., high-resolution images inside cells and more (Editorial, 2015; Dietze *et al.*, 2013; Mackechnie *et al.*, 2011; Seksik and Landman, 2015; Sleeman, 2013; Xu and Rhee, 2014; Kuhlbrandt, 2014; Aronson and Rehm, 2015). However, this does not solve all modeling challenges. Integrating big data into mechanistic models can be difficult or infeasible; critical model details sometimes exist only as diffuse and diverse datasets in myriads of formats, and unknown parameters may only be amenable to 'guesstimating.' Such challenges for an integrated understanding can be substantial; appropriate advanced modeling techniques may help, but without special measures it can be surprisingly difficult to reproduce results from increasingly complex computations and statistics (James *et al.*, 2015; Stodden, 2015). These trends matter for complex models of evolution and their corresponding sub-models.

Systems Genetics

The new field of systems genetics builds on genetics, genomics, 'phenomics,' more -omics, CSysBio, and other fields to better integrate insights about how genotypes shape phenotypes in different environments (Hughes, 2010; Markowetz and Boutros, 2015) with huge medical implications (Aronson and Rehm, 2015). It combines into genome-wide association studies (GWAS) as many triplets as possible, each containing an individual genome, respective quantitative phenotypes (molecular and organismal), and a record of environmental conditions that shaped the genotype–phenotype map of these organisms. It aims to obtain integrated models that highlight which genes have probably been affected by selection on organisms with a specified phenotype in a given environment. Advanced statistical techniques used in GWAS provide empirical estimates about how much a given gene might have contributed to a particular trait of interest, thus identifying candidate genes that may cause important phenotypic functions. Systems genetics methods work because patterns of DNA sequence diversity contain information on the strength of the environment's selection of certain phenotypic traits, which affects the survival probabilities of organisms with corresponding mutations. Patterns in modern genomes echo a long history of selection. Examples include studies in the fruit fly *Drosophila melanogaster* (Anholt and Mackay, 2015; Huang *et al.*, 2014; Mackay *et al.*, 2009; Harbison *et al.*, 2009; Ayroles *et al.*, 2009).

Systems genetics provides a different perspective on the complex gene regulatory networks that govern phenotypes, which are also studied in CSysBio; thus in principle, both could combine their strength. For example, many unknowns often exist in detailed, mechanistic models of complex gene regulatory networks that could, in principle, predict phenotypes also amenable to systems genetics. Thus, GWAS may constrain overly large parameter spaces in mechanistic models, while CSysBio simulations may add mechanistic details about genetic architecture by highlighting genes with effects too small to detect in GWAS.

Integration

Better understanding evolution weakens boundaries between diverse systems approaches in biology, as evolution is its biggest system. Molecular systems biology can only study systems shaped by evolution (excludes extinct species), while systems ecology and evolution critically depend on biochemistry; systems genetics relies on all to shape the statistical patterns of genetic diversity in populations, and initiatives in 'personalized precision medicine' aiming to predict clinical consequences of genetic variants (Aronson and Rehm, 2015) will hardly be able to do so without computational models (Iyengar *et al.*, 2015), which will both inform and be informed by a profound understanding of evolution (Stearns and Koella, 2008). Continuing progress on this integrative trajectory increasingly depends on modeling skills and leads to the mechanistic view of EvoSysBio discussed in the next section.

Systems Science: The Whole is Different from the Sum of Its Parts

The realization that a system as a whole can be very different from a heap of its parts is at least as old as Aristotle (-344 ± 22). It also has long guided Gestalt theory, arguably an early form of systems science (Wagemans *et al.*, 2012b) used to study sensory perception (Wagemans *et al.*, 2012a), which undoubtedly affects the evolution of species reliant on learned mental models for navigating their world. Investigating how elements combine into a 'Gestalt' also inspired the thought that – at least in principle – the whole world could be understood with a single mathematical equation, albeit of extravagant composite complexity (von Ehrenfels, 1890, p. 292).

Computational models in systems science essentially provide such 'world-equations,' if only for the small, closed worlds described in the composite formal structures called 'programs.' Modeling investigates the whole by studying relevant parts, their interactions, and systemic properties. Good models usually start with a curiosity, informal questions, and a hunch. They grow as researchers integrate more observations and are eventually formalized as small, computable worlds that mimic relevant interactions of parts and the emergent properties of their whole. The main results of systems science are empirically tested models that become more valuable as they survive increasingly difficult challenges to mechanistically predict non-trivial phenomena. To develop such models, it is essential to actually look at the whole repeatedly and from different perspectives, which seems to be challenging even for systems biologists (Cornish-Bowden, 2006). It helps to know what types of systems others have already seen and which models were developed as a result.

Types of models can be classified in many broad categories using various criteria, such as their approach to randomness in the system, how they are computed, which values they allow, etc.; the myriad modeling approaches needed for understanding evolution could easily fill an encyclopedia the size of this one (and indeed fill many pages in this Encyclopedia too). Examples include approaches that are

- *deterministic*: assumes no chance exists, so recomputing always produces the same result;
- *stochastic*: chance exists, so recomputing always produces variable outcomes as computers use random number generators to choose between possible outcomes;
- *probabilistic*: effects of chance are modeled as probability distributions that can form complicated networks, which can be analyzed deterministically or stochastically;
- *equilibrium*: no effective changes over time can be observed, for example, in steady-state balance;
- *nonequilibrium*: the system is dynamic and changes over time.

Additional incomplete and overlapping classifications of modeling approaches may serve as search terms, illustrating model diversity and jumpstarting further investigations for readers: backwards in time (e.g., coalescent models) ⇆ forwards in time (e.g., individual-based simulations); analytic ⇆ numeric ⇆ computational; binary ⇆ integer ⇆ continuous; constrained by input ⇆ output ⇆ equations; mechanistic ⇆ descriptive; absence of submodels ⇆ multilevel nesting; linear ⇆ nonlinear; time-based ⇆ event-based ⇆ static; graph-based ⇆ matrix-based ⇆ agent-based ⇆ logic-based (see Zeigler, 2012; Thiele *et al.*, 2012; E, 2011; Grimm and Railsback, 2005; Zeigler *et al.*, 2000; Law and Kelton, 2000). There are many more computer programming paradigms that also expand the list. In fact, Gödel's incompleteness theorem of mathematical formalisms suggests that infinitely many modeling and programming approaches exist, each of which is extremely powerful. Many different such approaches need to come together to enable the modeling of nested multi-scale evolution of diverse organisms from a rigorous probability theory perspective. We are very far from this goal; to make progress, we need a better grasp of the limits of various modeling approaches and how they might complement each other.

After a eureka experience of realizing how many questions could be answered by just one approach, researchers can be tempted to ignore its limits. However, even if the approach is a great hammer, not every problem is a nail. Ignoring these limits is dangerous when software assuming one model is applied to data for which the assumed model does not hold. This is particularly true for the many tools that do not automatically check for such problems (contributing much to the current reproducibility crisis). Biologists familiar with a broader range of modeling approaches are in a better position to choose a more reasonable approach for their biological question.

Model-building for any particular biological system is more efficient when researchers (1) have clear questions for their model, (2) can choose from a broad range of approaches to construct models, and (3) are well supported by corresponding tools that help to quantify uncertainty, detect modeling errors, and document results. Most approaches worth learning mirror *certain* modeling problems in biology extremely well and reasonably approximate others; however, all fail for *some* real-world systems, either due to unreasonable assumptions or computational intractability.

For any complex real-world problem, even the best models will always differ from reality and easily become misleading if interpreted from perspectives that violate their simplifying assumptions. Hence, the conclusion of the famous statistician George Box: "*All models are wrong, but some are useful*" (see Box and Draper, 2007, p. 414). Such usefulness either increases our mechanistic understanding or translates into success at prediction challenges in the real-world, where models are tested against empirical data. Estimating parameters for many biological models is a substantial challenge: the sloppy parameter sensitivities make it impossible to predict the importance of a given parameter without simulation, even in small nonlinear CSysBio models (Gutenkunst *et al.*, 2007). Difficulties compound for whole-cell models (Karr *et al.*, 2015), which suffer from the curse of dimensionality as the number of parameters increases. Aiming for reasonable reliability, complex models in CSysBio and elsewhere *must* quantify their uncertainty (Kirk *et al.*, 2015) and may want to manage their error budget (Parysow *et al.*, 2000). It is important for the reproducibility at the heart of science to avoid illusionary precision (Stodden, 2015). A combination of reasonable models and efficient tools can vastly expand a biologist's thinking capabilities and will be essential for reliable EvoSysBio analyses discussed below.

Systems Approaches Are Young

Systems modeling, big data and computational science (e.g., HPC university, see Section Relevant Websites) are young and lack the centuries of aggregated experience in weeding out unreliable results – unlike mathematics, physics, chemistry, and biology. The beginning of the previous section illustrates many problems in computational science simply by attempting to referencing Aristotle in the usual style: missing data (surname), data beyond an unreasonably narrow range (BCE), and uncertainty (year ± 22); if this were input for a scientific simulation, chances are that nobody would realize the hidden bias it introduces unless the model 'misbehaves' and produces 'unreasonable' results (as judged by competent, but not infallible experts).

The biggest challenge of the field is to improve the quality of models so they (1) describe causality chains that connect real-world input and assumptions to real-world output and conclusions in a reasonable way, (2) are complete, clear, and contradiction-free, (3) are readable and well documented for semantic reproducibility, (4) accurately quantify uncertainty of all claims for statistical reproducibility and explicitly define limits of applicability, (5) continually integrate new evidence as it becomes available, (6) run reproducible tests to reject claims that are not compatible with increasingly accurate observations, and (7) automatically trigger updates of other models that build on its output.

Reasonable models satisfy (1)–(4) for the data and evidence available at construction, but need (5)–(7) to *activate* them. State-of-the-art modeling is advanced enough to inspire such noble goals, but current model reliability is often much less clear. Much time, effort and organization will be required before such high-quality models can become standard in biology (Macklin *et al.*, 2014). Progress might be accelerated by dropping the principally impossible aims to 'validate' or 'verify' models of the natural world, which is not a closed system (Oreskes *et al.*, 1994). 'Validation' could more likely stifle further testing than encourage curious critical exploration of the 'valid.' The reliability of a model can be measured by its ability to withstand diverse and difficult tests that contrast model claims with the real world.

Questions are keys, models are locks. The key to assessing the reliability of a model is in the questions that motivated building it and that defined its purpose. This insight is both simple and profound. It is easy to see that the street map of a city (a type of model) does not help determine geological strata (wrong question for this model). It usually takes much more experience to identify similar model-data mismatches in 'big data' collections, which can be analyzed using unreasonable models to generate irreproducible results (Stodden, 2015). Hence remembering the question and relevant transformations is essential for avoiding results like '42,' also known as the answer to everything (Adams, 1979).

Questions that drive models are also essential for determining the level of details to include, which can be increased (*fine-graining*) or decreased (*coarse-graining*) in almost all cases. These decisions determine required parameters to be measured, indirectly estimated, collected from the literature, or imported from appropriate databases (such as BrendaEnzymes for kinetic parameters or BioNumbers for cell biology, see Section Relevant Websites). Much of the value of the abstractions developed by the New Evolutionary Synthesis since the 1920s is in their ability to guide coarse-graining and fine-graining across vast parts of biology. The formal EvoSysBio framework discussed below further refines this process.

Optimistically, and with some organization and standardization, the transition toward reliable computational modeling in biology will require far less time than the centuries it took to move from alchemy to chemistry. EvoSysBio could help catalyze these efforts, and the definitions of IOB, EOB, PGB, and TOB given in **Table 1** could be a step on the way.

Defining EvoSysBio

EvoSysBio, in its fullest form requires a union of insights from three very broad areas of research: evolutionary research (studies replicators, sometimes beyond biology), systems science (studies dynamic things consisting of parts), and biology (studies all aspects of carbon based life). Without such a union of insights and matching computational tools, progress in EvoSysBio remains a distant dream or a daunting yet diffuse challenge. This integrative reading of EvoSysBio reflects its trans-disciplinary mission to build bridges between many diverse disciplines, making it both extraordinarily broad and deep.

However, English syntax interprets combined terms as intersections, so that 'EvoSysBio' is less general than 'SysBio,' which already specializes 'Bio,' just as in 'House Keys.' This reading might see EvoSysBio as a highly specialized add-on with narrow applicability, a view hardly appropriate on principal grounds: virtually every 'thing' studied in biology can also be studied with the tools of systems science and has also been evolving or playing a role in evolution. Thus, EvoSysBio represents a panoramic perspective that matters for quantifying change over time in systems with self-replication.

This astonishing breadth of EvoSysBio implies that its results can depend on preceding breakthroughs in far-flung fields of evolution, systems biology, computing, and other disciplines, often developed by researchers without interests in EvoSysBio, evolution, or related topics. Once fully grown, EvoSysBio will be more keystone than add-on, as it brings together various tall pillars of evidence quantified with enough precision to meet in one place, essentially stabilizing the enormously complex structure of biology as a discipline.

Thus, EvoSysBio is much more than the occasional application of evolutionary insights in CSysBio or the occasional inclusion of metabolic network analyses in evolutionary genetics (see, e.g., Klipp *et al.*, 2009; Voit, 2013; Caetano-Anolles, 2010; Walhout *et al.*, 2013). Different attempts have been made to define EvoSysBio (Loewe, 2009, 2012; O'Malley, 2012; O'Malley and Soyer, 2012; Soyer and O'Malley, 2013; O'Malley *et al.*, 2015). To help clarify its contributions, the definition of EvoSysBio below integrates previous definitions and provides formal abstractions that can facilitate quantitative connections to the diverse, descriptive, experimental, and theoretical contributions necessary for building the rational models essential to EvoSysBio. Since EvoSysBio's core goal is to explain how evolution works mechanistically, it inherits the astonishing integrative abilities of evolutionary theory.

Table 1 Relationships among different focuses in biology

Focus of study	*Relationship to other focuses*
Intra-Organism Biology (IOB) *intra*, Latin for 'inside.' Relevant disciplines: *Biochemistry, molecular biology, gene regulation network biology, cell biology, cancer biology, developmental biology, physiology, neurobiology, and more.*	Focuses on actions, reactions, processes, parts, and patterns within a single, individual organism that enable it to live (grow, survive, reproduce, move, etc.) by changing its state in a given environment. IOB models simplify TOB models by assuming that genotypes and environmental interactions only matter where explicitly specified for a given individual organism and the nested organisms it may contain (e.g., gut bacteria, parasites, cancer cells). IOB details depend on environments; if the latter are known, then IOB allows in principle the prediction of all relevant fitness traits by constructing a complete Fitness Causality Network (see **Table 2**).
Extra-Organism Biology (EOB) *extra*, Latin for 'outside.' Relevant disciplines: *Ecology, biogeochemistry, climate science, population ecology, community ecology, ecosystem ecology, behavioral ecology, cognitive ecology, social ecology, and more.*	Focuses on actions, reactions, processes, parts, and patterns in ecosystems that alter states of individual organisms by interacting with biotic or abiotic environments. EOB models simplify PGB models by explicitly listing all relevant genotypic differences and assuming that no others matter. EOB models implicitly simplify IOB models by providing corresponding rates of interaction between individual organisms of the same or different types, even if these interactions are ultimately governed by biochemistry. Nested organisms have nested EOB ecosystems.
Population-Genetics Biology (PGB) *genetikos*, Classic Greek for 'generative' *γένεσις*, Classic Greek for 'origin.' Relevant disciplines: *Population genetics of single and multiple loci, quantitative genetics, breeding, coalescent theory, population genomics, population epigenetics, and more.*	Focuses on heritable information (genotypes, alleles, traits, methylation patterns, etc.) that can directly or indirectly affect fitness traits like survival and reproduction when passed on by individual organisms in evolving populations. PGB models simplify EOB models by using simple demographics (e.g., constant, exponential growth) to explicitly or implicitly summarize complex ecological mechanisms. PGB models also simplify IOB models to approximate genotype–phenotype-fitness maps for a given environment (e.g., multiplicative fitness models). An extreme focus on counting alleles in a population can turn PGB into efficient but abstract 'Bean-Bag Genetics' (Haldane, 1964). Nesting works as in EOB.
Trans-Organism Biology (TOB) *trans*, Latin for 'beyond' Relevant disciplines: *Evolutionary ecology, conservation biology, coevolution, sociobiology, metagenomics, phylogenetics, and more.*	Focuses on integrating EOB, PGB, and all direct or indirect actions, processes, and patterns that otherwise fall through the disciplinary cracks of EOB or PGB (excluding the inside of organisms). TOB models simplify IOB models. Examples range from the inclusive fitness of relatives (Gardner and West, 2014) to environmental DNA abundances in metagenomics (Wooley *et al.*, 2010) and phylogenetics at any nesting level (Felsenstein, 2004) or genotype by environment interactions (Pavlicev and Wagner, 2015). Nesting works as in EOB.
Evolutionary Systems Biology (EvoSysBio) Integrating 'keystone,' not 'add-on' Relevant disciplines: *All of above and more, including data science, computational modeling, network biology, information management, integrative biology or just 'biology'*	As a field, EvoSysBio has so far mostly used various informal definitions, which are not broader than the more formal definition given in the main text. *Formal EvoSysBio*: focuses on developing more reliable real-world 'flight-simulators for fitness landscapes' by integrating IOB and TOB using the five fundamental factors of evolution. Formal EvoSysBio drops the problematic aim to *directly* quantify the complex multi-dimensional dynamics of real-world fitness landscapes. Instead, it progresses by constructing **F**itness **C**ausality **Net**works (FCNets), which quantify and connect different types of **L**andscapes of **I**ncomplete **F**itness **T**raits (LIFTs; see **Tables 2** and **3**); formal EvoSysBio can then simulate evolving populations governed by dynamic, mechanistic IFT predictions from these FCNets. *Informal EvoSysBio:* builds bridges between IOB ('Current Systems Biology') and TOB ('Evolutionary Biology'). Even if using different terminology and not motivated by fitness landscapes, informal EvoSysBio results often contribute toward a better understanding of details that might directly or indirectly help to better quantify LIFTs.

Notes: The different interests of biologists often distinguish their focuses when studying organisms (see Table 2 for definitions). To create models relevant for their focus, researchers often simplify the models created by peers with other interests. EvoSysBio aims to integrate Intra-Organism and Trans-Organism Biology by using the five fundamental factors of evolution as shared abstractions to facilitate the construction of fitness landscapes. Other biological disciplines provide integration from different perspectives, for example, those of focal organisms (e.g., botany, virology, zoology), habitats (e.g., limnology), problems (e.g., cancer biology), organizational levels (e.g., cell biology), disciplines (e.g., biophysics), and more; but it is difficult to find biological theories more general than those evolutionary theories that enable, in principle, the construction of appropriately quantified fitness landscapes, thereby requiring the integration of vast amounts of biological results (Figure 2).

This integrative power can best be understood by reviewing how the definition of EvoSysBio developed in the past decade.

Partial Approaches

CSysBio has produced many analyses of molecular interaction networks; accordingly, 'evolutionary systems biology' has been used from the very beginning to denote the comparison of such molecular networks among different biological species (Stearns *et al.*, 2003; Medina, 2005). Such use might appear to contrast with more mechanistic frameworks (e.g., Loewe, 2009), which build on simulations that directly or indirectly include network interactions along with many other details affecting a system's dynamics. Network analyses often need only small subsets of the data required by corresponding mechanistic simulations. Therefore, collecting more data on the same system can, in principle, provide a common basis for using both approaches to ask the shared question of how life evolves. It seems unnecessarily confusing to fragment EvoSysBio into many different sub-definitions; one focusing on *network analysis*, another for *mechanistic simulations*, and many more for *different methodological approaches* (e.g., flux-balance-analysis (Ibarra *et al.*, 2002; Papp *et al.*, 2011), metabolic control theory (Kacser and Burns, 1981; Keightley, 1996), systems genetics (Markowetz and Boutros, 2015), etc.), others for *different biological levels of organization* (e.g., molecular functions (Dean and Thornton, 2007), cells (Lynch *et al.*, 2014), epigenetics (Hallgrimsson and Hall, 2011), development (Carroll, 2008), ecology (Pelletier *et al.*, 2009), etc.), and others for *different biological questions* (e.g., energetics (Watt, 1985), modularity (Wagner *et al.*, 2007), robustness (Payne and Wagner, 2014), game strategies (Pacheco *et al.*, 2014; Bohl *et al.*, 2014; Hummert *et al.*, 2014), etc.), or even focusing on *different perspectives provided by the five fundamental factors*, mutation (e.g., Loewe and Hill, 2010a,b), selection (e.g., Okasha, 2006), genetic drift (e.g., Lynch, 2007), recombination (e.g., Charlesworth *et al.*, 2009), and space (e.g., Westervelt and Cohen, 2012). All these contribute different important aspects to EvoSysBio, but where would the splintering stop?

Darwin and subsequent evolutionary geneticists have convincingly demonstrated the abstract elegance and unity of evolution based on the five fundamental factors. They demonstrated, in principle and with many examples, how these factors combined into a mechanism powerful enough to create the bewildering diversity of species and biological phenomena just by variations in the patterns of strengths of its fundamental factors. Ideally, the conceptual beauty of evolutionary genetics at the core of EvoSysBio will enable different important lines of EvoSysBio inquiry to sharpen evolutionary theory by extending a single consistent set of abstractions, which will make it easier for future researchers to build on its foundation. By comparison, defining different vital aspects of EvoSysBio in conflicting but partially overlapping terms seems less desirable if no clear conceptual integration can be provided.

Thus, the question of splintering EvoSysBio is related to the question of whether it is essential to extend evolutionary theory in any unusual way, for example, to integrate TOB interactions between genotypes and environments (Laland *et al.*, 2014). There is no doubt that evolutionary theory needs to be extended in many ways or else research would stop, but it seems unnecessary to extend it in any *unusual* way beyond its continued extension 'through relentless synthesis' (Wray *et al.*, 2014). EvoSysBio can add to this relentless New Synthesis in many ways, ideally by quantifying aspects that are necessary for mechanistically predicting evolution, one of the highest goals in evolutionary biology since Fisher, Wright, and Haldane laid the groundwork for the New Evolutionary Synthesis. The wish to avoid the splintering of disciplines has also inspired the wish to reintegrate all biological subdisciplines into a 'New Biology' (National Research Council USA, 2009).

Pragmatic Definitions

EvoSysBio has been defined as an effort to build bridges between CSysBio and evolutionary biology, integrating theoretical tools, experimental methods, and datasets from multiple disciplines into an evolutionary framework (see **Figure 2**; Soyer and O'Malley, 2013; Loewe, 2009). Comparable in breadth to CSysBio, this broad definition easily encompasses the diverse work associated with EvoSysBio (e.g. studies in the volume edited by Soyer (2012) and studies cited by Loewe (2009) and below). However, a more precise definition without loss of generality would be preferred, as it could facilitate creating powerful formal interfaces between EvoSysBio and other fields. These interfaces could help to interpret data collected elsewhere in its bigger evolutionary context. Conceptual advantages like these motivate a continued search for more precise definitions of EvoSysBio.

Formalizing EvoSysBio without Loss of Generality

Indeed, it turns out that EvoSysBio can be defined more precisely without loss of generality by building on the powers of abstraction offered by evolutionary theory. At the heart of this definition of EvoSysBio are evolving populations of *individual replicating organisms*, where evolution is governed by their *fitness landscapes*, a metaphor for mathematical causality statements about the traits of evolving populations in an abstract space with very many dimensions (see **Table 2** and Section Fitness Landscapes for more details).

To give EvoSysBio something to study, individual organisms have to exist and be near-perfect replicators of genotypic information, which affects phenotypic fitness traits like survival and reproduction in finite discrete populations. Hence, in these organisms all five fundamental factors of evolution can be active. This generic view includes viruses, microbes, and multicellular organisms (nested replicators), but also a fringe with exotic systems such as ribozymes (Martin *et al.*, 2015), prions (Li *et al.*, 2010), and data structures in computers (as exploited in biological individual-based simulations (Grimm and Railsback, 2005), evolutionary computation (De Jong, 2006), and artificial life studies (Adami, 1998)). Using the abstractions of evolutionary theory enables simulated populations with appropriately chosen parameters to closely mimic natural populations, facilitating their study. Organisms defined in this general way make it possible to see EvoSysBio as an integrating bridge between the great quantitative traditions of modern biology that complement each other through

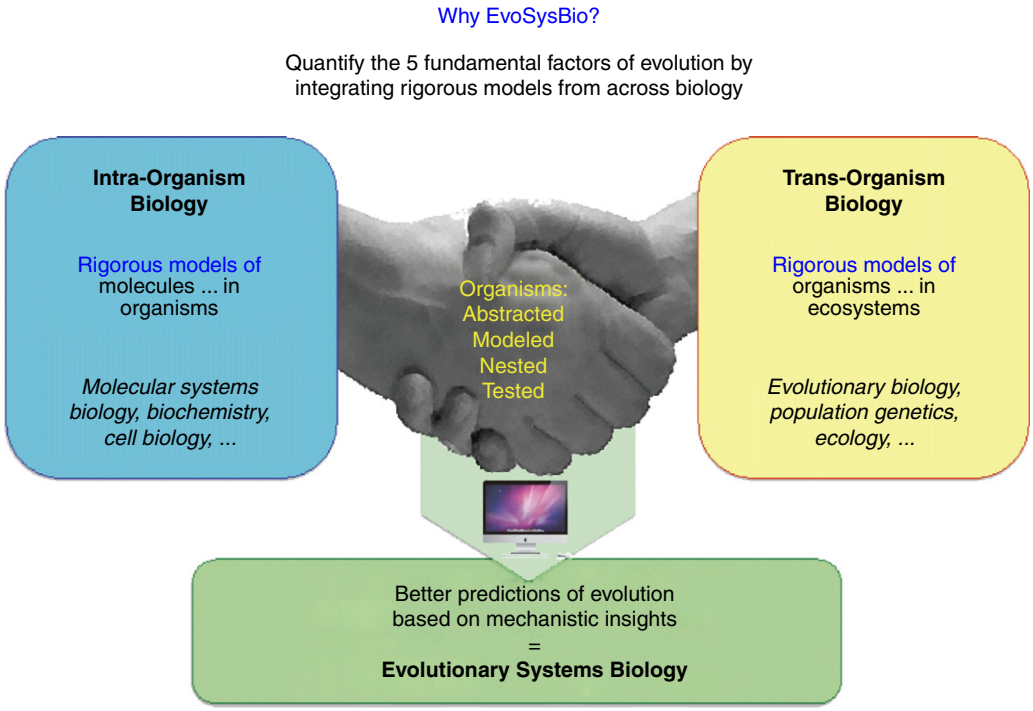

Figure 2 Overview of EvoSysBio integration. Traditionally, models from Intra-Organism Biology (IOB) and Trans-Organism Biology (TOB) have ignored each other to simplify rigorous modeling in their respective domains. This separation of concerns is facilitated by powerful abstractions such as the fitness of individual organisms and the five fundamental factors of evolution. When this separation fails at the cutting-edge of research, EvoSysBio offers a reversal that uses the same abstractions to bond branches of biology together. By measuring fitness traits of individual organisms and their contributions to the five factors, EvoSysBio enables asking new trans-disciplinary questions in addition to rigorously quantifying fitness landscapes. Fitness is either affected by – or itself affects – almost everything in biology, and 'nonquantitative' biology often breaks the conceptual ground for more precise studies; such observations have suggested: nothing in biology makes sense except *when properly quantified* in the light of evolution (Dobzhansky, 1973; Loewe, 2009). Picture credits: © Laurence Loewe (2015a), reuse under CC-BY 4.0, updated from previous versions (Loewe, 2009, 2012).

abstractions pioneered in population genetics (see **Table 1** and **Figure 2**):

(i) **I**ntra-**O**rganism **B**iology (IOB): biochemistry, molecular-, cell-, … -biology, … physiology; organisms may be nested (e.g., mitochondria in cells in humans; similarly gut bacteria, parasites, cancer cells, etc.). If nested, the IOB of the containing organism provides an environment for the smaller contained organisms, all of which come with their own IOB and TOB.

(ii) **T**rans-**O**rganism **B**iology (TOB): includes **E**xtra-**O**rganism **B**iology (EOB) disciplines such as ecology, **P**opulation **G**enetics **B**iology (PGB), the complementary genomics disciplines, and many diverse genotype-environment interactions (Pavlicev and Wagner, 2015). Nesting applies here too.

Over many decades, these traditions have produced outstanding empirical results and rigorous theory in their respective areas by using evolutionary fitness as a powerful abstraction for enabling an efficient separation of concerns. Both remain far from reaching their long-term goals: research in organisms ultimately aims to predict fitness and other phenotypic properties from genotypes (usually simplifying models of environmental changes), while population research ultimately aims to predict how populations evolve under a given set of environmental conditions (usually simplifying models of **IOB** to just a few numbers, such as fitness, epistasis, etc.). This mutual exclusion of broad areas of biology has enabled much progress, but the simplifications have started to break down for cutting-edge research. EvoSysBio can provide more detailed and explicit views of the relevant abstractions to facilitate more powerful evolutionary hypotheses that combine the strengths of both traditions. The elegance of these abstractions and the separation of concerns they enable between IOB and TOB are well illustrated by fitness landscapes, which provide a powerful intuition for explaining how evolution works (see Section Fitness Landscapes).

Based on these considerations and previous discussions (Loewe, 2009; National Research Council USA, 2009; Loewe, 2012; O'Malley and Soyer, 2012; Soyer, 2012; Calvert, 2012; Soyer and O'Malley, 2013; O'Malley, 2012; O'Malley *et al.*, 2015; Loewe *et al.*, 2015–2009), EvoSysBio can be defined in a way that leverages the integrative capabilities of fitness landscapes:

EvoSysBio is a trans-disciplinary **framework for constructing** reliable, testable, interactive **overviews of** nestable, dynamic, multi-dimensional **fitness landscapes**, **which mechanistically predict:** (i) **changes in fitness** of individual organisms when their states and environments change; (ii) **how populations evolve** when organisms traverse fitness landscapes.

This definition derives its formal power from IOB and TOB models of how individual organisms and populations change over time, respectively. These models can be efficient analytic summaries or approximations or computational models of much more complex underlying Continuous Time Markov Chains; they may require simulations to explore them. Reviewing relevant TOB theories (see e.g., Crow and Kimura, 1970, 2009; Charlesworth and Charlesworth, 2010; Kirkpatrick *et al.*, 2002; this Encyclopedia) and IOB theories (see e.g., Gillespie, 2007; Gillespie *et al.*, 2013; Klipp *et al.*, 2009; Gutenkunst *et al.*, 2007; Karr *et al.*, 2015; Kirk *et al.*, 2015; Section Systems Science: The Whole is Different from the Sum of Its Parts; **Table 3**) is beyond the scope of this article.

Informally, EvoSysBio and other work contributing to relevant computational models and appropriate visualizations might also be summarized as:

> EvoSysBio aims to improve the real-world reliability of 'flight-simulators for multidimensional fitness landscapes.'

This analogy is easily adapted to multi-dimensional data analyses, where researchers interactively explore interesting local features while relevant data is automatically selected, summarized, and presented by computers (as if 'flying' over the data; by comparison, manual coding for static snapshots feels 'pedestrian'). This requires a deep computational toolchain that integrates many diverse results extremely well; the approach thus enables EvoSysBio to leverage the full integrative power of *real-world* fitness landscapes, which are conceptually as powerful as evolution itself – known as the only theory general enough to unify biology.

In practice, this elegance comes at the steep price of requiring a deep mechanistic understanding of *real-world* fitness landscapes, an extraordinary challenge as relevant modeling expertise is often lacking in biology, statistics, formal modeling research, and more (see end of Section Systems Approaches to Genome Evolution).

This definition of EvoSysBio encourages developing more precise ways of defining fitness landscapes (see **Tables 2** and **3** and Loewe, 2009, 2012) to reduce the confusion surrounding the exact nature of these landscapes.

Fitness Landscapes

Sewall Wright (1932) introduced the now well-known concept of fitness landscapes to provide an intuitive understanding of how evolution works. These landscapes have been defined in various ways and labeled by different names, including adaptive landscapes, surfaces of selective value, and seascapes (Svensson and Calsbeek, 2012; Gavrilets, 2004; Loewe, 2009; Mustonen and Lassig, 2009). In statistics, similar landscapes have been described as response surfaces (Box and Draper, 2007).

Intuition behind Fitness Landscapes

Fitness landscapes provide a strong intuitive analogy for how evolution works. They are based on the familiar experience of walking in a hilly landscape. By representing potential genotypic states as points in a **plane** with fitness as the **height**, it becomes easy to see how evolution works:

> In a fitness landscape, *populations* evolve by moving toward higher (fitter) points whenever such points can be reached by the stochastic movement of *organisms* between positions in the plane (e.g., by mutation to other genotypic states); organisms tend to accumulate at these positions (genotypes) because there they are more likely to survive or reproduce (selection).

The strength of selection (selection coefficients) is governed by an organism's life history of survival and reproduction in comparison to other organisms in the same population. Selection and population size together govern the probabilities that the genotypes existing in one generation make it into the next, but these factors do not change genotypes (which is done by mutation and recombination). Thus, parents at higher points in the landscape have higher probabilities of generating offspring that also live at these higher points: the population evolves (see movie in **Figure 3**, Movie 1).

At a very high level, this intuition correctly captures the essence of what the complicated evolutionary mechanisms are all about: explaining why organisms are likely to exist in states of higher fitness. This intuitive nature of fitness landscapes has captured the imagination of many interested in evolution, from popular science writers to professional evolutionary biologists (Svensson and Calsbeek, 2012; Gavrilets, 2004; Loewe, 2009). Taken to one extreme, it leads to adaptationist interpretations of evolution that view populations *always* at the top of some adaptive peak and ignore the exceptions to the rule (e.g., quasi-species populations of RNA-viruses mutate at such high rates that they form 'bands around peaks' (Biebricher and Eigen, 2006); see **Figure 3**). Taken to another extreme, fitness landscapes become neutral networks, where all genotypes share the same fitness (Kimura, 1983).

Criticisms and Limitations

Critics of fitness landscapes as a concept highlight (1) the frequent lack of precise definitions, (2) its abstract nature, which does not easily lend itself to directly proposing new experiments, and (3) general human difficulties with navigating multi-dimensional spaces (Kaplan, 2008).

Little can be done about the limits of human imagination, which often fails when attempting to translate the multi-dimensional planes and heights of fitness landscapes into more familiar spatial dimensions. Different useful visualizations have been developed for highlighting various aspects of fitness landscapes (McCandlish, 2011), yet it is difficult to convey an undistorted picture. Visualizing landscapes from larger EvoSysBio projects may require interactivity, but appropriate 'flight simulators' are missing. These problems have not prevented numerous speculative drawings, which are prone to misinterpretation. It might be beneficial for the field to label them accordingly to contrast them with *attempts* that precisely define and measure the much less comprehensive real-world LIFTs (see below and **Figure 4**).

Table 2 Concepts related to fitness landscapes

Concept	*Description*
Fitness Landscape Ultimate EvoSysBio goal	An abstract 'landscape' defined by causal 'positions in a plane' and their consequential 'heights' as defined by a corresponding FCNet, mapping genotypic traits to IFTs (see below). Each position in a plane describes a potential causal state of an individual organism and its given environment; both govern its height(s). Fitness landscapes are near-impossible to measure, compute, or visualize, since both planes and heights often have very many dimensions and their 'points' often turn into fuzzy distributions due to stochasticity. Nevertheless, Evolutionary biology studies them due to their importance (even if resorting to MOCA-LIFTs, see below), and EvoSysBio formalizes these studies to make them more efficient.
Fitness Causality Network FCNets define fitness Landscapes	A network of nodes (data) and links (functions), together describing causal influences on consequential IFTs over a given time period. Causal influences include genotypes, environments, and initial states (e.g., maternal methylation patterns of DNA). Nodes in FCNets are defined by IFTs and links between nodes by LIFTs. A complete FCNet of a single organism links all LIFTs (see **Table 3**) relevant for predicting the fitness consequences of mutations and environmental changes.
Landscape of Incomplete Fitness Traits LIFT, LIFTs are always context specific	An abstract representation of a fitness landscape that maps causal IFTs to consequential IFTs, thus providing a causality statement about how input governs output, comparable to probabilistic mathematical functions. Inputs are distributions of causal states of organisms ('positions in a plane,' e.g., mutations), and outputs are distributions of consequential IFTs ('local heights,' e.g., survival, reproduction); both only apply to a given context and time duration. IFT designations 'causal' and 'consequential' are relative to a LIFT, sometimes indicated by adding 'more' to distinguish 'more causal IFTs' from the 'most causal IFTs' (e.g., DNA). If complete and rigorous, these representations of fitness landscapes can be viewed as existence probability theorems that facilitate predictions of the probability that a given number of organisms will exist in a given set of locations of the plane. Such results are mathematically related to the analysis of Continuous Time Markov-Chains, which is extremely useful for defining and analyzing such fitness landscapes. This technical aspect makes LIFTs markedly different from the MOCA-LIFTs often used for illustration (see below). The strength of LIFTs is in their simplicity, which enables precise measurements and simulations. See **Figures 7** and **8** for examples.
Incomplete Fitness Trait IFTs never quantify fitness in full	A phenotypic trait that impacts aspects of the fitness of an organism, its offspring, or its genetic relatives. Traits are IFTs when they, at least occasionally, affect rates of survival, reproduction, merging, etc., or when they modify evolutionary factors (mutation, migration, etc.) in one or more environments, at least to a small degree.
Organism Single individual replicating system	A single, individual system that consists of different parts that builds a whole, which – in biology – must be able to replicate and may be nestable. Intra-Organism Biology (IOB) investigates how these parts work together. Extra-Organism Biology (EOB) studies how each individual organism interacts with its environment and other organisms of any type. If organisms (i) use hereditary information to grow on environmental resources and (ii) can produce identical or similar descendants, then their populations necessarily evolve as modeled in Population-Genetics Biology (PGB). Remaining indirect interactions are captured by Trans-Organism Biology (TOB), which also integrates EOB and PGB at its level of replication (but not IOB). Note the simplification when a huge nested stack of replicators is denoted as a 'single multi-cellular organism.'
Nesting (in Organisms) Organisms in organisms	A nest organism is a single, individual organism that contains one or more populations of different types of nested organisms, which replicate(d) by themselves. A nested organism is a single, individual organism encapsulated by a larger nest organism, which is the smaller nested organism's environment. Organisms can be both nest and nested organisms at the same time. Examples of nesting include mitochondria in cells in multi-cellular organisms, microbes in the gut, viruses in cells, parasites in hosts, cancer in patients, and more. In each case, the smaller contained ('nested') organism is nested in the larger containing ('nest') organism. The Trans-Organism Biology (TOB) of evolving populations of nested organisms and the Intra-Organism Biology (IOB) of the nest organism affect each other, but the IOB of a nest organism has to describe the TOB of its nested organisms well enough to exclude relevant surprises (e.g., evolution of tumors). Each population of nested organisms needs its own EvoSysBio analysis, where the IOB of the nest organism plays the role of the ecological environment in the TOB of the nested organism. The complex TOB of nested organisms can sometimes be simplified by assuming nested populations are homogeneous and incapable of mutation.
MOCA-LIFT 'Instant' LIFT cartoons	**M**assively **O**versimplified **C**artoonish **A**bstract **LIFT**s are essentially like LIFTs, but without much clarity on how their various dimensions map to reality. Their cartoon-like distortions can be caused by complex transformations, lack of data, imprecise definitions and other problems that are often difficult to resolve. The value of a MOCA-LIFT can range from useless speculation to useful stop-gap (capturing some aspects of reality) to ground-breaking 'Gedankenexperient.' While researchers put in the enormous effort to measure more rigorous LIFTs, they will likely construct increasingly realistic MOCA-LIFTs, since their cartoonish nature facilitates smooth transitions from fact-free figures to high-precision result repositories with varying degrees of usefulness. As a rule of thumb: LIFTs that are (i) not fully defined by measurements or simulations, (ii) are not quantified in defined units, and (iii) do not specify their real-world uncertainty are MOCA-LIFTs (see **Figures 3**, **4**, and **6** for examples).

Notes: A fitness landscape provides an intuitive understanding of how complicated evolutionary mechanisms impact the fitness of an organism. To comprehend the complexities of fitness landscapes and use them to predict the fitness consequences of mutational and environmental change, it is important to understand (i) the concept of an organism as it relates to biology, (ii) how researchers use Incomplete Fitness Traits (IFTs) to construct Landscapes of IFTs (LIFTs) that represent different aspects of the fitness landscape of an organism, and (iii) how IFTs and LIFTs form FCNets, which mediate finely tuned trade-offs in response to the environment. Only when complete FCNets are fully quantified is it possible to compute a complete fitness landscape, which can predict the distributions of phenotypes and fitness from an organism's distributions of genotypes and environments. See Table 3 for the types of LIFTs that can collectively bridge the whole gap from DNA to fitness.

Table 3 **Fitness predictions from genotypes** are, in principle, enabled by the types of **L**andscapes of **I**ncomplete **F**itness **T**raits (LIFTs) shown here

PT: Plane Type	→	***LT: Landscape Type***	→	***HT: Height Type***
Compute: Input type	→	Compute: Function type	→	Compute: Output type
FCNet: causal Node type	→	FCNet: Link type	→	FCNet: consequential Node
More causal IFT type	→	*LIFT: Landscape of IFT type*	→	*More consequential IFT type*
Type of multidimensional point in the causal plane of a FCNet node is given by its input data type that governs height as calculated by its LIFT type.	→	*Type of LIFT that represents a mapping function type that accepts a point in its causal plane as input and computes a consequential height as output. LIFTs define types of links between FCNet nodes in a larger FCNet.*	→	*Type of multidimensional point of consequential height that integrates all effects from replication (with all existing nested levels). It is computed as output type by its LIFT from a given input point in its plane type.*
PT7: Organism life history of fitness traits: survival, reproduction, etc.	→	LT7: **Summarizing** statistics of survival, reproduction, etc. for relevant groups of organisms over a given duration of time in a specified environment	→	HT7: Fitness summary statistics for organism genotypes in specified environments per time interval
PT6: Real-world Incomplete Fitness Traits (RIFTs)	→	LT6: **Balancing** trade-offs in IFT networks of organism life-history and physiology	→	HT6: Organism life history: survival, reproduction, etc.
PT5: Simulated Incomplete Fitness Traits (SIFTs)	→	LT5: **Mapping** simulations (*in silico* IFTs) to observed real-world IFTs	→	HT5: Real-world Incomplete Fitness Traits (RIFTs)
PT4: Time-series of phenotypic traits	→	LT4: **Extracting** fitness relevant traits (IFTs) from time-series analyses	→	HT4: Simulated Incomplete Fitness Traits (SIFTs)
PT3: Molecular functions network	→	LT3: **Simulating** dynamic time-series predictions (over multiple scales) for a given initial state in a given environment	→	HT3: Time-series of phenotypic traits
PT2: Molecular structures	→	LT2: **Abstracting** the structural biology of structure–function relationships	→	HT2: Molecular functions network
PT1: Hereditary information	→	LT1: **Folding** of expressed hereditary information	→	HT1: Molecular structures

Note: All LIFTs contribute some aspect to constructing the full **F**itness **C**ausality **Net**work (FCNet) that governs the dynamic distributions of various incomplete fitness traits of an organism. Each FCNet node connects at least two LIFTs (In → Out; see header row for terminology illustrative for different contexts). The fitness of an individual organism depends on the structure of its FCNet and on its genotype (broadly defined as any hereditary material), its environment (abiotic and biotic), its initial state (immediately after being 'produced'), and the time period over which the expected change in organism numbers is to be measured. Real-world examples exist for all LIFT types in this table, albeit spread across different organisms (see Loewe, 2009). Defining a full FCNet for any *single* organism will be a major milestone for EvoSysBio and requires the computational integration of all LIFT types in this table that are required for representing the organism. These LIFTs require many independent studies of Simulated IFTs (SIFTs) and Real-world IFTs measurements (RIFTs). The huge costs and high risks of pursuing this integrative vision are matched by the high rewards of enabling rational, *in silico* analyses of arbitrary mutations. Hence, such EvoSysBio work is at the core of personalizing medicine and reliably predicting evolution.

Fitness Landscapes Map to Real-World Concepts

Criticism of the notorious lack of quantitative and semantic precision in depictions of fitness landscapes does not imply that they are not real or cannot be defined rigorously.

(i) As introduced above, real-world fitness landscapes *are by definition* all possible states of real-world populations of replicating organisms, which *by definition* are subject to the five fundamental factors of evolution that describe how organisms traverse fitness landscapes.

(ii) *Models* of fitness landscapes *aim to approximate* real-world fitness landscapes by choosing features deemed relevant by modelers; thus, human reconstructions of fitness landscapes are *by definition not perfect*.

(iii) The quality of these approximations is determined by *testing* how useful their predictions and/or insights are for navigating their real-world counterparts.

(iv) Neither existence nor quality of these approximations influences real-world fitness landscapes, as long as they do not affect how humans shape their world.

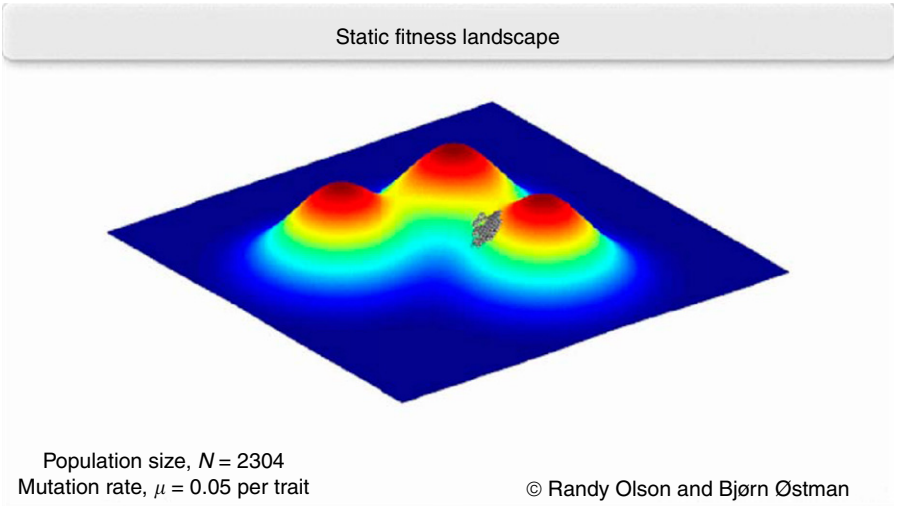

Figure 3 Future EvoSysBio 'flight simulators for fitness landscapes' might provide dynamic overviews of how populations evolve on measured data-rich Landscapes of Incomplete Fitness Traits. This MOCA-LIFT (see **Figure 4**) shows a snapshot from a cartoonish movie of a population that evolves on a static MOCA-LIFT (for more details, see also links in Section Relevant Websites). Picture credits: © Østman and Olson (2014a), reusable under CC-BY-SA 3.0.

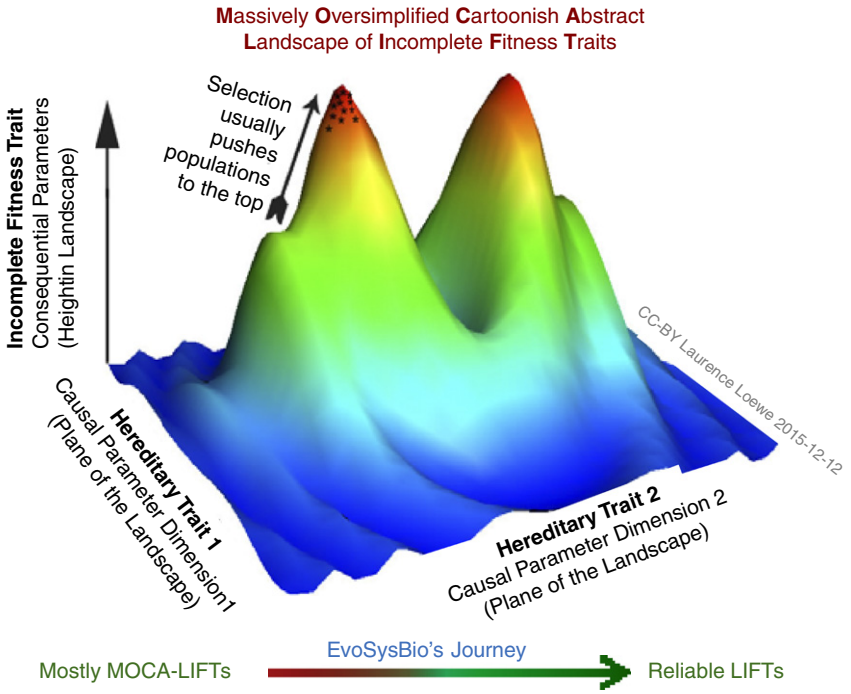

Figure 4 **M**assively **O**versimplified **C**artoonish **A**bstract **L**andscape of **I**ncomplete **F**itness **T**raits. Such a MOCA-LIFT captures the central causality statement of a fitness landscape: each position in the causal plane determines its consequential height. Its value usually ends here, as real-world LIFTs tend to differ substantially. Picture credits: © Laurence Loewe (2015a), reuse under CC-BY 4.0, updated from previous versions (Loewe, 2009, 2012).

(v) Human measuring or managing can severely limit the ability of EvoSysBio to improve model quality if these actions substantially perturb real-world fitness landscapes.

Fitness landscapes play a central role in the EvoSysBio definition because they integrate all fundamental factors of evolution. Quantifying fitness landscapes accurately is a key long-term goal for EvoSysBio (and other systems approaches to genome evolution, even if they have other priorities).

Though compelling, the mechanistic goals of EvoSysBio can be extraordinarily difficult to achieve. In such cases, less-than-ideal fitness landscape definitions can enable important progress. These may (1) *only describe* observed phenomena without requiring explicit rational hypotheses on real-world

causal mechanisms, or (2) are *only system-specific* and so enmeshed with their particular context that abstraction obscures their meaning, or (3) are so *highly abstracted* from the real world that they are essentially void of interpretable biological facts.

How to Avoid Misreading Fitness Landscapes

The following simple principles might help to keep the useful aspects of **fitness** landscapes while reducing the confusion:

1. **Fitness landscapes exist** for all organisms that produce offspring at all nested levels of replication, even if the landscapes are impossibly difficult to define.
2. **Pictures mislead.** A single picture of a fitness landscape is usually misleading, as it can rarely be created without distorting vital views of the underlying model when distilling the potentially many dimensions of a plane and height down to the three dimensions humans are used to.
3. **Uncertainty quantifies accuracy.** As elsewhere, quantifications of fitness landscapes are at most as reliable as the uncertainty quantifications performed for them from diverse perspectives to obtain reliable error bounds. Hence, the qualities of uncertainty measures of a landscape indicate the accuracy with which it was measured.
4. **Fitness has many measures**. The definition of fitness landscapes is intricately linked to the definition of fitness, which has its own challenges. While it first appears easy enough to measure (survival, reproduction), crucial complications appear when the subtle timing and indirect effects of real life are accounted for as well. Life history theory has compiled a substantial body of theory on how multiple traits combine in order to contribute to fitness (Stearns, 1992, 2000; Brommer, 2000; Houle *et al.*, 2011; Gardner, 2015; Gardner and West, 2014).
5. ***Incomplete F*itness *T*raits (IFTs) are easily defined**. The current inability to define or compute *complete* fitness measures does not prevent the definition and measurement of IFTs, which are defined as having at least some probability to impact survival and/or reproduction in at least one known environment (irrespective of whether the traits are continuous or not). Defining such IFTs is comparatively easy, and predicting them computationally, as well as observing them in the lab, is already possible for some IFTs. Thus, researchers can accumulate now the observations and prediction capabilities required for later EvoSysBio analyses.

From MOCA-LIFTs to Reasonable LIFTs

Intuitive appeal with difficulties of measurement and visualization have generated many speculative depictions of 'fitness landscapes'; most are probably misleading and deserve a name that distinguishes them from real-world measurements. Figure 4 shows such an entirely fact-free ***M*assively *O*versimplified *C*artoonish *A*bstract *L*andscape of *I*ncomplete *F*itness *T*raits**, or in short, a **MOCA-LIFT**:

- *Massively Oversimplified*: the *many* dimensions of plane and height are arbitrarily distilled to one or two dimensions, often without defined mapping or appropriate justification;
- *Cartoonish*: the landscape only captures a causality statement; a lack of real-world data precludes any further statements;
- *Abstract*: plane and height do not represent any biological systems in the real world.

Unless spoiled by 'MOCA properties,' LIFTs represent real-world progress in EvoSysBio, as LIFTs measure, model, simulate, and/or summarize real-world biology in a reproducible way:

- *Landscape*: a function that maps a multi-dimensional point in a plane of *more causal* IFTs (input) to its computable multidimensional height, which is a *more consequential* IFT (output);
- *Incomplete*: the recognition that IFTs are not complete and do not even attempt to be; IFTs may or may not affect other traits or be affected by other traits; all LIFT statements are conditional on 'all else being equal';
- *Fitness*: the statement that some aspect of this trait affects survival, replication, or some other evolutionary factor directly or indirectly at least with a small probability in some environments;
- *Trait*: a type of property of an organism; if traits do not affect fitness, they may be only phenotypic (e.g., gene expression without impact on fitness is irrelevant), or neutral (e.g., DNA sequences never expressed and without any impact on fitness).

It often makes more biological sense to investigate LIFTs than to attempt direct predictions of fitness (which often require abstracting too many complex processes at once, increasing the likelihood of failure). At their core, LIFTs can be thought of as the basic building blocks of fitness landscapes; they provide the most direct link between real-world biology and EvoSysBio simulations, either by interpolating real-world measurements or by simulating known processes. Deliberately ignoring the bigger picture of fitness in favor of simplicity makes basic LIFTs much easier to use and provides two additional conceptual advantages, one experimental and one conceptual.

Experimentally, the artificial nature of many LIFTs can remove them far enough from the finely tuned fitness trade-offs that dominate in the wild; it might thus be easier to find IFT mutants that are measurably different from wild-types, enabling tests of *in silico* prediction quality (see Loewe, 2009; Figure 3 there, read IFT for Candidate Fitness Correlate).

Conceptually, large collections of basic building-block LIFTs may facilitate the construction of **F**itness **C**ausality **Net**works (FCNets, see Table 2), which capture all causal influences that govern the expected distribution of fitness values for an individual organism over a given time interval in a given environment. Table 3 gives an overview of the diverse LIFT types and fitness causality node types that enable, in principle, the construction of a full causality chain, which ranges from DNA to fitness. Biological examples for each particular LIFT type have been given elsewhere (see discussion of Table 2 in Loewe, 2009) and details of linking them into full FCNets are beyond the scope of this article.

An alternative to mechanistic predictions of LIFT networks is to observe evolution by empirically measuring the speed at which different genotypes grow in a given environment. Such direct IFT measurements can be conducted for each individual genotype, as has been done for bacterial genotypes with respect to their ability to survive antibiotics (see below, EvoSysBio Milestone 5: Antibiotics Resistance Evolution). Several empirically observed LIFTs (Weinreich *et al.*, 2013) have been compiled for visualization with the MAGELLAN tool (Brouillet *et al.*, 2015). These measurements can provide important high-resolution views of very small local LIFT areas, assuming the context of otherwise constant fitness landscapes.

It is also possible to mix empirical observations of IFTs with statistical modeling in order to predict fitness landscapes with very many points from measurements of much fewer genotypes. As more big data sets become available, this approach becomes increasingly powerful, fueling a revival of the fitness landscape paradigm (Schuster, 2012). It has been used to computationally explore the complexity of HIV for *in vitro* LIFTs (Kouyos *et al.*, 2012). Statistical methods and quasi-species theory have also been employed to infer fitness landscapes *in vivo* using sequenced HIV samples from patients (Seifert *et al.*, 2015).

As evolutionary geneticists have struggled with the question of how to best capture relevant glimpses of fitness landscapes, they have developed a number of useful abstractions. Some of these quantify particular aspects of fitness landscapes and build on formalisms, which produce general complex landscapes of different types (Orr, 2005; Gravner *et al.*, 2007). Others analyze fitness landscapes from particular angles, including speciation (Gavrilets, 1997, 2004), game theory (Nowak and Sigmund, 2004), and more (e.g., Svensson and Calsbeek, 2012; Richter and Engelbrecht, 2014). Also, evolutionary geneticists have defined evolutionary parameters that, in principle, could be measured in the real world or be computed from fully known fitness landscapes. These provide excellent summaries of particular aspects of fitness landscapes.

Abstractions for Aspects of Fitness Landscapes

The complexity of multi-dimensional fitness landscapes and the notorious difficulties of exploring them have long been motivating evolutionary biologists to develop concepts that quantify more limited aspects of fitness landscapes, sometimes empirically or without requiring a full understanding. Such incomplete empirical summary statistics of fitness landscapes include:

- *distributions of mutational effects on fitness*: pick a point on the landscape as wildtype starting point, jump into all directions that represent genotype changes from naturally occurring mutations, then compare fitness to observe selection coefficients (e.g., Schenk *et al.*, 2012; Eyre-Walker and Keightley, 2007; Loewe and Charlesworth, 2006; Loewe and Hillston, 2008);
- *epistasis*: interactions between mutations that increase or decrease the effects of additional mutations play a major role in evolution, but nomenclature can be confusing (Wolf *et al.*, 2000; Phillips, 2008; Loewe and Hill, 2010a); epistasis captures the gene-regulatory and biochemical reaction network complexity of IOB (Phillips, 2008), so it is no surprise that higher-order epistasis can result in surprising changes of fitness (Weinreich *et al.*, 2013); it can sometimes be measured (Schenk *et al.*, 2012; Schenk and de Visser, 2013); it can determine the accessibility of certain evolutionary paths (Poelwijk *et al.*, 2007; Weinreich *et al.*, 2006) and affect robustness, even within a protein (Bershtein *et al.*, 2006); epistasis is also important for understanding the evolution of antibiotic resistance (e.g., MacLean *et al.*, 2010; Hall and MacLean, 2011; Schenk *et al.*, 2012; Schenk and de Visser, 2013);
- *robustness*: helps developmental or simpler processes produce patterns that reduce observable changes in phenotypes (e.g., Wagner, 2014, 2012; Payne and Wagner, 2014);
- *fragility* or *capacitance*: is the opposite of *robustness*; increases variability beyond usual amounts (e.g., Bergman and Siegal, 2003).

These measures can be used for investigating the evolvability of a system (e.g., Wagner, 2005) or its mechanisms of adaptation (e.g., Wagner, 2011). More on summary statistics of fitness landscapes can be found elsewhere (see Loewe, 2009 and in this Encyclopedia).

Practical Relevance of EvoSysBio

Ideas for applying EvoSysBio to solve practical problems are easy to conceive. From agriculture to medicine to zoos, replicating organisms are everywhere. For some, replication is desirable (e.g., rare species in zoos), for others not (e.g., cancer cells, agricultural pests, superbugs). Humans can sometimes shape the impact of these organisms as planned by increasing growth of desired organisms and blocking growth of undesired ones. Success usually requires the ability to predict growth with some reliability, which in turn often requires a deeper understanding of evolution, as also needed for computationally exploring potential management decisions and their side effects. EvoSysBio models can help by providing a rich framework that facilitates the integration of all important aspects of IOB, EOB, PGB, and TOB (see Table 1). If not overwhelmed by statistical prediction errors or numerical rounding errors, such mechanistic models might reliably predict important practical aspects of very rare, high-impact events, such as the extinction of endangered species, catastrophic virus epidemics, the evolution of superbugs resistant to all known antibiotics, or the origin of tumors.

The next milestones on the long journey to formal EvoSysBio analyses mirror many models in mixing mechanistic and descriptive aspects. Using their motivating questions and chosen levels of abstractions, most models combine known cause and effect mechanisms with a coarse-grained phenomenological basis that merely describes statistical estimates of empirical data. For example, modeling metabolic regulation neither requires a simulation of the full quantum mechanics of ribosomes (too fine-grained) nor empirically observed cell-division rates (too coarse-grained). Summarizing individuals as fitness values is the ultimate coarse-graining in IOB; however, to merge IOB and TOB requires the inclusion of substantially more details than currently possible. The following milestones mark important points in building the capabilities for such broad integration.

EvoSysBio Milestone 1: Observed LIFTs

This marks the definition and observation of a new consequential IFT for a set of diverse causal traits that mechanistically specify how consequential IFTs are computed from causal traits, irrespective of how many LIFT types from **Table 3** are implicitly integrated. For example, consider the low probability that light-colored **mice** will be caught by birds of prey on sandy hills (Linnen *et al.*, 2009). It is easy to map genotypes to phenotypes if coat color is controlled by few known genes and mutations of coat color can be recognized in DNA sequences. Thus, a LIFT for survival in such an environment can be trivial to predict if good measurements of predation risks are available for different coat colors. Full measurements of fitness are much more complicated, as mice can die from many causes and reproductive success requires essentially a prediction of everything a mouse can do in that environment. The complexities of such measurements are beyond what most researchers would be willing to contemplate, let alone adding similarly complex models for the corresponding birds of prey, whose survival may depend on their ability to learn how to detect the mice (adding complex neuronal and evolutionary feedback loops). This example illustrates key reasons behind the 'Incomplete' in IFTs: measurements of mouse survival are clearly fitness relevant; yet they are also clearly incomplete and need to be complemented by additional studies that might never be conducted if 'fitness has been measured in this mouse.'

A simpler example uses flux-balance analysis to predict *in silico* how *Escherichia coli* evolves by adapting to a certain environment (Edwards *et al.*, 2001). It only needs a network of relevant metabolic reaction stoichiometries and the assumption of flux-balance equilibrium (influx = efflux everywhere). Such models are easily coupled with genomic datasets that indicate the presence of genes for particular enzymes that catalyze certain metabolic reactions; thus, inferring relevant metabolic networks becomes much easier. Additional empirically observed LIFTs are listed elsewhere (Brouillet *et al.*, 2015).

EvoSysBio Milestone 2: Fragmented LIFTs

Biology has now conceptually mapped much of the most causal LIFTs in the FCNet of model organisms (DNA sequences available). It has also followed diverse LIFTs in the network, spanning all the way to direct fitness contributions. At least since 2009, it has become possible to provide specific realistic examples for *all* LIFT types specified in **Table 3** (see discussion of **Table 2** in Loewe, 2009). Thus, each critical step on the full path from DNA to fitness can, in principle, be modeled; such LIFT-type models have been developed independently in different model-organisms, greatly complicating a potential integration. As biological research continues, the addition of increasing numbers of LIFTs will simply connect different types of LIFTs in the same organism.

EvoSysBio Milestone 3: Simulate a Whole Cell

While the first two milestones were passed some time ago, somewhere on the EvoSysBio journey toward integrating IOB and TOB it must become possible to mechanistically simulate a whole cell in a simple lab environment. This milestone is well aligned with the goal of Evolutionary Cell Biology (Lynch *et al.*, 2014), which requires the quantitative integration of a very large number of diverse quantitative models of cellular subsystems. It has recently been demonstrated that a very simple (but complete) single cell in a simple growth medium can be simulated at the level of biochemical interaction over a whole cell cycle (Karr *et al.*, 2012), though much work remains before such simulations become robust enough and available for more complicated cells. While organizing and curating the thousands of parameters required for simulating the complete biochemical reaction network of a single cell remains challenging (Macklin *et al.*, 2014), obtaining reasonable values for them can be even harder (Karr *et al.*, 2015). New experimental methods provide a wealth of diverse information about cellular processes that was previously difficult to conceive: genomic ontologies are being constructed to list all genes, types of RNAs and proteins (many with functional annotations); advances in microscopy are approaching imaging at atomic resolution in cells (Kuhlbrandt, 2014); fluorescent proteins allow the recording of live single-cell time series of intracellular amounts or tracking the movement of individual molecules. In fact, for some types of cells, it is no longer clear if EvoSysBio is more limited by the need to observe relevant data or the need to organize, interpret, and integrate data that has already been collected (Macklin *et al.*, 2014). This growing need for more efficient data analysis can also be seen in sequencing, where the cost of analysis can substantially exceed the cost of obtaining raw data.

EvoSysBio Milestone 4: Predict Mouse Cancer

Successfully predicting the growth of diverse, complex cells on controlled growth media might encourage addressing the challenges posed by more complex ecologies. The need to understand the full-scale ecology of an animal or plant can be postponed by focusing on the small-scale 'internal ecology' of the body of a mouse from the perspective of cancer cells. In cancer cell biology, cells cannot grow outside their 'mouse-body-ecosystem'; from an experimentalist perspective, mice with known cancer mutations are about as well-controlled and well-studied as ecosystems can be. While predicting the full IOB of a mouse will remain very unlikely for some time, it is easily replicated with high accuracy by growing more mice, all of which can be analyzed with the tools of modern biology. Thus, in comparison to the full-scale EOB of wild mice, it is relatively easy to quantify the IOB of mice for the purposes of describing the environment that controls much of the possibilities of mouse cancer cells. Such mice facilitate addressing a number of interesting challenges in EvoSysBio and cancer research, simply because they can be so extraordinarily well characterized. Given the big interest in mouse cancer research, important breakthroughs are more likely to occur here first.

Cancer therapy resistance remains the most difficult challenge in the diagnosis and treatment of cancer. It is enabled by diverse populations of cancer cells, some of which keep surviving therapy to grow back. Populations of cancer cells are, in principle, governed by the same complex TOB processes studied in ecology and evolutionary biology. Measurements of survival and reproduction rates of cells enable

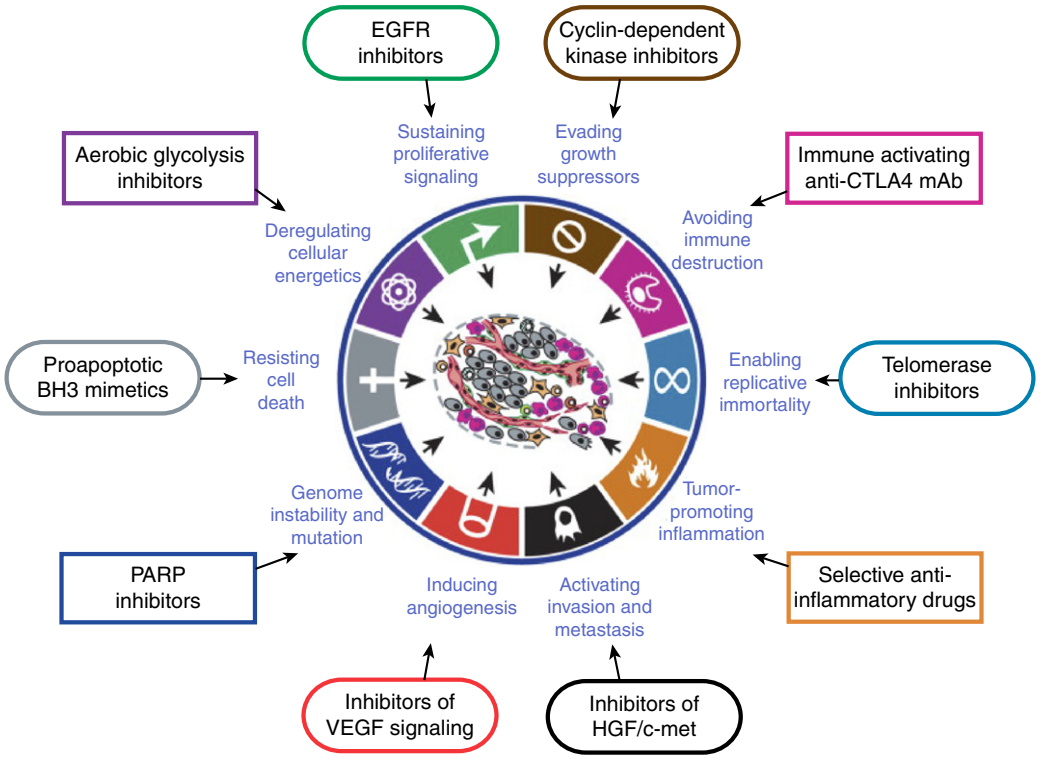

Figure 5 The hallmarks of cancer have strong links to either survival incomplete fitness traits (IFTs) or reproductive IFTs, hence the evolutionary processes triggered by cancer cell biology are interesting test cases for EvoSysBio. Picture credits: Hanahan and Weinberg (2011) © Elsevier, reuse now allowed under CC-BY-SA 4.0 (http://creativecommons.org/licenses/by-sa/4.0/).

predicting evolutionary outcomes of a population in a given environment. Survival of cancer cells in therapy depends on the dynamics of intracellular reaction networks, which can, in principle, be studied with the modeling tools of CSysBio. EvoSysBio aims to combine the theoretical modeling with the real-world observations needed to simulate evolutionary outcomes for cancer populations with increasing precision.

Cancer is an evolutionary process of a population of cells that replicate too much and can grow outside of their normal boundaries. A substantial number of survival and reproductive traits in cancer cells provide a target for natural selection among them (see **Figure 5**): Evolutionary factors like surviving attacks from the immune system, mutating, migrating to new tissues, and other such traits have vexed cancer biologists for a long time (Hanahan and Weinberg, 2011). It is not difficult to define corresponding IFTs for cancer cells and all fundamental factors of evolution are active (Gerlinger *et al.*, 2014).

Mouse cancer as a milestone for EvoSysBio is reasonable due to high independent interest, a wealth of existing information, and readily applicable methodologies. Once it becomes routinely possible to simulate the biochemistry of whole cells with reasonable accuracy, these techniques could be used to quantify relevant mouse IOB and define the TOB for a type of mouse cancer that is comparatively well understood. It is relatively easy to compare predicted numbers and sizes of a given tumor type with actual observations. This could even be done in many replicate mice to explore the impact of chance and necessity in cancer evolution at a very fine-grained level. Such analyses are impossible in large-scale ecology, since 're-running' the world is impossible. Mouse cancer provides unique opportunities for learning about the dynamic aspects of LIFTs in a context where real-world checks are conceivable, as cancer cells are affected by dynamics in their environment (rhythms of mouse life; attempts to cure cancer, etc.) and in return affect their environment (eventually killing the mouse). This might eventually allow turning the dynamic MOCA-LIFT in **Figure 6** into a LIFT (Movie 2).

Ongoing discussions between evolutionary biology and cancer biology (Horne *et al.*, 2015) include work in evolutionary modeling (Nagy, 2005), ecology (Korolev *et al.*, 2014), investigations of life-history trade-offs (Aktipis *et al.*, 2013), evidence for positive selection (Crespi and Summers, 2006), evidence for the role of mutations during development (Frank, 2010), interactions between cancer and viruses (Brandon Ogbunugafor *et al.*, 2013), and epigenetics (Swanton and Beck, 2014).

EvoSysBio Milestone 5: Antibiotics Resistance Evolution

The first part of this milestone predicts how fast bacterial model-organism cultures evolve resistance to well-understood antibiotics under defined laboratory conditions without nested organisms, a challenge comparable to Milestone 3. Adding nested organisms increases difficulties to **the level of** Milestone 4 by requiring the specification of a TOB for the bacteria. However, in simple applied real-world contexts, it is no longer possible to repeat the 'world history' for improving the model

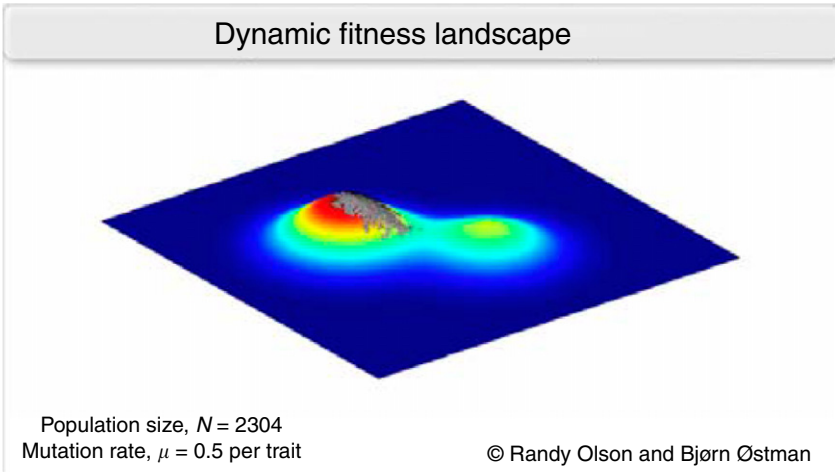

Figure 6 Fitness always depends on the environment. Frequent environmental changes can trigger perpetual changes in fitness, resulting in a constant need for adaptation. Dynamically changing Landscapes of Incomplete Fitness Traits pose additional challenges to EvoSysBio 'flight simulators,' which now also have to predict environmental properties and how they might be affected by populations of organisms over time. Here a snapshot is shown from a cartoonish movie of a population that evolves on a dynamic MOCA-LIFT (for more details, see also links in Section Relevant Websites). Picture credits: © Østman and Olson (2014c), reuse under CC-BY-SA 3.0.

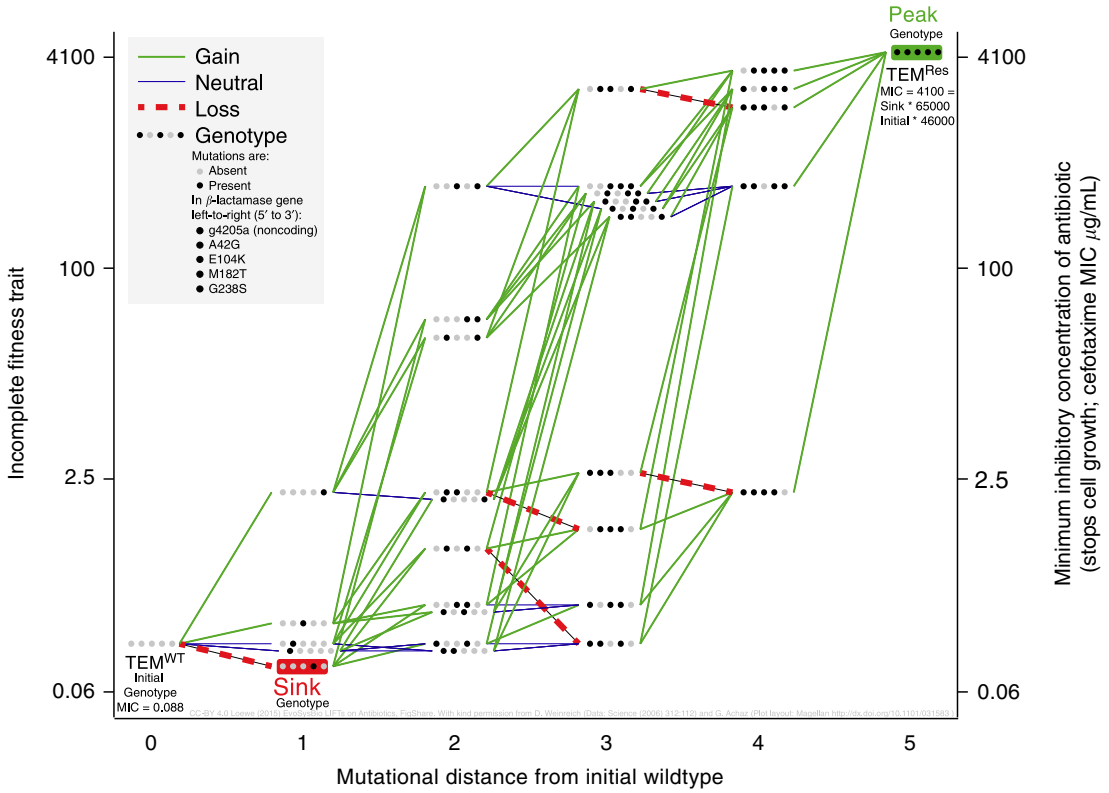

Figure 7 Evolution can take different paths to increase an IFT, such as in this experimentally observed LIFT snapshot of antibiotics resistance from five point mutations in a bacterial gene, where they can accumulate from the original TEM^{WT} genotype to the TEM^{Res} with different trajectories of fitness increases, which are followed with different probabilities as shown elsewhere (Weinreich *et al.*, 2006; DePristo *et al.*, 2007). This LIFT uses MIC, a cellular IFT that depends on a corresponding protein based LIFT where IFTs are determined by molecular features, which can be measured independently (Meini *et al.*, 2015). Picture credits: © Laurence Loewe (2015b), reusable under CC-BY 4.0. Data (Weinreich *et al.*, 2006) was plotted by the MAGELLAN fitness landscape mapping tool (Brouillet *et al.*, 2015), both shared with kind permission of Daniel Weinreich and the MAGELLAN authors.

(tumor evolution is repeatable by using new mice; but hospitals are difficult to sterilize, turning their resistance evolution into a historic process). Nested organisms, reservoirs, and very complex ecologies further complicate the picture by introducing many unknowns of potential importance. Unfortunately, these difficulties are matched by the urgency of the problem.

Prescribed doses of antibiotics have been climbing and now some bacterial strains are resistant to all known antibiotics. The evolution of antibiotic resistant bacteria has been discussed since antibiotics were first used (Neu, 1992; Normark and Normark, 2002; Choffnes *et al.*, 2010; Perros, 2015; Baker, 2015): How can we use these antimicrobial 'super-drugs' in a way that reduces the ability of 'superbugs' to evolve complete resistance? If we do not succeed, bacterial infections will become more deadly until one of the biggest medical advances of the twentieth century will have lost practical relevance. Predicting antibiotic resistance evolution (Martinez *et al.*, 2007) might allow us to find ways to use antibiotics that minimize the evolution of resistance.

Challenges of dynamic, multi-level simulations of evolution need to be mastered for truly understanding antibiotics resistance evolution. At the molecular level, questions include how many mutations a bacterial protein needs to accumulate to confer a higher level of resistance to the cell that produced it. The molecular features of such a protein can be interpreted as IFTs that define a protein-based LIFT (Meini *et al.*, 2015). The interplay of new mutations with the other content in crowded cells leads to an IFT at the cellular level: the Minimum Inhibitory Concentration (MIC) of an antibiotic above which a given bacterial cell is not longer able to grow. **Figure 7** shows the results of experimentally measuring a small, but combinatorially complete LIFT for an antibiotic-based IFT defined by a corresponding MIC (Weinreich *et al.*, 2006). At intermediate levels, events in organisms need to be modeled, since bacteria evolve during an infection. On geographic scales, the diverse use of antibiotics contributes to resistance evolution in many unexpected places (e.g., soil in agriculture, biofilms in hospitals). Despite progress, it is not clear how to optimize the use of antibiotics overall in hospitals and agriculture (where infected humans can carry resistant super-bugs between both, unwittingly exposing others). International travel adds further complications by moving infections over large distances. The challenges of both dynamic and static fitness landscapes (**Figures 6** and **3**) apply to antibiotics resistance evolution, as changes in antibiotics usage policies can easily generate either.

Complex evolutionary phenomena, such as epistasis, mutation rates in the stationary phase of bacteria and many other effects are important for understanding the evolution of antibiotics resistance (Loewe *et al.*, 2003; MacLean *et al.*, 2010; Hall and MacLean, 2011; Schenk and de Visser, 2013; DePristo *et al.*, 2007; Poelwijk *et al.*, 2007; Weinreich and Knies, 2013). For example, resistant bacteria often pay a fitness cost unless it is mitigated by compensatory mutations (Andersson, 2006). Unfortunately, antibiotics are often used in a way that facilitates the evolution of antibiotics resistance by creating environments where sublethal concentrations of antibiotics can select for resistant bacteria over longer periods of time (e.g., in sewers or agriculture; see **Figure 8**, (Gullberg *et al.*, 2011)). To preserve one of the biggest medical success stories of the twentieth century requires continually solving the riddles that bacteria pose by evolving antibiotics resistance (Choffnes *et al.*, 2010; Perros, 2015; Baker, 2015). Given that prokaryotes rule the world (by numbers and by speed of growth), it will require the best, most precise, and most integrated understanding of evolution to outsmart them – with the help of many computers.

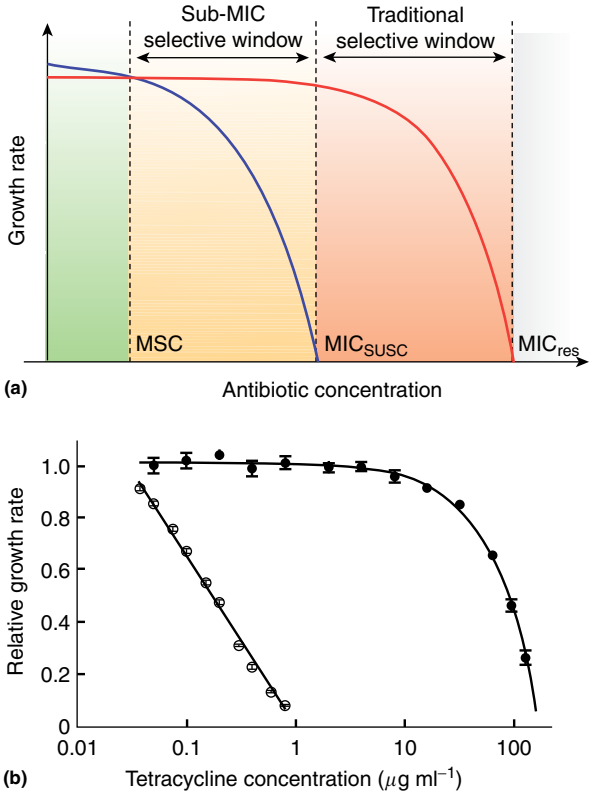

Figure 8 Small selective advantages can combine over a longer period of time to substantially accelerate antibiotics resistance evolution (Gullberg *et al.*, 2011). (a) Schematic overview, *MIC*: Minimal Inhibitory Concentration for susceptible (*susc*) and resistant (*res*) bacteria, *MSC*: Minimal Selective Concentration. (b) Experimental measurements for the antibiotic tetracycline. Picture credits: © Gullberg *et al.* (2011), reusable under CC-BY.

Conclusions

Even though the New Evolutionary Synthesis started in the 1920s and biological progress has continued at a phenomenal pace, much biology remains to be discovered and integrated. The New Synthesis is being renewed each day when researchers integrate new results into its framework through relentless synthesis (Wray *et al.*, 2014). The explosion of expertise on evolution and the complexity of systems science require reliable computational tools to give researchers a chance to keep up with the pace of the New Synthesis. Success critically depends on the strength of underpinning abstractions, which may be measured by intangibles such as conceptual clarity, completeness, simplicity, and elegance. The power of good abstractions is the best defense against the swamp of

complexity that otherwise mires researchers in endlessly redundant repeats of researching, reinventing, and rediscovering. There is nothing more practical than a good theory.

The ambitious aims of EvoSysBio for understanding and reliably predicting evolution depend on accelerating the pace of the relentless New Evolutionary Synthesis to which EvoSysBio is ultimately contributing. This can be done by making it easier to connect the five fundamental factors of evolution to results from models of complex Intra-Organism and Trans-Organism Biology. Toward this end, this article provides a high-level overview of FCNets and LIFTs, but many details remain to be worked out.

The relentless New Synthesis can benefit from lessons learned by programmers in their struggles in the swamp of complexity: aim for 'as simple as possible, but not simpler' (Raymond, 2003). To make EvoSysBio efficient, this view of Occam's Razor needs to inspire new computational approaches for knowledge organization, provenance, modeling, reliability, precise uncertainty quantification, ease of automation, abstraction management, automatic testing, efficient debugging, and reproducibility. Without such innovations, EvoSysBio researchers will either get stuck in the complexity swamp of constructing FCNets (and debugging their dependencies) or get lost in the fog of finding signals drowned by noise (due to missing uncertainty quantification).

Biology's complexity is so pervasive that efficient approaches for solving these problems often require semantic architectures with the strength of general programming languages that can integrate most biological research, if not all. Developing such general semantics is usually slow and challenging, but enables great leaps forward once the right concepts are ready for automation. Given the slow pace of developing semantics and the complexity of evolution, it may take ≥ 30 years until integrated computational pipelines can enable the use of all state-of-the-art expertise to mechanistically predict the effects of unknown mutations in well-studied model organisms. Analyzing resulting fitness landscapes with interactive 'multidimensional flight simulators' may sound like science fiction today – perhaps like 'population genomics' would have sounded in the 1970s. Yet it took only about 30 years to go from a method of DNA sequencing to population genomics. Maybe it is possible to start transitioning from a formal definition of EvoSysBio to usable flight simulators for fitness landscapes as the New Evolutionary Synthesis approaches its 100th year of relentless integration service. A bit of strategic long-term thinking might help to avoid swamps and fogs to make evolutionary research more efficient in the long run.

See also: Adaptive Landscapes. Evolutionary Medicine I. An Overview and Applications to Cancer. Gene Interactions in Evolution. Modularity and Integration. Modularity and Integration in Evo-Devo

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Transposable Elements, Population Genetics of

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Glossary

Beta distribution A continuous probability distribution, between 0 and 1, whose shape is determined by two parameters, α and β.

Gamma function A generalization of the factorial function, allowing for non-integer numbers.

Hill–Robertson effect The phenomenon in which the efficacy of selection acting on an allele is reduced by genetic linkage to a locus that is also the target of selection.

Muller's ratchet The accumulation of deleterious mutations that occurs in a finite population in the absence of recombination. In a finite, asexual population, fitness will decline due to stochastic loss of the most fit genotype that cannot be recovered by recombination.

Rolling circle amplification A mechanism of TE amplification in which a circularized DNA molecule is used as a repeated template for the generation of multiple DNA copies.

Synergistic epistasis A form of fitness function in which the fitness effects of additional mutations are stronger than the prior ones.

Introduction

Transposable elements (TEs) are selfish elements, typically the size of a gene, that reside in genomes and encode the machinery of their own proliferation. There are two dominant classes of TEs: Retrotransposons and DNA transposons (Figure 1). Autonomous retrotransposons are commonly designated 'copy-and-paste' elements. They encode reverse transcriptase and generate new DNA insertions off an RNA template either in the cytoplasm, in the case of long terminal repeat (LTR) elements, or in the nucleus, in the case of non-LTR elements. Because they replicate via an RNA intermediate, retrotransposons are capable of making many copies of a single insertion. DNA transposons replicate via DNA intermediates and the most well-understood class of DNA transposons are the 'cut-and-paste' transposons. Autonomous cut-and-paste'

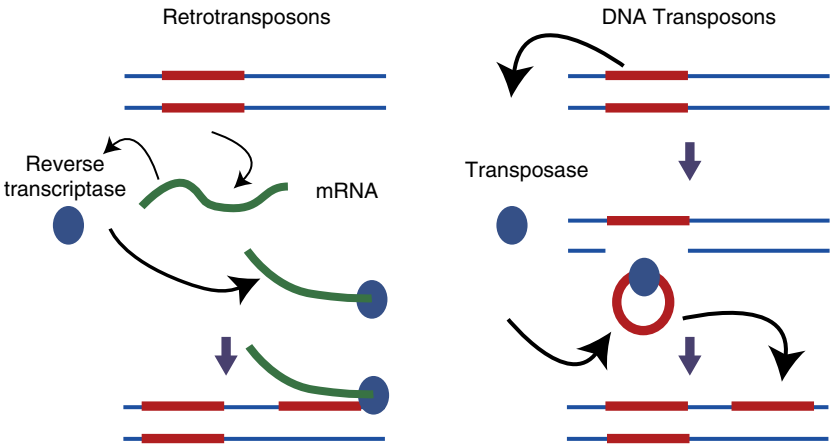

Figure 1 Most transposable elements can be classified as either retrotransposons or DNA transposons. 'Copy-and-paste' retrotransposons encode reverse transcriptase and can copy themselves via cDNA. 'Cut-and-paste' DNA transposons encode transposase and can increase in copy number when transposon excisions are repaired by the host repair machinery using retained copies as a template.

transposons code for transposase proteins that excise and reinsert DNA elements elsewhere in the genome. Such elements are able to proliferate in genomes when the gaps left by excised copies are refilled by the host DNA repair machinery using sequences that correspond to retained insertions. A second poorly understood class of DNA transposon known as a *Helitron* does not proliferate through a 'cut-and-paste' mechanism. Rather, such elements proliferate through rolling circle amplification (Kapitonov and Jurka, 2007).

Across species, TEs play a significant role in determining differences in genome size. For example, salamanders are known for some of the largest genome sizes and much of this can be attributed to significantly greater numbers of retrotransposons compared to other vertebrates (Metcalfe and Casane, 2013; Sun *et al.*, 2012). TE insertions also contribute significantly to variation between individuals within species because TE insertions are frequently polymorphic within populations. These polymorphic insertions may disrupt gene function and be harmful, but occasionally they may be beneficial. It also appears that TEs play an important function in shaping the evolution of gene function and gene networks. For example, the V(D)J recombination mechanism in the immune system evolved by taking advantage of transposase encoded by a DNA transposon (Hencken *et al.*, 2012).

Genetic analysis in maize by Barbara McClintock was the first work to identify TEs. After their molecular characterization in the late 1960s and through the 1970s, the dynamics of TE proliferation in species became of significant interest because it was clear that the genome could no longer be viewed as static. Rather, genomes were shaped by dynamic processes of TE mobilization within and across generations. Moreover, TEs provided an explanation for the C-value paradox, the observation that genome size does not correlate with organismal complexity. The population dynamics and genetics of transposable elements became a topic of significant interest in the early 1980s and continues to this day as genome sequencing has revealed significant complexity in TE dynamics within species.

Critical Parameters and the Role of Sex and Recombination

A central question driving population genetics research in transposable elements is: What determines variation in TE proliferation in populations? Answering this question may allow us to understand why TE profiles differ greatly among species. There are several critical parameters to consider: the rate that TEs enter genomes, the rate of movement (transposition), host mechanisms that modulate the rate of transposition, the strength and form of selection acting on TEs, the population size of the host, and the amount of recombination in the genome. The latter parameter – the amount of recombination – is critical. This is because in species with effectively no recombination – either due to asexuality or complete selfing – TEs are unlikely to spread because a lack of genetic exchange means that TEs cannot escape the genome in which they reside (Hickey, 1982; Charlesworth and Charlesworth, 1983). In species with no sex at all, the fate of TEs is completely determined by the success of the clonal host lineage. Clonal lineages lacking harmful TEs will outcompete clonal lineages that carry TEs, thus jointly driving TE lineages residing within asexual lineages to extinction. In contrast, sex and sexual reproduction allow TEs to proliferate within species even if they are harmful. This fact was first pointed out by Donal Hickey in 1982 (Hickey, 1982). A selfish element may proliferate within a species, even if detrimental, if the rate of movement to new genomes through sexual reproduction is greater than the rate of removal due to selection. More generally, the rate of recombination plays a key role in determining the fate of TEs because linkage leads to an association between original insertions and potentially harmful descendant copies (Charlesworth and Charlesworth, 1983). When the rate of recombination is low, selection can limit the spread of an original insertion because it will become linked to the harmful mutations that it causes. When the rate of recombination is high, harmful descendant insertions are uncoupled from progenitor copies, allowing progenitor copies greater opportunity to make new copies.

While recombination and sex are critical for the spread of TEs from genome to genome, recombination can jointly play an important role in limiting the increase of individual insertion alleles in populations that already carry a burden of elements. This is because asexual lineages in finite populations are susceptible to Muller's ratchet. When a sexual population undergoes a transition to asexuality, all individuals may carry proliferating elements that have high transposition rates. However, in the absence of sex, recombination cannot reestablish insertion free chromosomes. Since TE lineages will continue to proliferate, newly asexual species are likely to experience a great increase in copy number. In fact, simulations have shown that this proliferation can become unbounded, likely driving asexual species to extinction when populations are small (Dolgin and Charlesworth, 2006).

This effect can also be seen in regions of the genome with low recombination rates. Since Hill–Robertson effects can limit the efficacy of selection where recombination is low, regions of the genome with little exchange are also expected to accumulate TE insertions. This has been born out by simulation (Dolgin and Charlesworth, 2008) and is also clearly seen in empirical analysis of genomes. For example, in *Drosophila*, there is no recombination in males and neo-Y chromosomes that never recombine quickly accumulate TE insertions (Bachtrog and Charlesworth, 2002).

The Equilibrium Model of TE Population Genetics

Assuming an infinite population size, one may ask what allows TEs to proliferate and what limits their spread. Without constraint on copy number, we might expect TEs to amplify exponentially and entirely fill the genomes they reside in. This is not plausible, so we may consider two forms of constraint that limit unbounded TE proliferation – a diminishing transposition rate and natural selection against TE insertions.

Does a Diminishing Transposition Rate Constrain TE Proliferation?

Evidence suggests that the per copy transposition rate is not independent of copy number. With increasing numbers of TEs,

the per insertion transposition rate may decrease. Mechanistically, this form of regulated transposition may be due to saturation of the transposition machinery, host defense mechanisms that limit TE spread or encoded TE self-regulation. If we consider a transposition rate that decreases with copy number, is an equilibrium possible without selection?

Considering the average number of elements in each individual (Charlesworth and Charlesworth, 1983; Charlesworth and Langley, 1989) equal to $\overline{n}$, the per generation change in copy number is given by:

$$\Delta\overline{n} \approx \overline{n}(u_{\overline{n}} - v_{\overline{n}})$$

where u_n and v_n are the respective per copy transposition rates and excision rates of insertions in an individual with n copies. An equilibrium will be achieved when the transposition rate equals the excision rate since the rate of increase will be equal to the rate of loss. However, there is little evidence that the rate of excision (which is mostly relevant to DNA transposons that encode transposase) would ever equal the rate of transposition. Most evidence shows that the rate of excision is likely quite low. For this reason, it is unlikely that a diminishing transposition rate with increasing copy number would be sufficient to entirely constrain TE proliferation. Instead, another force is required to limit unbound TE proliferation. This force is most likely natural selection.

How Can Selection Prevent Runaway TE Proliferation?

In 1983 Brian Charlesworth and Deborah Charlesworth developed the standard model for TE dynamics with selection that allows us to determine how an equilibrium may be established when the rate of transposition is greater than the rate of excision (Charlesworth and Charlesworth, 1983). To do so, they used the theory of selection on a quantitative trait (see Charlesworth and Charlesworth, 2010, p. 579) to show that the selective change in the mean number of elements per individual $\overline{n}$ is given by:

$$\Delta\overline{n} \approx V_n \frac{\partial \ln \overline{w}}{\partial \overline{n}}$$

where V_n is the variance in copy number and $\frac{\partial \ln \overline{w}}{\partial \overline{n}}$ is the derivative of log fitness w with respect to $\overline{n}$. The interpretation here is that the mean number of elements per individual is a quantitative trait and the rate of removal is equal to the variance in copy number multiplied by the slope of the log-fitness function, which indicates how strongly selection is acting against insertions in individuals with n copies. If we assume that the rate of recombination is sufficient such that linkage disequilibrium between elements can be ignored and that the frequency of each insertion is low enough, it can be shown that copy number is Poisson distributed, with variance simply equal to the mean. Thus, also including the addition of new elements arising from the excess of transposition over excision, the change of copy number is given by (Charlesworth and Charlesworth, 1983):

$$\Delta\overline{n} \approx \overline{n} \frac{\partial \ln \overline{w}}{\partial \overline{n}} + \overline{n}(u_{\overline{n}} - v_{\overline{n}})$$

and an equilibrium is established when

$$-\frac{\partial \ln \overline{w}}{\partial \overline{n}} = u_{\overline{n}} - v_{\overline{n}}$$

Stability in this equilibrium, in which TEs are maintained in the population, will be obtained when

$$\frac{-1}{\hat{n}} < \frac{\partial^2 \ln \hat{w}}{\partial \hat{n}^2} < 0$$

Here one can see that the second derivative of the log-fitness function at equilibrium must be less than zero. In other words, there must be synergistic epistasis, where the log-fitness function is concave, or decreasing more sharply than linearly, at the equilibrium point for copy number. Additional copies are required to decrease fitness by an increasing magnitude at equilibrium. This balance can be understood given the fact that with even a modest transposition rate, the rate of increase of insertions is exponential when there is no selection. An increasing strength of selection acting against increasing TE copy number is required to limit TEs that are exponentially proliferating.

Behavior of Insertions in a Finite Population

The analysis given above establishes the equilibrium copy number in an infinite population, but it does not describe the dynamics of insertions that might be expected to drift to moderate frequency in finite populations. Here, Charlesworth and Charlesworth (Charlesworth and Charlesworth, 1983) also described the steady-state distribution for TE insertions that are selected against, but for which drift may allow an increase to modest frequency. This steady-state distribution $\phi(x)$ is approximated by a beta distribution for which the two shape parameters correspond to the joint effects of transposition and drift (α) and excision and selection coupled with drift (β):

$$\alpha = \frac{4N_e u\hat{n}}{T - \hat{n}}$$

$$\beta = 4N_e(s + v)$$

where T is the number of possible sites in the genome for inserts, u and v are transposition and excision rates respectively, and s is the selection parameter equal to:

$$-\frac{\partial \ln \hat{w}}{\partial \hat{n}}$$

when the equilibrium copy number, $\hat{n}$, is obtained.

This gives

$$\phi(x) \approx \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} x^{\alpha - 1}(1 - x)^{\beta - 1}$$

where Γ is the gamma function.

The influence of the coupled parameter β – through either the effects of drift in smaller population sizes or stronger selection – can be seen in **Figure 2**. When β is high, selection is stronger and elements only segregate at low frequencies. As β decreases, the stationary distribution shifts and some elements can fix. One may fit observed TE insertions frequencies from natural populations to obtain estimates of β. This has been fruitful in *Drosophila* where the site frequencies of TE insertions

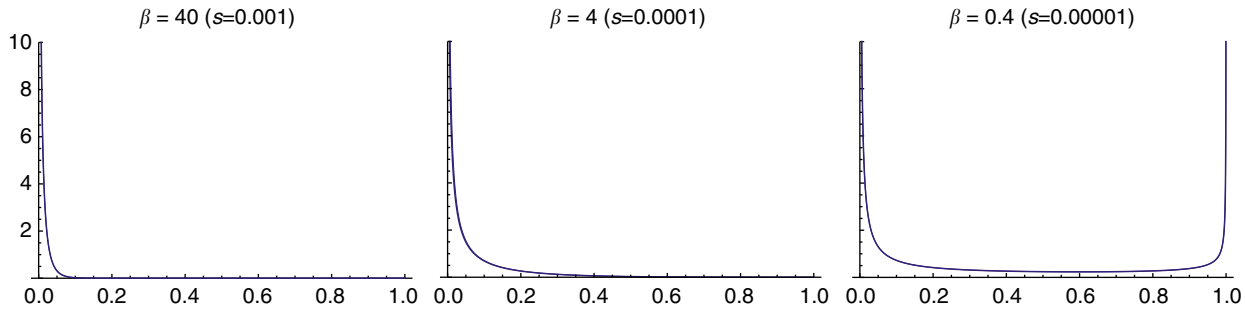

Figure 2 The equilibrium frequency distribution for deleterious TE insertions in a finite population. The β term captures the selection coefficient of insertions. With higher values of β, insertions remain at low frequency. With lower values of β, selection is weaker and some are allowed to fix.

are typically very low and estimates of β can be above 1 (Charlesworth and Langley, 1989). However, in other species, insertion frequencies can be high. In these cases, some of the assumptions outlined above – such as copy number being Poisson distributed – may be violated. In addition, studies have shown that TEs may proliferate in bursts and be recent introductions to genomes. In this case, the assumptions of equilibrium are violated. For these scenarios, simulation has been a critical tool for describing TE dynamics.

Regulation of Transposition Rates: Host and TE Lineage Selection

As indicated previously, unless the transposition rate becomes equal to the excision rate, selection is required to constrain TE proliferation in genomes. This is true if the transposition rate decreases with increasing copy number, as long as it is always higher than the excision rate. However, when regulated transposition and selection act together, the requirement for a concave log-fitness function becomes relaxed (Le Rouzic and Capy, 2009). Thus, regulated transposition can be critical when selection is insufficient for constraining runaway TE proliferation. How might we expect natural selection to act on regulation of the transposition rate? We must consider two forms of selection.

In the first case, we can consider selection acting on the TE lineage to modify the transposition rate of all members of the TE family. As previously mentioned, the amount of recombination is a critical parameter. If recombination rates are low, or zero in the case of asexuals, selection will favor low transposition rates if insertions are harmful. This is because TE lineages become burdened by their own harmful effects without recombination. In contrast, with high levels of recombination, high transposition rates will be favored because progenitor copies can become unlinked from the harmful consequences of descendant copies. Generally, when recombination rates are high, selection for lower transposition rates is weak, but this depends on how harmful insertions are. When insertions cause dominant lethal or sterile alleles, there is no opportunity for recombination to disassociate the source element from these effects. In this case, selection will favor variants in the TE family with lower transposition rates (Charlesworth and Langley, 1986). Early invasion of TEs may also place significant constraints on transposition rates. If the transposition rate is too low, element loss by drift during early invasion will occur nearly all the time. However, if the transposition rate is too high, rapid amplification within the genomes of infected individuals will cause significant loss of host fitness, and loss of the TE lineage. Overall, invasion appears favored by elements that have a high transposition rate during the initial phase of invasion, but soon have a reduced transposition rate that does not result in TE loss due to excessive TE load imposed on the host (Le Rouzic and Capy, 2005).

In addition to selection on TE lineages, we may also consider selection on host alleles that limit transposition rates. As before, the amount of recombination is an important parameter, but the conditions that lead to selection for a reduced transposition rate are not as restrictive. The selection coefficient for a host allele that reduces the transposition rate u by δu is given by (Charlesworth and Langley, 1986; Nuzhdin, 1999):

$$s \approx \delta u \frac{\overline{n}u}{2\tilde{H}}$$

Here, H is a summary statistic for the amount of recombination genome wide and is defined as the harmonic mean of $(r+u)$ for all pairs of loci, r being the recombination rate between any two loci. With standard transposition rates greater than 10^{-7}, u has only a modest influence on $\tilde{H}$ and $\frac{1}{2\tilde{H}}$ is at most as high as 10 to 20 when recombination rates are low. With completely free recombination the recombination frequency between any two random loci is 0.5 and $\frac{1}{2\tilde{H}} = 1$. If we consider transposition rates of 10^{-4} (a typical estimate for *Drosophila* (Nuzhdin *et al.*, 1997)), 20 copies in the genome, and $\frac{1}{2\tilde{H}} = 5$, an allele that completely represses transposition ($u = \delta u$) will have a selection coefficient of 1×10^{-6}. Since selection is limited with coefficients less than the reciprocal of the population size, in this scenario selection on host repressor alleles would be restricted to population sizes on the order of one million or more. However, a transposition rate of 10^{-3} and 100 copies yields a selection coefficient of $s = 5 \times 10^{-4}$. In *Drosophila* transposition rates of 10^{-4} may underestimate the transposition rates of early invasion because they may have been measured in backgrounds in which host repressor alleles may have already been selected for. Thus, it appears that selection for host repression may evolve under reasonable conditions.

Deviations from Equilibrium Assumptions

To consider non-equilibrium dynamics, simulation approaches have been critical. Such approaches allow relaxation

of many of the assumptions of the standard model. For example, non-equilibrium dynamics may occur in the early stages of invasion when copy number is below the equilibrium value. In addition, over the long term, there may be heterogeneity among TE copies. Le Rouzic and Capy have paid special attention to these long non-equilibrium TE dynamics through simulation (Le Rouzic *et al.*, 2007). In particular, they considered a TE lineage that could periodically cause mutations beneficial to the host but was also itself susceptible to mutations that reduced the activity of the insertion. Depending on a balance between beneficial effects and disabling mutations that occur within the lineage, several different outcomes were observed over the long term.

As suggested by previous work, when there are few beneficial insertions and a very low rate of disabling mutations on the TE lineage, a long-term steady-state equilibrium is observed. Copy number remains fairly stable and TEs impose a continuous fitness burden. In contrast, when the rate of disabling mutations is increased significantly, non-autonomous copies lacking the ability to catalyze their own movement begin to accumulate and these non-autonomous copies lead to lower levels of activity. Beneficial insertions, however, act as a reserve. As non-autonomous elements drive activity levels down, elements begin to disappear, but then autonomous beneficial insertions can drive a new wave of activity, leading to cycling. Depending on the rate of disabling mutation and the proportion of insertions that are beneficial, heterogeneity among TE copies can also result in persistence through beneficial insertions or complete loss. Interestingly, when there is heterogeneity in the selective effects of TE insertions, larger populations may maintain more active TEs than smaller populations. This contradicts the standard expectation that smaller populations, most influenced by drift, should harbor a greater TE burden. This is because slightly beneficial insertions, that act as a reserve for maintaining an active lineage across generations, are more likely to be lost to drift in smaller populations. In contrast, such reserve insertions may persist in larger populations and maintain a higher TE burden.

Forms of Selection

These analytical and simulation-based studies all agree that natural selection is critical for constraining TE proliferation in genomes. However, it is not clear what form of harm is most likely to limit TE copy number. Three standard forms have been proposed: (1) disruptive effects of insertion into genes, (2) the cost of expressing the transposition machinery, and (3) the chromosome damage caused by ectopic recombination events between insertions at non-homologous positions. Under the standard model, equilibrium is only achieved when the log-fitness function is curved downward. However, it is not apparent that gene disruption can yield this form of synergistic epistasis. In addition, there does not seem to be significant selection against expression of the transposition machinery because TEs carrying promoter sequences achieve similar frequencies compared to TE insertions lacking a promoter (Yang and Nuzhdin, 2003). Thus, the cost of expression is also unlikely to constrain copy number. In contrast to these two forms of selection, the damage caused by ectopic recombination may be the most plausible selective force that constrains TE proliferation (Lee and Langley, 2010). This is because the probability of ectopic recombination between elements will increase quadratically with copy number. This meets the equilibrium requirement that the log-fitness function must be concave and decreasing at a rate that is more than linear.

Empirical Studies of TEs

Genetic studies show clear evidence that TEs are harmful. About half of all visible mutations arise from TE insertions in *Drosophila* (Tenhave *et al.*, 1995). While TEs are not very active in humans and transposition events are rare, cases of hemophilia and cancer caused by TE insertions have been found (Belancio *et al.*, 2009). To further characterize TE dynamics in species and populations one may measure the genomic distribution and abundance of different TE classes and families. In addition, a population genetic approach can be used to measure the frequency of TE insertion alleles in natural populations. Empirical studies have confirmed many of the theoretical predictions outlined above.

Evidence for Selection

Theory suggests that TEs can spread in populations even if they are harmful. Therefore, many studies, especially in *Drosophila*, have employed population approaches to estimate the strength of selection acting on TE insertions to test this hypothesis. *Drosophila* has a large population size and studies have consistently shown that single TE insertion alleles segregate at low frequency (Charlesworth and Langley, 1989). This is consistent with selection acting to limit TEs. Interestingly, in species with smaller populations, the evidence for selection is weaker. For example, pufferfish (Neafsey *et al.*, 2004) and *Anolis* lizards (Tollis and Boissinot, 2013) both carry TE insertion alleles that segregate at higher frequency than in flies. Nonetheless, *Anolis* lizards from larger populations display lower insertion frequencies for harmful full-length elements. The overall trend of an inverse population size effect on TE abundance and frequency strongly supports a model in which natural selection against their harmful effects is a limit on TE proliferation. Studies have also shown that TEs with promoters tend to segregate at similar frequencies compared to TEs without promoters (Yang and Nuzhdin, 2003). This suggests that the expression costs of TEs are not significant. In contrast, smaller TE insertions segregate at higher frequencies than larger TE insertions (Petrov *et al.*, 2003). Since the likelihood of ectopic recombination will be higher among larger copies, this supports the premise that ectopic recombination is a key form of selection that limits TE copy number. In addition to comparing patterns of polymorphism across species, studies that examine the distribution of elements across the genome within a species also find that TEs can accumulate in regions of low recombination. Because of Hill–Robertson effects, natural selection is less effective in regions of low recombination. This further supports the idea that TEs are harmful, though a reduced rate of harm mediated by lower rates of ectopic

recombination in these regions of the genome can also contribute to this observation.

The Role of Mating System

Because harmful TEs can proliferate only to the extent that they can escape their harmful effects, theory suggests that mating system can have a significant effect on TE dynamics. Over the long term, selfing is predicted to select for TEs with reduced transposition rates. Plants provide a test of this model because there are multiple transitions to selfing. So far, however, results suggest that there is a complicated relationship between mating system and TE proliferation (Agren *et al.*, 2014). This is because the earliest stages of selfing can lead to a reduced effective population size. Thus, a transition to selfing can be associated with an *increased* TE burden due the reduced efficacy of selection limiting TEs (Nuzhdin and Petrov, 2003). Only in the long term might we expect evolution or extinction of TE lineages to lead to a decline of TE movement in selfing species. Studies suggest that this time effect must be more thoroughly investigated (Agren *et al.*, 2014).

Host Mechanisms of TE Repression

Until recently, natural selection was considered the dominant force limiting TE proliferation. However, recent studies have shown that host encoded mechanisms of genome defense by DNA methylation and small RNAs also contribute (Aravin *et al.*, 2007). Theory has shown that when transposition rates are sufficiently high, mechanisms of host control can evolve, but little is known about the population dynamics of TE control by genome defense in actual populations. Studies of the P-element in *Drosophila melanogaster* demonstrate that alleles that repress transposition can accumulate rapidly in populations soon after genome invasion (Ajioka and Eanes, 1989; Ronsseray *et al.*, 1991). Since small RNA mechanisms of genome defense rely on TE insertions to generate the repressor molecules, the strength of TE repression may increase with increasing copy number. If so, genome defense mechanisms may play a crucial role in limiting runaway transposition in the genomes of sexually reproducing species (Lee and Langley, 2010; Blumenstiel, 2011). Further studies are required to determine the significance of genome defense mechanisms in limiting TE proliferation over the long term.

See also: Genome Plasticity, Bacterial. Recombination and Selection. Sex and Selfish Genetic Elements

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U

Unikonts, Evolution and Diversification of (with Emphasis on Fungal-Like Forms)

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Glossary

Amoeba A cell of a eukaryote that does not have a set shape. Rather, it varies dynamically in outline as it produces temporary extensions called pseudopodia. *Amoeba* is a formal name for a genus of organisms that have amoeboid cells, but not all amoebae belong to that genus.

Amoebozoa One of the two major monophyletic lineages of the unikonts. It contains many of the classical amoebae, including the genus *Amoeba*, and many of the slime molds. Note: Not all amoeboid eukaryotes are members of Amoebozoa.

Bikont A flagellated cell where the flagellar apparatus contains two basal bodies, from each of which extends a flagellum. Thus, a bikont cell is one with two flagella that arise from the same point on the cell.

Dikaryon A kind of cell found in certain members of the Fungi that contains two genetically different haploid nuclei (di – two, karyo – nucleus). A dikaryon originates when gamete nuclei enter into a cell but do not undergo nuclear fusion, karyogamy. Karyogamy is delayed for several cell generations during which each of the two nuclei undergo synchronized divisions. A dikaryon is genetically equivalent to diploidy except that the homologous chromosomes are housed in separate haploid nuclei rather than in one diploid nucleus.

Karyogamy The final event of the fertilization stage of a sexual life cycle. Two haploid gametic nuclei fuse to form a single diploid nucleus, the zygote nucleus.

Lysotrophy A form of heterotrophic nutrition in which the cells of an organism secrete digestive enzymes to the outside environment. These enzymes break down biological macromolecules into their component subunits, and these subunits are absorbed back into the organism's cells to be used as sources of energy or as building blocks for the organism's own macromolecules.

Mycelium The vegetative body of many of the groups of fungi and fungus-like protists. It consists of a system of branching filaments that grow longer from their tips. The cells of a mycelium are surrounded by a cell wall. Each individual filament of a mycelium is called a hypha.

Obazoa The second major monophyletic lineage of the unikonts. It consist primarily of the Opisthokonta and two protist lineages that do not branch within the opisthokonts.

Opisthokonta The most species-rich monophyletic lineage in Obazoa. It has two major monophyletic subgroups. One subgroup (Nucletmycea) includes the true Fungi plus some groups of protists, including the parasitic Microsporidia. The other subgroup (Holozoa) includes the animals (Metazoa) and several groups of protists.

Phagocytosis The process by which individual cells engulf particles of dead or living organisms to use them as food. A food particle is ingested by the cell such that it becomes contained in a food vacuole surrounded by cytoplasm. The cell secretes digestive enzymes into the food vacuole, and the digestion products are absorbed by the cytoplasm through the food vacuole's membrane. Parts of a food particle that are not digested are released to the outside environment by exocytosis.

Phagotrophic, s.s. Where phagotrophy (see below) relies solely on the ingestion, phagocytosis, of food particles by single cells.

Phagotrophy Any form of heterotrophic nutrition where a eukaryotic organism takes food into its body to be digested. When an organism is unicellular, phagotrophy is essentially synonymous with phagocytosis. When an organism is multicellular, food is taken in by a mouth and enters the digestive system. Together, the mouth and the space within the digestive system form a pocket or tube of the outside environment that is surrounded by the organism. Food may get from the digestive system to the individual cells either by the cells using phagocytosis, as in sponges, or by lysotrophy, as in all other Metazoa.

Protist Any eukaryote that is not a member of the following monophyletic groups: Metazoa (animals), Embryophyta (land plants), or Fungi.

Pseudopodium A transitory extension from a cell that may be extended or retracted either to effect motility or to engulf food particles. Sometimes both activities occur at the same time. A few protists have fixed body shapes from which a pseudopodium can arise only from a particular

point. Most pseudopodial eukaryotes are amoeboid and can, therefore, extend pseudopodia from any place on the cell surface.

Slime mold An informal term used to designate organisms who spend their active life as some kind of amoeba. At some point in a slime mold's life cycle, it will develop into a spore-bearing dispersal structure, often called a fruiting body. There are two kinds of fruiting bodies (that unfortunately have very similar names), sporocarp and sorocarp.

Sorocarp Cellular slime molds produce spore-bearing structures following the aggregation of lots of amoebae into a multicellular mass. The spore-bearing structures built by multicellular masses are called sorocarps. Sorocarpic development is found in both major lineages of unikonts and in all but one of the major lineages of bikonts.

Sporocarp Sporocarps are spore-bearing structures that develop from a single amoeboid cell. This kind of differentiation is found only among several lineages of Amoebozoa.

Unikont A flagellated cell from which a single flagellum is associated with each flagellar apparatus. In strict unikonty, the flagellar apparatus contains only the basal body from which the flagellum arises. Note: There are a number of kinds of flagellate cells in eukaryotes where there is only one flagellum per flagellar apparatus and there is a second, flagellum-less basal body present. Whether to consider such cells bikont or unikont is subject to various intepretations. However, it is generally agreed upon that such flagellar apparatuses are derived by reduction from ancestors that had bikont flagellar apparatus.

Introduction

At some point in time, probably between 1 and 2 gigayears ago (see Eme *et al.*, 2014; Sharpe *et al.*, 2014), a population of eukaryotes became the foundation, the Last Common Ancestor, of a major lineage of eukaryotes that is often informally designated as unikonts (following Cavalier-Smith, 2002, 2003), although the formal names Amorphea (Adl *et al.*, 2012) and Opimoda (Derelle *et al.*, 2015) have recently been proposed, as well as the informal designation, podiates (Cavalier Smith, 2003). For the sake of brevity, this ancestral population will be referred as LunCA (Last unikont Common Ancestor) from here forward.

The surviving descendants of LunCA include two major eukaryote lineages, or 'supergroups,' Amoebozoa and Opisthokonta (see Adl *et al.*, 2012). In addition, it has recently been suggested that the descendants of LunCA also include some groups of protists that are not members of either of these 'supergroups' (**Figure 1**; **Table 1**). Amoebozoa (**Figure 2**) (see Adl *et al.*, 2012) contains many of the classic 'naked' amoebae, including the namesake genus *Amoeba*, the amoebae that are partially enclosed in a shell (arcellinid amoebae), and several groups of slime molds (mycetozoans). Slime molds are amoeboid organisms that produce 'fungus-like' spore dispersal structures as part of their life cycles (see Spiegel *et al.*, 2004; Olive, 1975).

Opisthokonta (**Figure 3**) (see Adl *et al.*, 2012) has two major lineages: (1) Holozoa (Lang *et al.*, 2002) which includes animals (Metazoa), unicellular and colonial eukaryotes considered the closest relatives of Metazoa (choanoflagellates), and several protist groups, and (2) Nucletmycea (Brown *et al.*, 2009), which includes Fungi, a small group of ameoboid protists called nuclearids, and a varied but monophyletic group of intracellular parasites (see James *et al.*, 2013; Karpov *et al.*, 2014). This latter group is sister to Fungi. It has recently been proposed that the clade that includes the opisthokonts also includes two groups of flagellate protists (breviates and apusomonads) that are in separate lineages from

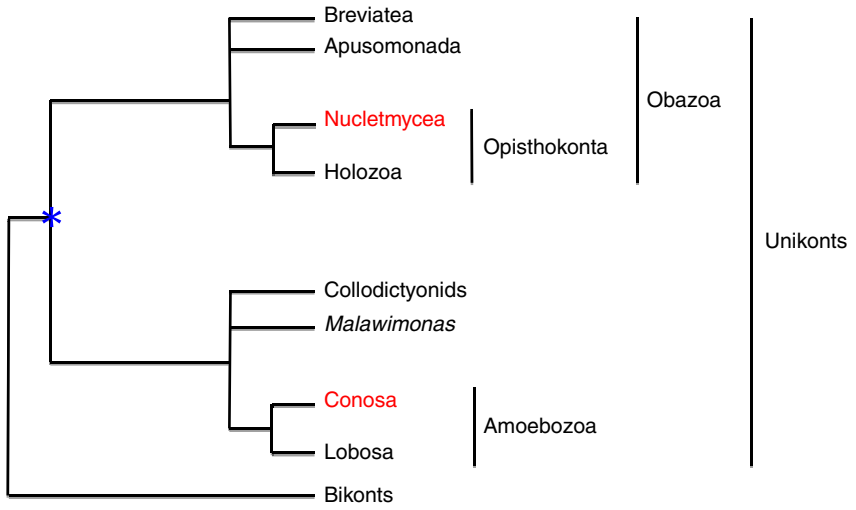

Figure 1 Diagram of phylogentic relationships among unikonts. Taxa in red font are the primary subjects of this article. * = LunCA.

Table 1 Higher level relationships among the living descendants of LunCA

Unikonts (=Amorphea, =Opimoda, =podiates), the living descendants of LunCA					
Status uncertain	***Amoebozoa***			***Obazoa***	
	Conosa	***Lobosa***		***Opisthokonta***	
				Holozoa	***Nucletmycea***
Collodictyonids, *Malawimonas*	**Important members:** **Sporcarpic species of amoebae, Some sorocarpic species of amoebae**	**Important members:** **Some sorocarpic species of amoebae**	Apusomonadidae, Breviatea	**Important members:** **Fungus-like Eccrinales** Choanoflagellates (=Chonomonada) Metazoa	**Important members:** **Some sorocarpic species with amoeboid somatic cells** **Fungi** Rozellids (=Opisthosporidia), includes Microsporidia

Note: Members that are emphasized in this article are in larger, bold font.

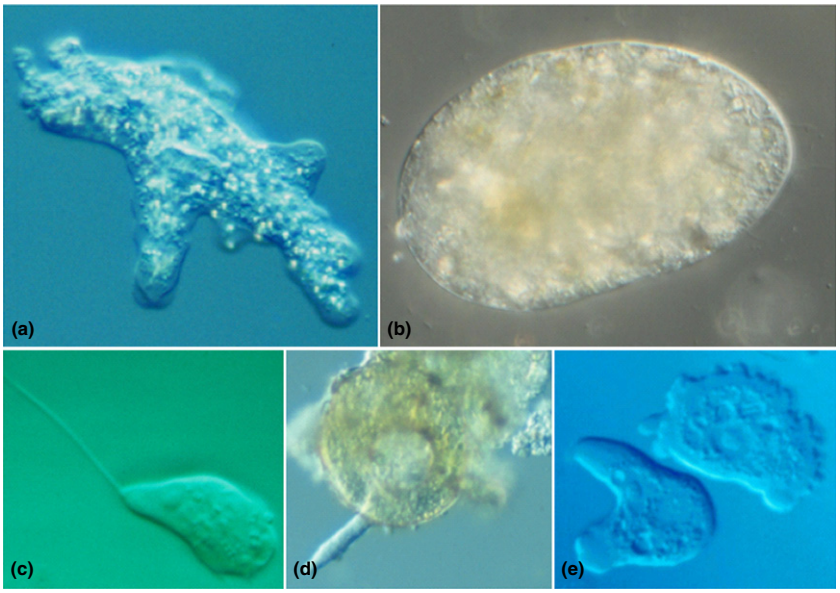

Figure 2 Examples of Amoebozoa. (a) *Amoeba proteus*. (b) *Pelomyxa* sp. (c) Typical flagellate cell of a myxogastrid slime mold. (d) Testate (shelled) amoeba, *Arcella* sp., single pseudopodium extends from opening in yellow shell. (e) Amoebae of the protosteloid amoeba, *Protostelium mycophaga*.

Opisthokonta. The name applied to this more inclusive group is Obazoa (Brown *et al.*, 2013). Therefore, Obazoa (**Table 1**; **Figure 3**) is sister to Amoebozoa.

Finally, Derelle *et al.* (2015), among others, present evidence that two flagellated protist groups (collodictyonids plus the genus *Malawimonas*) form either a monophyletic sister group to Amoebozoa or a monophyletic sister group to Amoebozoa + Obazoa. In either case, they are considered to be among the extant descendants of LunCA. **Table 1** and **Figure 1** summarize the most recent suggestions of relationships among the descendants of LunCA. The groups of unikonts on which this article focuses are highlighted.

Depending on which eukaryotic Tree of Life one accepts, the sister group to the unikonts is either all the other eukaryotes (**Figure 1**) – a group informally referred to as bikonts (Cavalier-Smith 2002, 2003) or more formally, as Diphoda (Derelle *et al.*, 2015) – or, alternatively, unikonts are sister to all other eukaryotes except the Excavata, a group of unicellular protists that often lack typical mitochondria (He *et al.*, 2014). This latter unikont sister group, called Diaphoretickes (Adl *et al.*, 2012) or, informally, corticates (Cavalier-Smith, 2003), along with unikonts, forms a monophyletic sister group to excavates (He *et al.*, 2014). It should also be noted that Katz *et al.* (2012) have argued that opisthokonts are sister to all other eukaryotes, in which case unikonts would be a paraphyletic assemblage, and LunCA would also be LECA (Last Eukaryote Common Ancestor). However, even if that were to be true, the following discussion would still be sound.

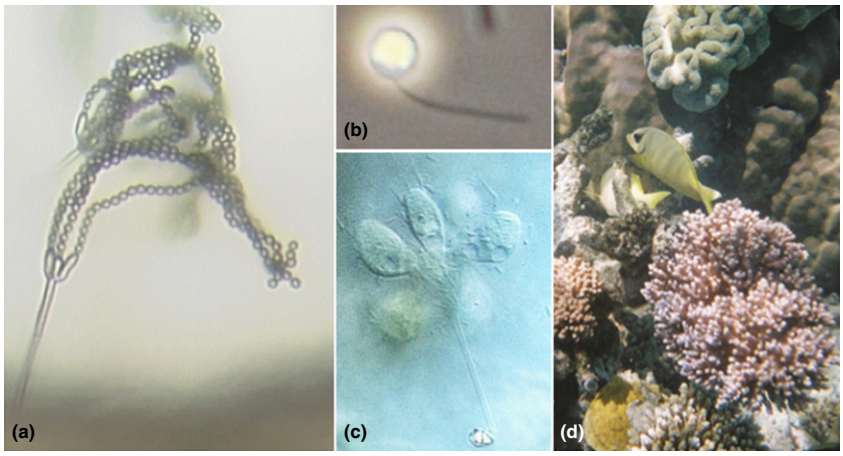

Figure 3 Examples of Opisthokonta. (a) A sporulating member of Fungi, *Penicillium* sp. (b) A typical flagellated cell of opisthokonts, a zoospore of a species of Fungi in the Chytridiomycota. (c) A colonial choanoflagellate. (d) A Metazoa-rich community on the Great Barrier Reef.

When LunCA was alive, it represented the entire unikont lineage that is now represented by its highly diversified descendants. That is a point that should go without saying, but it is often ignored. It is likely that LunCA was not terribly different from many of the other eukaryotes that were extant when it was, but it would have carried some unique traits it could potentially pass on to its descendants that it did not share with any of its contemporaries. Thus, the basic unikont 'toolkit' that LunCA could have passed on to its descendants would have consisted both of the traits that it shared with other eukaryotes, i.e., the symplesiomorphies of the unikonts, and traits that were unique to it, i.e., the synapomorphies of the unikonts. In part, how the elements of that 'toolkit' were passed on, or not, to its descendants is a very important aspect of understanding the subsequent diversification of unikonts. This will be the focus of the rest of this article.

Because there are myriad approaches to discussing the diversification of unikonts from LunCA to the present, and because that would require more space than is available for this article, discussion is limited to the spread of certain sets of characters that were potentially passed from LunCA to its descendants. In particular, the examples focus on unikonts that have traditionally been considered as fungi, s.l. These include the taxon Fungi and the various slime molds found throughout the Amoebozoa and Opisthokonta.

What Traits Could LunCA Have Transmitted to Its Descendants?

By comparing contemporary unikonts with each other, and also by comparing them with other eukaryotes, it is possible to reconstruct some of the traits that had to have been present in LunCA. The following list gives several examples.

1. It had a flagellated stage in its life cycle that consisted of flagellar apparatus that supported two flagella, one extending from each basal body. Because of the complexity of the flagellum, the basal bodies, and the flagellar apparatus (see Yabuki and Leander, 2013), it is extremely unlikely that these structures evolved more than once. Because flagellated cells are found in all lineages of eukaryotes, it must be the case that LECA was flagellated during at least part of its life cycle. Eukaryotes, including many unikonts, that lack any flagellated stage in their life cycle, are non-flagellate because of the loss of flagella.

The term unikont, which means 'single pole (flagellum)' was coined (Cavalier-Smith, 2002) because phylogenetic evidence available at that time suggested that opisthokonts and amoebozoans formed a monophyletic group, and these groups were thought ancestrally to have a single flagellum. Essentially all flagellated cells of opisthokonts have a single flagellum arising from one of a pair of basal bodies, and that flagellum pushes the cell through water, a trait unique to opisthokonts. The opisthokont flagellar apparatus, i.e., the cytoskeletal apparatus associated with the basal bodies, is very reduced compared to the rest of the eukaryotes (Cavalier-Smith 2002; Adl *et al.*, 2012; Yabuki and Leander, 2013). Many amoebozoans have a flagellar apparatus with only a single flagellum (see Adl *et al.*, 2012; Spiegel 1990, 1991) that arises from a single basal body (true unikonty). In amoebozoans that swim, the beating of the flagellum pulls the cell through the water, in contrast to opisthokonts. The flagellar apparatus in amoebozoans is structurally complex, unlike the reduced structure in opisthokonts, and it is relatively easy to recognize homologies shared with other eukaryotes outside the unikonts (Adl *et al.*, 2012; Spiegel, 1990, 1991; Yabuki and Leander, 2013).

However, the assumption that unikonts were ancestrally unikont (Cavalier-Smith, 2002) was based on a number of misconceptions, including observations that had been published prior to 2002 (Spiegel, 1990, 1991). Subsequently, the flagellated protists in Obazoa that are sister to the opisthokonts (apusmonads and breviates, **Figure 3**; **Table 1**) (Brown *et al.*, 2013; Cavalier-Smith, 2013) were shown to have a pair of flagella that arise from a pair of basal bodies, as do several amoebozoans (see Adl *et al.*, 2012; Spiegel, 1990, 1991), and aspects of the flagellar

apparatus of these bikont unikonts have been proposed to be homologous with those found throughout the non-unikont eukaryotes (Spiegel, 1991; Yabuki and Leander, 2013; Spiegel, 2012). Thus, it seems likely that LunCA was bikont and that the unikont condition has arisen separately several times among descendants of LunCA. Because the complexity of the homologies proposed among the flagellar apparatuses of bikonts and unikonts are so structurally intricate, convergence is highly unlikely.

An important point to consider, however, is that the presence of a flagellated cell in its life cycle does not provide evidence to conclude that LunCA was an organism with its sole cell type being a flagellate (i.e., a monad). Whether LunCA had other states, somatic or reproductive, that were not flagellated is presently unclear. There are extant unikonts that are monads, and there are unikonts whose flagellated stages are limited to propagules and/or gametes. Until there exists much more comparative genomic data on a broad range of unikonts, it will be difficult to be certain how LunCA used flagellated states in its life cycle.

2. It was able to feed by phagocytosis. Zuck (1953) coined the term 'phagotroph' to describe organisms that ingest their food, but he did not distinguish carefully between organisms whose cells acquire their own food by phagocytosis and multicellular organisms that bring food into their digestive systems to be digested extracellularly as is the case with most Metazoans. The term phagotrophy here is limited to only those organisms whose cells acquire a significant portion of their nutrition by phagocytizing other organisms. A strong case can be made that LECA was phagotrophic, s.s. (see Cavalier-Smith, 2013) and the presence of phagotrophic members in all major lineages of unikonts is entirely consistent with LunCA being phagotrophic.
3. LunCA was able to produce pseudopodia. All members of all major lineages of unikonts contain species that produce pseudopodia, at least in association with phagocytosis (see Derelle *et al.*, 2015; Adl *et al.*, 2012). Whether LunCA had cells that regularly moved using pseudopodia, i.e., ameoboid cells, as a part of its life cycle is not clear. Ameoboid cells are present in most major lineages of eukaryotes (see Adl *et al.*, 2012), but the work has not yet been done to evaluate whether the underlying genetics of being ameoboid is a result of a single origin or multiple origins. Therefore, more work is necessary to determine whether all pseudopodial cells in unikonts share homologies.
4. LunCA had stages in its life cycle in which cells were surrounded by cell walls. Walled cells as cysts, spores, or somatic cells are found in all groups of unikonts (see Adl *et al.*, 2012). About the only group of unikonts lacking a walled-cell stage is the Metazoa. Walled-cell stages are also present in almost all groups of non-unikont eukaryotes as well. So, being able to produce a cell wall might be a universally present trait of all eukaryotes that has been retained by unikonts. However, sufficient work has not been done to determine whether the ability to produce cell walls in eukaryotes, in general, and unikonts, in particular, is homologous or if it has arisen multiple times.
5. It was sexual. Almost all eukaryotes, including the major lineages of unikonts, that have been examined carefully enough, have a life cycle in which haploid nuclei produced by meiosis eventually give rise to diploid nuclei via karyogamy (see Spiegel, 2011; Lahr *et al.*, 2011a; Adl *et al.*, 2012; Cavalier-Smith, 2013). Meiosis is so complex and stereotypical that it is extremely unlikely that it had multiple origins (Spiegel, 2011). However, evidence is still lacking to determine whether the somatic stages of LunCA (here defined as those stages in which mitosis occurs) were haploid, diploid, dikaryotic, or a combination of these. Thus LunCA's life cycle is open to speculation.

There are other aspects of LunCA that could be hypothesized, but these examples provide a framework to develop hypotheses concerning how the unikonts have diversified from their start as LunCA. It should be noted that no characters of LunCA yet mentioned are proposed to be synapomorphies of unikonts. At this point, that would be premature since not enough evidence exists on which to base such hypotheses.

There follow some examples of how certain, but not all, aspects of diversification could have been manifested in some of the mycological descendants of LunCA.

Diversification of the Mycological Descendants of LunCA

Cellular slime molds, a.k.a. sorocarpic amoebae (Brown *et al.*, 2011; Brown and Silberman, 2012; Spiegel *et al.*, 2004; Olive, 1975), are amoeboid organisms in which individual amoebae, upon starvation or some other signal, aggregate to form a multicellular fruiting body, or sorocarp, containing walled, dormant spores. Sorocarpic amoebae are found in all supergroups of eukaryotes (Amoebozoa, Opisthokonta) except Archaeplastida (red algae, green algae, and land plants) (see Brown and Silberman, 2012) and thus sorocarpy represent the most phylogenetically diverse kind of multicellularity found in eukaryotes. It is found in three major sublineages of the unikonts, two within Amoebozoa (dictyostelids in the Conosa lineage, **Figure 4**; and copromyxids in the Lobosa lineage, **Figure 5**), and one within Opisthokonta (the nucleariids in the Nucletmycea lineage, **Figure 6**) (see Adl *et al.*, 2012). It seems unlikely that either LunCA or, more distantly, LECA were sorocarpic amoebae. However, as pointed out above, LunCA could well have had an amoeboid stage in its life cycle, and since amoebae are found in almost all of the major lineages of eukaryotes, LECA may also have had an ameoboid stage in its life cycle.

In examining the diversification of unikonts, two questions, at least, seem obvious. (1) To what extent are the amoebae of unikonts homologous to each other? (2) To what extent are cell signaling and sorocarp development homologous among the diverse groups of cellular slime molds? In other words, how many times did these traits arise?

We know that amoebae are widespread among eukaryotes, but the genetic underpinnings of amoeba development and physiology have not yet been well studied to determine if the ameoboid condition has a single or multiple origins in eukaryotes. Unikonts offer an ideal system from which to begin to address the possible homology of amoebae. Briefly, if there exists some subset of genes that are expressed in the amoeboid stages of all unikonts, then that would suggest that LunCA had

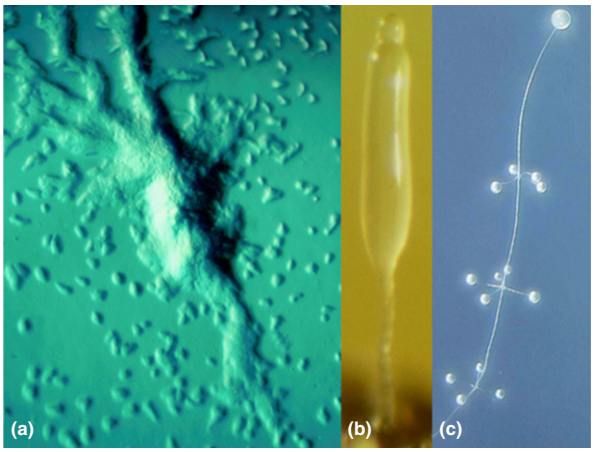

Figure 4 The stages in sorocarpic development in the amoebozoan dictyostelid sorocarpic amoebae. (a) Aggregation of individual amoebae into a central, multicellular mass. (b) Differentiation of the multicellular mass such that it has some cells that become stalk and some that will become spores. (c) A mature sorocarp in which masses of spores, propagules, are suspended in the air by multicellular stalks.

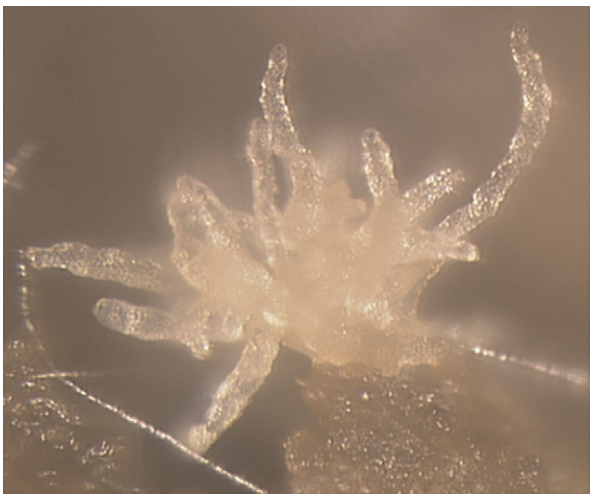

Figure 5 The mature sorocarp of the amoebozoan sorocarpic amoeba, *Copromyxa*. In this case spores develop as amoebae aggregate and pile on top of one another. Photo by Matthew W. Brown.

an ancestral amoeboid state. If those same genes, or a subset of those genes is found in amoeboid stages of non-unikont eukaryotes, that potentially provides support for an ancestral ameoboid state of LECA.

While it seems unlikely that sorocarpy, the aggregation of amoebae into a fruiting body containing walled dormant spores, had a single origin in unikonts (see Brown and Silberman, 2012), it is quite possible that some aspects of cell signaling necessary for both aggregation and triggering cell dormancy and cell wall development could have been present in LunCA or, more broadly, in LECA. Studies in comparative genomics and gene expression during development may possibly be able to reconstruct the evolution of developmental

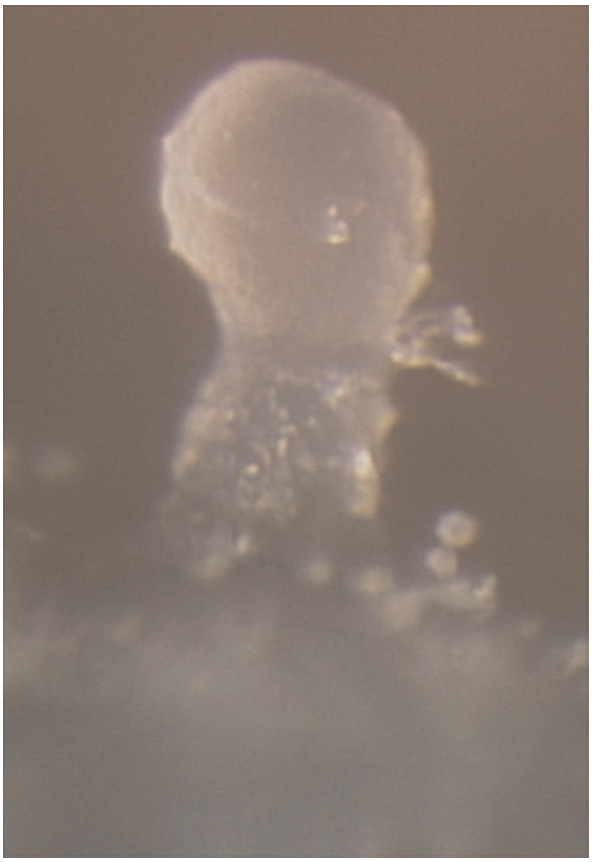

Figure 6 The mature sorocarp of the opisthokont sorocarpic amoeba, *Fonticula*. In this case the aggregate secretes an extracellular stalk through which the cells move to form a ball of spores at the apex. Photo by Matthew W. Brown.

events that are universal or phylogenetically limited in aggregation and spore development, shedding light on the number of origins of this complex trait. Also, as mentioned in Brown *et al.* (2009), the aggregation of cells in opisthokonts can be found in Fungi and in Metazoa even though the result of aggregation is not a sorocarp. The evolution of aggregation-related mechanisms could possibly be more widespread than just for cellular slime molds.

Sporocarpy, in which a single amoeba differentiates into a spore-bearing fruiting body, called a sporocarp (see Adl *et al.*, 2012; Spiegel *et al.*, 2016; Shadwick *et al.*, 2009, Spiegel *et al.*, 2004) is found solely in Amoebozoa among two groups: The plasmodial slime molds (Myxogastria), which have a life cycle in which a large, multinucleate amoeba, the plasmodium, eventually differentiates into a sporocarp containing many spores (**Figure 7**), and various groups of microscopic protosteloid amoebae, which individually round up, secrete a stalk, and then differentiate into one to a few spores (**Figure 8**).

Until recently, sporocarpy, because of its similarities, was considered to be a synapomorphy of a monophyletic subset of Amoebozoa (called Eumycetozoa) (see Olive, 1975; Spiegel *et al.*, 1995). However, Shadwick *et al.* (2009) and subsequent molecular phylogenetic studies, for example, Lahr *et al.* (2011b), clearly show that protosteloid amoebozoans form a para/polyphyletic assemblage in Amoebozoa. Sporocarpy may

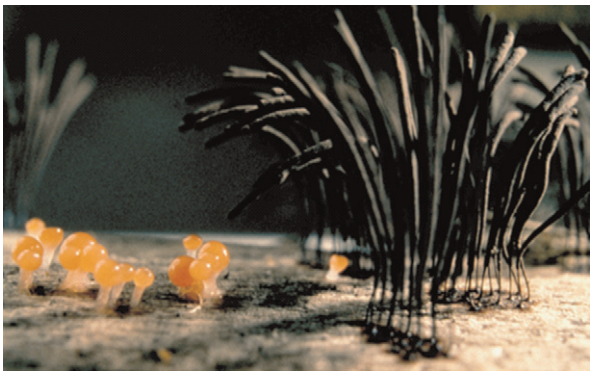

Figure 7 Sporocarpy in two different macroscopic amoebae in the myxogastrid amoebozoans. The species of *Hemitichia* on the left still consists of multinucleated, single cells that are secreting an extracellular stalk. The species of *Stemonitis* on the right has matured fully such that the multinucleate cell has divided up into uninucleate spores that are suspended above the substrate by the stalk. Each *Stemonitis* sporocarp is about 2–2.5 cm tall.

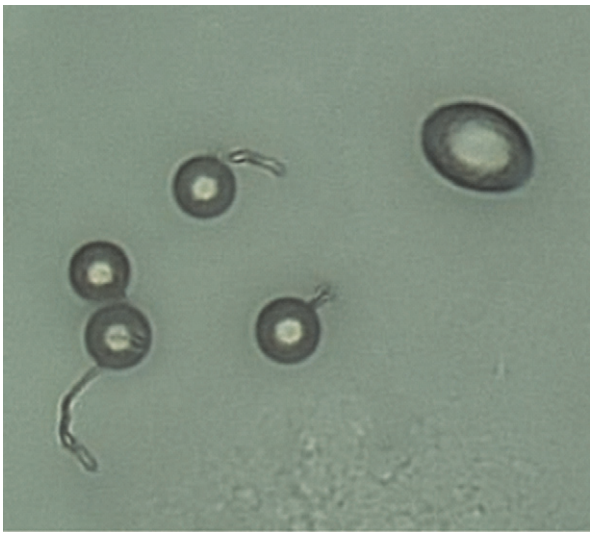

Figure 8 Microscopic, stalked, single spored sporocarps of the protosteloid amoebozoan, *Protostelium mycophaga*. The cell at the upper right is an amoeba that has rounded up prior to synthesizing a stalk. The cell will become a spore once stalk synthesis is completed.

prove to have one origin or it may have evolved independently several times. More work is needed to demonstrate if the genetic underpinnings of sporocarpy are homologous or not. Should they prove to be, that would suggest that the Last Common Ancestor of Amoebozoa was sporocarpic. Since nothing similar to sporocarpy is found in Obazoa, it would appear to be unlikely that LunCA was sporocarpic.

The taxon Fungi is the best-known group in the Nucletmycea lineage of Opisthokonta. It is sister to a group of protists that are intracellular parasites (see James *et al.*, 2013; Corsaro *et al.*, 2013). Two related characteristics of Fungi that will be discussed here in relation to their place in the unikonts are the fungal mode of nutrition and the presence in many

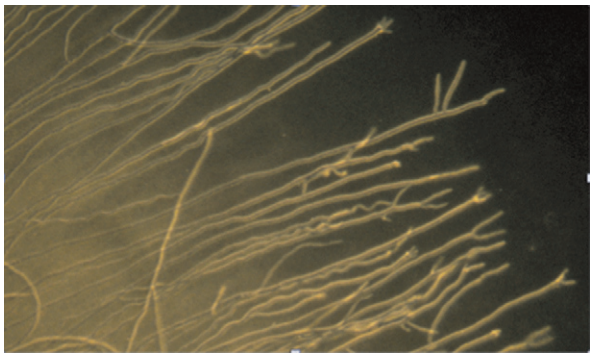

Figure 9 A portion of a mycelium of a member of Fungi in the genus *Penicillium*. Notice that it consists of many branching, filamentous hyphae.

lineages of Fungi of a somatic stage that is filamentous, the state called the mycelium (see Dee *et al.*, 2015).

Fungi are lysotrophic, a term also coined by Zuck (1953). This means that they release digestive enzymes into the extracellular medium, and absorb the digestion products. This is a form of absorptive nutrition. All organisms may be able to use lysotrophy to some extent, but few rely on it entirely. Two major groups of opisthokonts are lysotrophic, Fungi and Metazoa except the sponges. Also within the Holozoa, the fungus-like Eccrinales may be lysotrophic. While most people would call animals phagotrophs, they excrete extracellular enzymes into the topologically external space in the digestive tract and absorb the digestion products. Some fungus-like members of the bikont lineage Stramenopila, for example, water molds (oomycetes), are also lysotrophic (see Adl *et al.*, 2012).

By carrying out comparative studies of the physiology and genetic basis of lysotrophy in Fungi, Metazoa, and Stramenopila, it should be possible to determine whether the kind of lysotrophy found in Fungi is homologous with that in Metazoa or stramenopiles. If only the former, that would be the evidence that this process is not something that occurred in LunCA. If it is homologous in all exclusively lysotrophic eukaryotes, then it must have been present in LECA and, therefore, also in LunCA. Obviously, it is possible that some elements of lysotrophy could be more universal than others.

Fungi are the only group of unikonts, with the exception of the Eccrinales, whose cells are walled during the part of the life cycle during which they take in nutrients. Because of that, they cannot use phagotrophy, nor can they move around actively when in this state. That puts constraints on how they can successfully acquire nutrients. Many fungi are mycelial (**Figure 9**). That is, they have filaments, hyphae, that grow from their tips and penetrate and surround the substrates they are digesting (see Dee *et al.*, 2015). A mycelium has a high surface-to-volume ratio that makes absorption of digestion products efficient. In Fungi, the phylogenetic distribution of mycelial morphology suggests that mycelial growth arose at least three independent times (Dee *et al.*, 2015).

Among unikonts, mycelium-like growth is found only in Fungi and the Eccrinales, both opisthokonts (see Adl *et al.*, 2012). It is not present in Amoebozoa. The only other place it is found among the eukaryotes is in the oomycetes, the

stramenopile analogues of Fungi. In order to test the hypothesis of Dee *et al.* (2015), comparative studies of gene expression during hyphal growth initiation and growth of established hyphae in the mycelial lineages of Fungi, Eccrinales, and oomycetes should be carried out in addition to microscopic and ultrastructural examination. These would establish the levels of homology among the underlying mechanisms of mycelial growth. Only then will it be safe to conclude if any mycelium-specific traits are limited exclusively to certain groups, and only then will it be possible to conclude that there were multiple independent origins of mycelial growth. In essence, then, it will be possible to determine whether the last common ancestor of Fungi, the last common ancestor of opisthokonts, or LECA, and of necessity LunCA, had the potential to grow as a mycelium as part of its life cycle.

Acknowledgments

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See also: Archaeplastida: Diversification of Red Algae and the Green Plant Lineage. Endophytic Microbes, Evolution and Diversification of. Fungal Evolution: Aquatic–Terrestrial Transitions. Lichen-Forming Fungi, Diversification of. Mycorrhizal Fungi, Evolution and Diversification of. Protist Diversification

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V

Vertebrates, the Origin of

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You are a member of the group 'Vertebrata,' as is every creature with a backbone: cats, eagles, poison dart frogs, salmon, great white sharks, lamprey, etc. (Figure 1). If you look at the base of the phylogenetic tree shown in Figure 1, you will see the species highlighted as 'the ancestor of the vertebrates'. About 530 million years ago, this species was flourishing in early Cambrian seas. What did the world of the vertebrate ancestor look like? The supercontinent of Rodinia had begun to fragment (Figure 2), leaving the massive continent of Gondwana (South America, Africa, Australia, Antarctica, India) to the

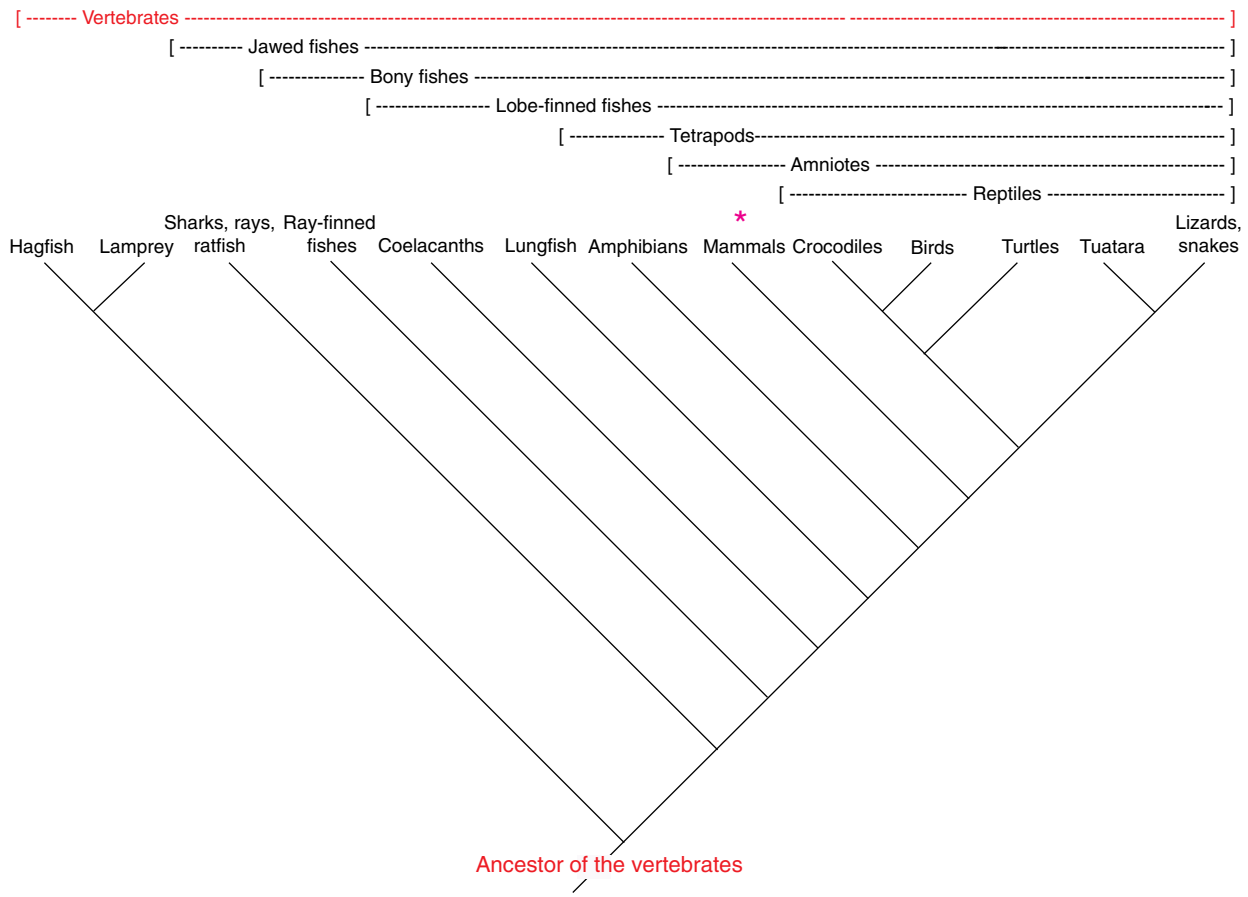


Figure 1 'Wave your fins in the air'. Family (phylogenetic) tree for major extant (=living, fossil groups not included) groups of animals with backbones (Vertebrata). *=you! If you trace your ancestry back from the small group to which you belong (mammals) you can see that you are a jawed, bony, lobe-finned fish with a backbone, four legs and an amniotic egg. To understand this diagram better, listen to *The Devonian Blues* by Ray Troll (www.youtube.com/watch?v=g9j0Mq0knDo). In the song, the following terms refer to the common names on this figure: Sarcopterygian=lobe-finned fish; Dipnoi=lungfish.

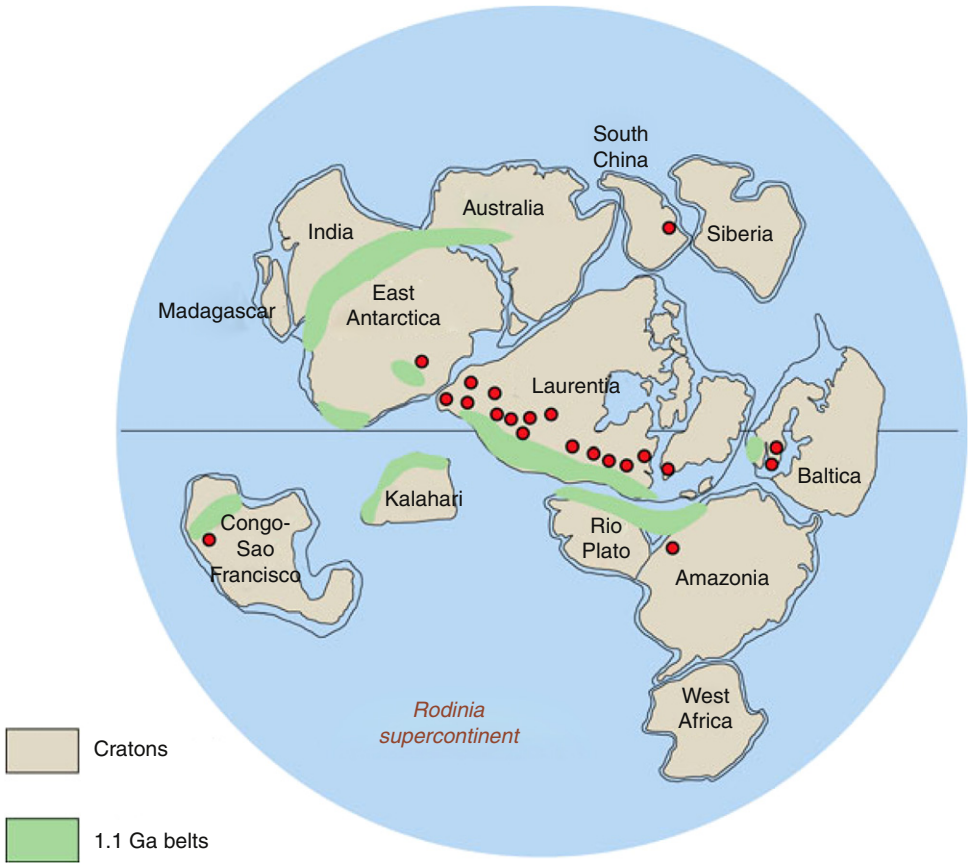


Figure 2 The supercontinent of Rodinia beginning to break apart about 750 million years ago. Author: John Goodge (commons.wikimedia.org/wiki/File:Rodinia_reconstruction.jpg).

south. Laurentia (North America) was drifting northwards to the Equator; Baltica (northern Europe), Siberia and South China were drifting northeastwards away from Laurentia and each other (for an image see www.ucmp.berkeley.edu/cambrian/cambrian.php). Much of the smaller, northerly landmasses were covered by shallow seas, formed as glaciers from the late Proterozoic ice age melted and sea levels rose. These seas were undergoing a transformation as the levels of oxygen (allows more efficient metabolism), calcium (along with phosphorus, necessary to produce hard tissues such as bone and teeth), and phosphorus (necessary for metabolic processes, building cell membranes and DNA/RNA) began to increase, while the sea itself became less saline (Zhang *et al.*, 2014 and references therein). It was a time of great biological exuberance; the majority of invertebrate phyla became established during the Cambrian explosion (540–520 Ma). By 530 million years ago, the shallow, warm seas were teeming with life, with complex ecosystems comprising single celled bacteria, phytoplankton and zooplankton, and multicellular burrowers, filter feeders, detritivores and predators. Part of this complexity was the ancestor of the vertebrates.

What exactly did this ancestor look like? To answer this question we must first establish what exactly a vertebrate is. Oddly, this is not as easy as it seems.

Who Are the Vertebrates, Really?

Historically, there have been three extant (living) groups involved in this discussion, hagfish, lamprey, and gnathostomes (jawed fishes; **Figure 1**), along with a bevy of fossil 'fishes,' both jawed and jawless. Their relationships one to another have been the basis of heated debate for nearly three centuries.

Linnaeus (1958) is widely credited for recognizing that creatures with backbones formed a group distinct from other animals. But where exactly did the hagfish (**Figure 3(a)**) and lamprey (**Figure 3(b)**) fit in this scheme? Artedi (1738) grouped lamprey in the Chondropterygii with sturgeon, dogfish, and rays. Linnaeus (1758) agreed but added the chimera and renamed the group Nantes. He placed hagfish, which Artedi had not discussed, in Vermes (along with leeches, earthworms, and some parasitic flatworms). Duméril (1806) was the first biologist to classify lamprey and hagfish together, in the family Cyclostomes, still related to sharks and rays. Cope (1889) dramatically restructured the whole picture, oddly characterizing the Vertebrata as containing the invertebrate groups Hemichorda (acorn worms), Cephalochorda (lancelets) and Urochorda (tunicates) plus the Craniata. The Craniata, in turn, was subdivided into the Agnatha (jawless fishes: Ostracodermi (extinct) + Marsipobranchii (=Duméril's

Figure 3 (a) Hagfish are unusual looking creatures, to say the least. Reproduced with permission of the photographer, Jan Halvarson, and the Vancouver Aquarium (www.aquablog.ca/2012/04/the-hagfish-a-face-only-its-mother-could-love-2/). (b) Lamprey doing what it was named for, suckering onto a stone (Petromyzontiformes: *petros* (stone) + *myzon* (sucking)). Reproduced with permission of the photographer, Juanjo Alonso (freefins.com).

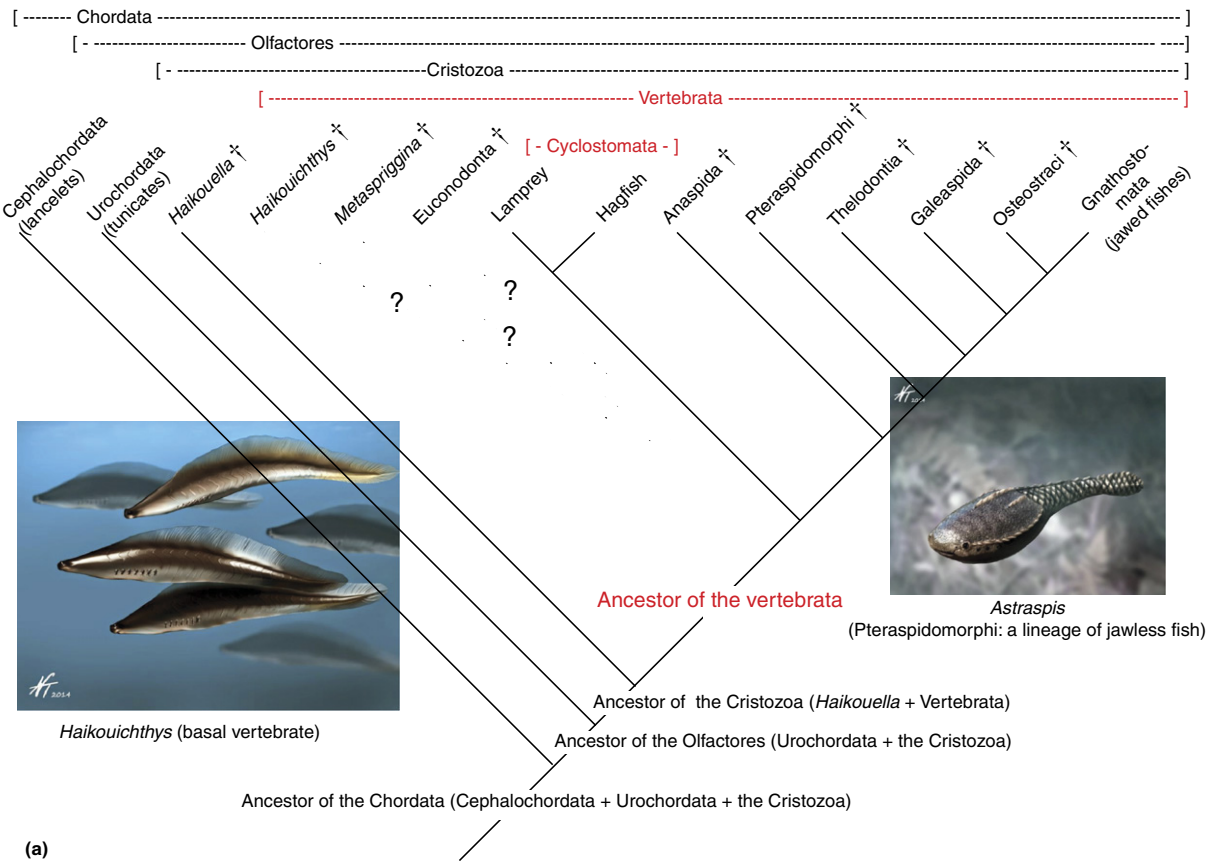
Cyclostomes)) and 4 classes of jawed animals (In 1898, Hans Gadow wrote a book (Gadow, 1898) that seems to have been largely ignored. In it he proposed that the vertebrates consisted of the Acrania (lancelets) and the Craniota, which was subdivided into three superclasses, Cyclostomata (name attributed to Richards; hagfish + lamprey), Hypostomata (various extinct groups) and the Gnathostomata (name attributed to Haeckel).). Cope's Ostracodermi was a grab bag of fossil fishes placed together solely because they had no jaws. As more fossil taxa were added to (and removed from) the agnathans, disputes over the relationships among these taxa escalated, but most authors still supported a monophyletic Cyclostoma. In 1927, this view was shaken when Stensiö (1927) concluded that lamprey and hagfish were more closely related to different extinct jawless fishes than to each other (see discussion in White, 1935).

Debates concerning the relationships of the jawless fishes to one another and to jawed fishes continued among the paleontologists, but most neontologists continued to support the sister-group relationship between lamprey and hagfish. This consensus vanished when Løvtrup (1977) published a phylogenetic tree based on morphological and physiological traits indicating that lamprey were more closely related to jawed fishes (Gnathostomata) than to hagfish, leading Janvier (1981) to propose that systematic terminology should be changed to reflect the new hypotheses of relationships: Vertebrata, diagnosed by the presence of vertebral elements, included gnathostomes, lamprey and some jawless fossil fishes, while the term Craniata would be applied to the larger group characterized by a skull and otic, optic and olfactory capsules (hagfish, other jawless fishes, vertebrates). Subsequent morphologically based studies overwhelmingly supported the Vertebrata–Craniata distinction (Maisey, 1986 and references therein) and for a time, relative peace reigned again.

Fifteen years later, however, the newly emerging field of molecular systematics began to cause uneasiness once more, when the first phylogenetic systematics-based tree resurrected a monophyletic Cyclostomata (Stock and Whitt, 1992). This uneasiness was amplified as subsequent studies provided evidence both for, and against, the phylogenetic hypothesis. But with the dawn of the new millennium, molecular analyses began to show overwhelming support for monophyly (reviewed in Near, 2009), although many morphologists remained, paradoxically, unconvinced. A study by Heimberg *et al.* (2010) based on microRNAs (highly conserved, noncoding genes) seems to have tipped the balance as Janvier (2010), echoing the sentiments of many biologists, wrote "I am impressed by the evidence … and prepared to admit that cyclostomes are, in fact, monophyletic". After four years and no further data sets emerging to challenge the hypothesis of monophyly, it appears that molecular data have finally returned us to the initial intuitions of morphologists: lamprey and hagfish are united in a group (Cyclostomata) that forms the sister-group to the remaining jawless and jawed fishes (**Figure 4**).

Why does all this matter? There are two ways to answer this question. First, science is based on the search for the hypothesis that is best supported by the data, so there will always be periods of intense debate as new data are discovered. So the preceding discussion is a textbook example of why the scientific process 'works'. Second, and more importantly for this article, hypotheses of evolutionary relationships form the framework upon which questions of character evolution and speciation are investigated (Brooks and McLennan, 1991, 2002 and references therein). If we want to understand more about the ancestor of the vertebrates, we need to know where that ancestor fits on a larger phylogenetic tree. Any conclusions we draw from those genealogical patterns are only as strong as the phylogenetic hypothesis – so this is why the decades of debate, which have gradually begun to converge on a consensus, are important.

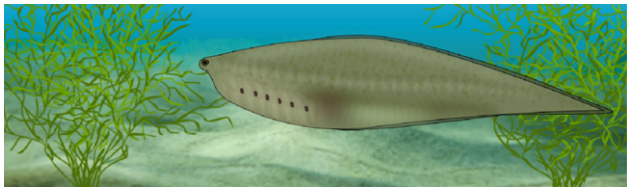
Why was it so difficult to reach consensus in the first place? Aside from the obvious problems with character description and data analysis, the answer to this question is simple – hagfish (~29 species with at least four awaiting description); creatures that appear at once as extremely simplified (for a 'vertebrate') and extremely odd (**Figure 3(a)**). The idea that hagfish were very simplified arose because they evidently had poorly developed eyes with no lens, only one semicircular canal in their inner ears, no vertebral elements and no lateral line (Contrary to the common wisdom, recent studies have shown that some species of hagfish do develop rudimentary vertebral elements (Ota *et al.*, 2014) and an embryonic lateral



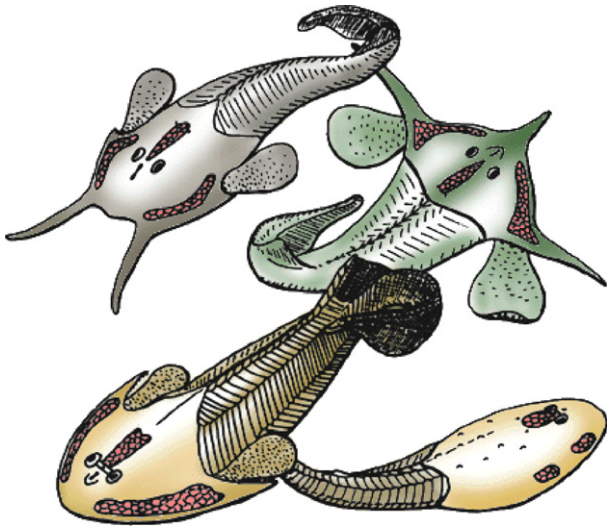
Haikouichthys (basal vertebrate)

Astraspis
(Pteraspidomorphi: a lineage of jawless fish)

(a)



(b)



(c)

line (Wicht and Northcutt, 1995).). Their oddness came from their ability to eject streams of mucins that coagulate into a wall of slime upon contact with seawater (hence one of their less complimentary names of 'snot eels') and their skill at tying themselves in a knot during feeding or when grabbed by a predator (or biologist). The unusual traits are unique to hagfish. It is the 'simple' traits that are problematic because they can be interpreted in two ways. Either (1) hagfish represented an early stage in vertebrate evolution, in which case the evolutionary scenarios for many characters would appear to be a straightforward linear progression from 'simple-complex' or (2) hagfish were secondarily simplified, in which case the evolutionary scenarios for character evolution might be more complicated. The current configuration of the phylogenetic tree, coupled with recent studies on the genetics and development of hagfish, support the latter scenario.

All of this is very interesting in terms of investigating hagfish biology. But what does it mean in terms of reconstructing the vertebrate ancestor?

Meet Your Ancestors (Some of Them)

All organisms are a mosaic of conserved (plesiomorphic) and novel (apomorphic) traits, there is no such thing as an 'all plesiomorphic' species, although many people often mistakenly equate the term 'ancestor' to mean 'all plesiomorphic'. So, in order to reconstruct what the vertebrate ancestor (=VA) might have looked like, we have to go further back in time to disentangle the conserved old traits from the unique new ones.

The following discussion is based on the origin and diversification of morphological traits depicted in **Figure 5** (see **Figure 4** to identify the various ancestors discussed in the following paragraphs). Phylogenetic trees are hypotheses of the genealogical (evolutionary) relationships among taxa. You read them like you would any genealogical diagram – the characters are mapped onto the ancestor in which they are hypothesized to originate. So, for example, say all your ancestors were blue-eyed until your great grandfather married a brown-eyed woman, thus introducing a change in the trait 'eye color' into your family. From that point onwards your family tree will include both blue-eyed and brown-eyed descendants, brown being traced back to that one ancestral event.

Based upon characteristics of the oldest chordates (Cephalochordata (lancelets) and Urochordata (tunicates)), we hypothesize that the following traits evolved before the origin of the vertebrates: caudal, dorsal, and ventral fins; numerous slits in the pharynx; a dorsal, hollow nerve cord expanded anteriorly into a rudimentary two part brain; a collagenous notochord providing support for somatic muscles (myomeres); a rudimentary light sensing eye (ancestor of the Chordata); a one-chambered heart; and type 1 mechanosensory cells (whether these cells functioned as a precursor to the lateral line, or as hydrostatic balance is currently unknown; ancestor of the Olfactores). In the ancestor of the Cristozoa, the pharyngeal slits, which had functioned solely in filter feeding up to this point in time, were modified to serve more of a respiratory role (possibly true gills: Chen, 2008 and references therein). Importantly, this is also the point at which large, paired eyes appear. These small, fish-like creatures are thought to have prowled the shallow seas, sucking up small soft-bodied invertebrates from the substrate by means of a more powerful muscular pumping system that drew water into their mouths and out over their gills. (Interestingly, there is evidence that some of that musculature is present in lancelet larvae, disappearing when the larva goes through a complicated metamorphosis to become a filter-feeding adult (Yasui *et al.*, 2014). If so, then the precursor to vertebrate muscular ventilation was already established in the ancestor of the chordates). Prior to this, water had been moved by the beating of small, hair-like structures called cilia lining the pharynx. The change in pharyngeal slit morphology and the way in which water was moved from the environment across those openings was thus associated with two major ecological shifts – from a predominantly low activity, filter feeder, to a much more active, free-swimming benthic feeder; something that would have been promoted by the development of a larger two-part brain and paired eyes.

When you look at **Figure 5** it is obvious that the 'vertebrate ancestor' is characterized by numerous morphological innovations. These traits, however, are only depicted in this way because as of yet there is no consensus about the relationships among the putative basal-most vertebrate species (e.g., *Haikouichthys* and *Metaspriggina*) and, even when that gets resolved, the level of detail described (so far) from the fossils is not sufficient to resolve the state of many characters (Donoghue and Keating, 2014). Ignoring the perplexing euconodonts, for which very little soft tissue characters have been preserved, it appears that the common ancestor of *Haikouichthys* + *Metaspriggina* + everyone else possessed, minimally, rudimentary vertebrae (which, as shown on **Figure 5**, may be even older), paired eyes with a lens, a nostril (possibly), nasal sacs (possibly), rudimentary fin-rays and, in *Metaspriggina* at least, a rudimentary skull (Shu *et al.*, 2003; Zhang and Hou, 2004; Conway and Caron, 2014, references therein and, at the end of the article, an artistic depiction of *Metaspriggina* by M. Collins). The exact point in time in which the remaining characters evolved will be difficult to determine – molecular studies place the potential for trait development in the vertebrate ancestor based on data from extant species – but that reconstruction does not contain molecular information from the basal extinct animals. Still, it is obvious

Figure 4 (a) Simplified phylogenetic tree for the Chordata (see e.g., Near, 2009; Goudemand *et al.*, 2011; Conway and Caron, 2014 and references therein), identifying ancestors of various groups discussed in the text. ?=There is no consensus on the exact relationships among these extinct groups. Researchers do agree that *Haikouichthys* and *Metaspriggina* are basal vertebrates, but exactly where the Euconodonts fit is still a mystery. The Anaspida, Pteraspidomorphi, Thelodontia, Galeaspida, and Osteostraci are an assemblage of extinct (indicated by †) jawless fishes (they are not a monophyletic group). The *Haikouichthys* and *Astraspis* illustrations are reproduced with the permission of the artist, Nobu Tamura (paleoexhibit.blogspot.ca/2014/04/early-vertebrates-myllokunmingiidae.html and paleoexhibit.blogspot.ca/2014/04/jawless-armored-fish-from-ordovician_20.html). (b) *Metaspriggina*. Author: Giant Blue Anteater (en.wikipedia.org/wiki/File:Metaspriggina_and_Maripolia.png). (c) Morphological diversity within the Osteostraci. Author: Philippe Janvier (https://commons.wikimedia.org/wiki/File:Osteostraci_Janvier.gif).

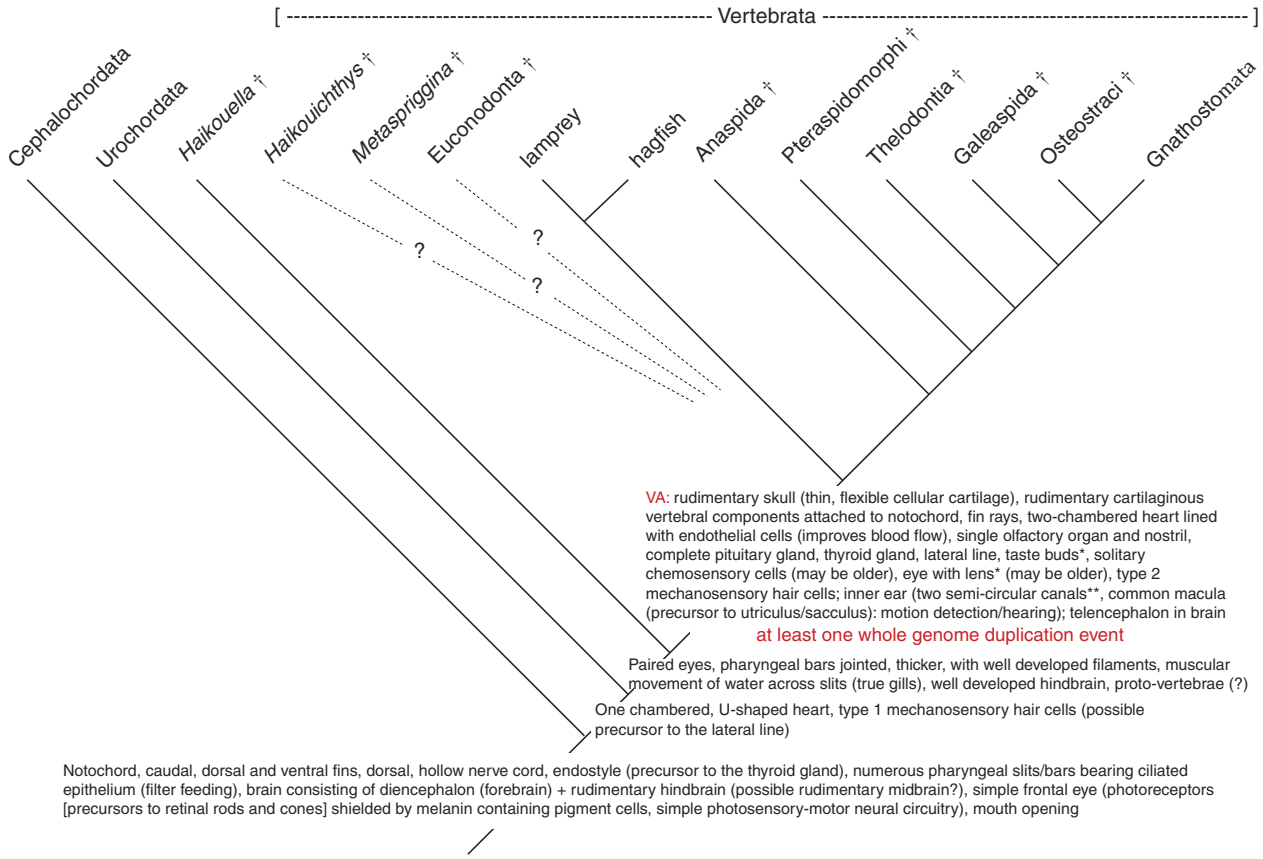


Figure 5 The vertebrate ancestor (VA) is a mosaic of morphological traits that are older than, and unique to, vertebrates. Characters are placed on the phylogenetic tree in the ancestor in which they are thought to have originated. ?=Data are not robust for this trait. ∗=lost or poorly developed in hagfish; ∗∗=hagfish only have one semicircular canal. The information shown on this phylogenetic tree is based on the following references: Brain: Holland and Short, 2008; Sugahara *et al.*, 2013; Cañestro *et al.*, 2013; Eye: Vopalensky *et al.*, 2012; Slingsby *et al.*, 2013; *Haikouella*: reviewed in Chen, 2008; Heart: Davidson, 2007; Monahan-Earley *et al.*, 2013; Lateral line: Wicht and Northcutt, 1995; Mechanosensory cells/inner ear: Hammond and Whitfield, 2006; Manni *et al.*, 2006; Gasparini *et al.*, 2013; Fritzsch and Straka, 2014; Mouth: Soukup *et al.*, 2013; Olfaction: Oisi *et al.*, 2013; Skull: Shu *et al.*, 2003; Mallatt and Chen, 2003; Jandzik *et al.*, 2014. Taste buds and solitary chemosensory cells: Kirino *et al.*, 2013; Vertebrae: Shu *et al.*, 2003; Ota *et al.*, 2014.

that a great deal of morphological innovation was happening in a very brief (evolutionarily) period of time. How is that possible?

Generating Novelty: How Did the Vertebrate Ancestor Get To be So Different?

In 1970, Susumu Ohno proposed that duplication of the entire genome provided an important source of raw material upon which evolutionary processes could act. Based upon comparing genome sizes across various groups, Ohno (1970) suggested that two such widespread duplication events had occurred before and during vertebrate diversification. Subsequent research, primarily on Hox gene clusters (**Figure 6**), moved the timing of these events somewhat; two rounds of whole genome duplication (WGD) were hypothesized to have occurred in vertebrates, the first in an early ancestor of the group (**Figure 7**), followed by a second round sometime after the split between the cyclostomes and origin of the jawed vertebrates (Holland, 1992; Holland *et al.*, 1994). Although the 2R (two rounds) hypothesis was initially the subject of intense debate, support has since come from a wide variety of gene families (see, e.g., Panopoulou and Poustka, 2005; Steinke *et al.*, 2006; Huang *et al.*, 2010; Huminiecki and Heldin, 2010; Tostivint *et al.*, 2013; Hartmann *et al.*, 2014).

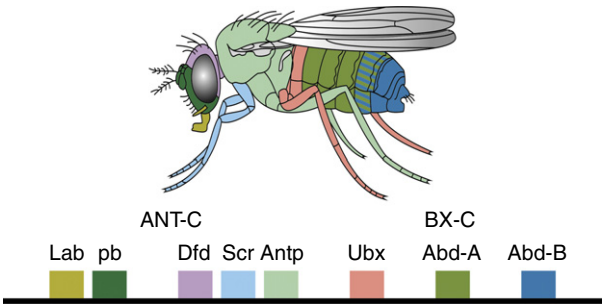


Figure 6 Diagram showing the areas whose development is influenced by eight Hox genes in the fruitfly, *Drosophila melanogaster*. For a discussion of Hox gene functions, see https://en.wikipedia.org/wiki/Hox_gene. Author: PhiLiP (https://commons.wikimedia.org/wiki/File:Hoxgenesoffruitfly.svg).

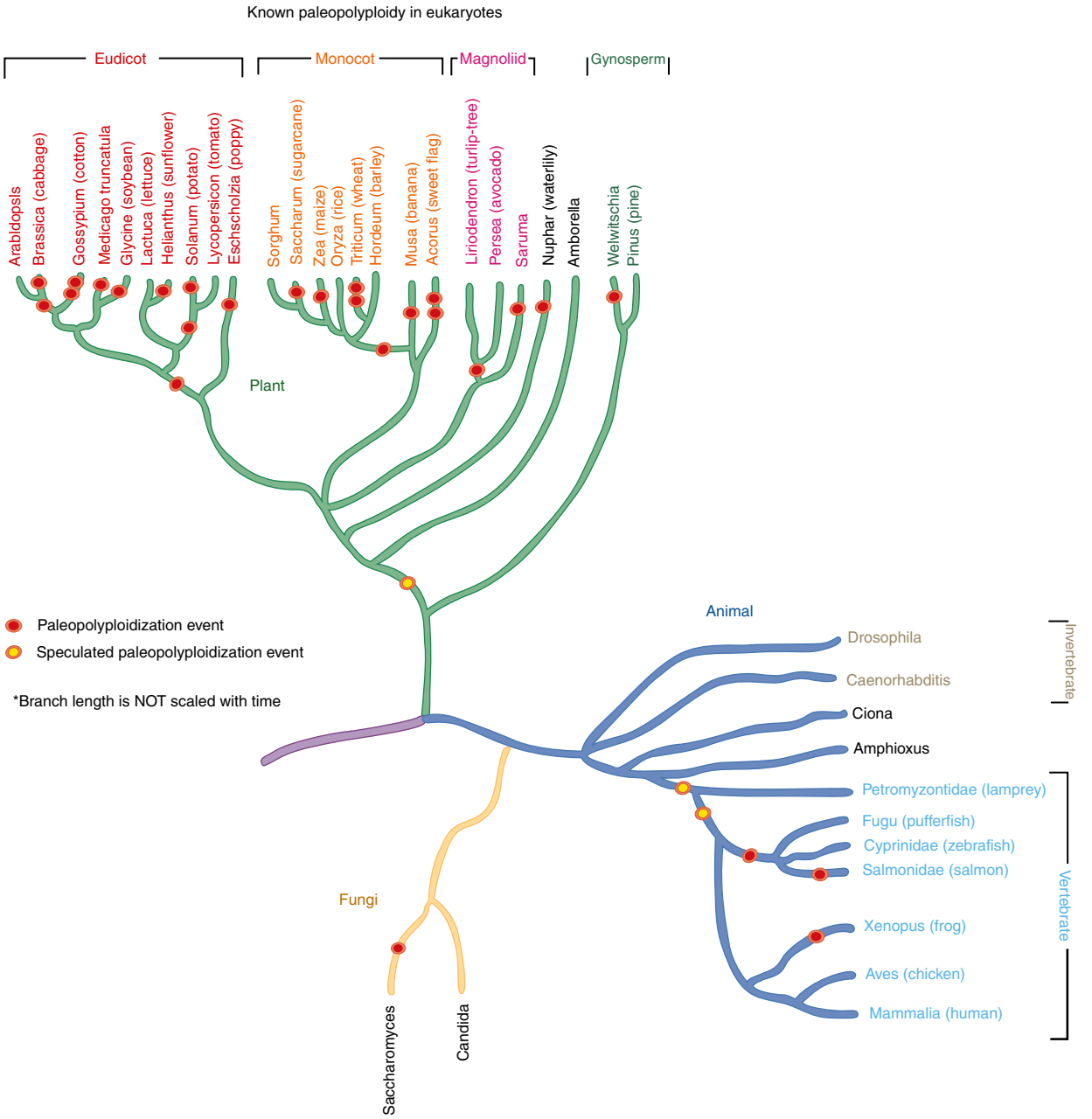


Figure 7 Summary of genome duplication events within the eukaryotes (organisms whose cells contain a nucleus and various organelles bounded by membranes). The event we are interested is occurred in the ancestor of the vertebrates (on this tree, the ancestor of the group comprising the lamprey + fishes + frog + chicken + human). Author: 5dPZ (https://commons.wikimedia.org/wiki/File:PaleopolyploidyTree.jpg).

At the moment, it is impossible to tell whether both of those events occurred rapidly in the vertebrate ancestor near the timing of the split between the Cyclostomata and its sister group, or whether the events were separated in time, the second possibly happening, as originally suggested, after the Cyclostomata diverged from its sister-group (but before the origin of the gnathostomes: Putnam *et al.*, 2008 and references therein; Decatur *et al.*, 2013).

Why are such duplication events important? The most obvious answer is that increasing the number of copies of a particular gene increases the scope for evolutionary change because you can get a novel function (neofunctionalization) appearing in one copy while retaining the original function of the gene in the other copy (**Figure 8**; for beautifully written reviews of this topic see Hurles, 2004; Cañestro *et al.*, 2013). So, for example, globins are oxygen-binding proteins, widespread throughout the animal kingdom, that fulfill a variety of roles from O_2 sensing to the detoxification of harmful reactive oxygen species (Ebner *et al.*, 2010). Hemoglobin and myoglobin, vertebrate specific molecules that evolved from duplicates produced during the WGD events (albeit through a very complicated pathway), took their oxygen-binding capacities to

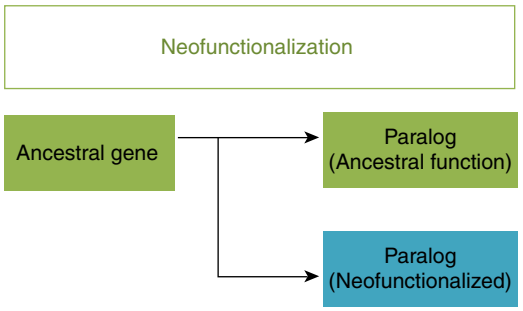


Figure 8 Neofunctionalization following a gene duplication event. Author: Jennonita (https://commons.wikimedia.org/wiki/File:Neofunctionalization_after_a_gene_duplication_event.png).

new areas in the body – oxygen transportation within red blood cells (hemoglobin) and oxygen storage in the muscles (myoglobin). These two novel functions allow oxygen to be delivered more efficiently to tissues.

Why was this important? Cephalochordates are capable swimmers, so clearly neither myoglobin nor hemoglobin are necessary for an animal to be active. Cephalochordates, however, are very small and thin and are active only in short bursts. They possess a closed circulatory system in which the colorless blood (no globins) is moved throughout the body by the pulsing of contractile blood vessels. Gas exchange is accomplished by the passive diffusion of oxygen across the skin or coelomic cavity (Schmitz *et al.*, 2000), a very slow process that can only occur over short distances. The ability to more effectively deliver and store oxygen was thus a critical prerequisite to the evolution of larger, more active animals (Storz *et al.*, 2013 and references therein). Overall then, gene duplications resolve the seeming paradox of novelty – the purported requirement for a gene to undergo a series of gradual changes that maintain functionality while, at the same time, promoting change – because 'conservation' of function and 'evolution' of function are partitioned into two separate (but initially identical) gene copies.

The Critical Vertebrate Developmental Innovations

If you look at a photograph of a lancelet and compare it to any vertebrate, even hagfish, one thing is immediately obvious – vertebrates have a very complex head. Northcutt and Gans (1983) postulated that this 'new head' was formed by two uniquely vertebrate embryological tissues, the neural crest and the cranial placodes. Both of these tissues arise in the developing neural plate, as it folds to form the dorsal hollow nerve cord (recall this character itself appeared in the ancestor of the chordates: **Figure 5**).

The neural crest consists of a population of multipotent neuroepithelial cells that originate in the inner walls of the folding neural plate, transform into mesenchymal cells and then begin to migrate outwards (green cells in **Figure 9**). Streams of these cells move to various places in the body, forming, among other things, the mineralized facial skeleton (cellular cartilage, bone, teeth), pigment cells, cranial ganglia, valves and walls of the heart, sensory and autonomic ganglia of the peripheral nervous system, and the enteric nervous

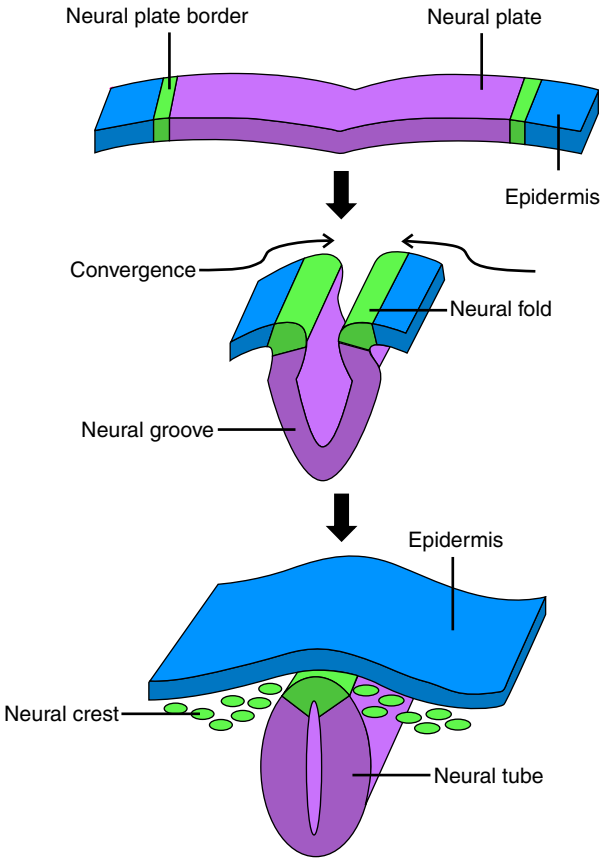


Figure 9 Diagram showing the formation of neural crest cells during neural plate folding. Author: NikNaks (https://commons.wikimedia.org/wiki/File:Neural_crest.svg).

system that innervates the gut (Simões-Costa and Bronner, 2013). It is no wonder the neural crest has been hailed as one of the most important vertebrate developmental innovations. But, what exactly was the innovation? All of the genes involved in neural crest formation were present in the ancestor of the chordates. Some of those genes (e.g., *Zic1*, *Pax3/7*, *Dlx3/5*, *Msx*) still play the same role today, specifying the development of the neural plate border, while others (e.g., *slug/snail*, *Foxd3*, *AP-2*, *Sox9/10*, *Twist*) are expressed in different tissues. Early in vertebrate evolution the latter group of genes became active in the preexisting neural plate border (changes in the timing and location of gene expression), changing the properties of their new target cells; this was the vertebrate innovation. Molecular studies indicate that these genes were not co-opted simultaneously, but rather that some were integrated into the developing neural crest before the genome duplications, while others were recruited afterwards (Green and Bronner, 2013; Ono *et al.*, 2014 and references therein). These studies may explain why some of the purported neural crest functions (e.g., the formation of bone) are not found in the basal-most vertebrates.

Cranial placodes, like the neural crest, develop from the folding neural plate. These placodes, which participate in the formation of various sensory systems in the vertebrate head (e.g., lens of the eye, olfactory receptors, inner ear sensory hair cells (otic), lateral line sensory hair cells, cranial nerve V

(sense of touch, pain, temperature in the face), neurons innervating the taste buds) have also traditionally been hailed as another major 'vertebrate innovation'. Recent studies, however, indicate that some of these placodes (olfactory, otic, lateral line) may have originated in the common ancestor of the Olfactores. Indeed the adenohypophysis placode may be even older (Graham and Shimeld, 2013). Interestingly, these stepwise patterns are largely congruent with a study of 5592 zebrafish genes (2222 of which are expressed in the neural crest or placodes) that uncovered three pulses of dominant adaptive evolution in sensory structures within the chordates. The third of these pulses occurred in VA (Šestak *et al.*, 2013).

So, did the neural crest and cranial placodes originate in VA? The answer to that question is 'No'. Genetic precursors to these structures were already in place by the time that ancestor appeared. This does not mean that the neural crest and cranial placodes are not important vertebrate innovations because all of them were highly modified in VA. What we are converging upon, then, is a more realistic picture of vertebrate origin based upon the stepwise assembly of genetic/developmental toolkits rather than the sudden appearance of complexity (for a detailed discussion of how complicated this assembly was, see Schlosser *et al.*, 2014). Indeed, this stepwise pattern is a recurring theme throughout the evolution of life (see e.g., de Mendoza *et al.*, 2013). The take-home message here is simple: the genomic/developmental architecture underlying the morphological exuberance displayed in the ancestral vertebrate species did not appear *de novo* in that species. It is not so much that new genetic material evolved in that ancestor (although this may have played a minor role; Khalturin *et al.*, 2009; Smith *et al.*, 2013), but that new developmental patterns emerged involving changes in timing and interactions among preexisting genes.

Putting It All Together

More than half a billion years ago, the vertebrate ancestor was flourishing in warm, shallow Cambrian seas. Current evidence suggests that VA was a small, jawless fish-like creature, with (1) moderately well-developed tail, dorsal and ventral fins (no paired fins yet), gills supplied with water by muscular ventilation and a two-chambered, endothelial lined heart, all of which contributed to increased swimming performance. In other words, VA was an active animal; (2) well-developed sensory capabilities: image forming eyes and the ability to detect chemosensory cues (smell and taste), the movement of water particles (lateral line) and sound/body position (inner ear); (3) a well-developed brain, including the vertebrate novelty – the telencephalon. The telencephalon contains the basal ganglia, which is responsible for the control of motor patterns that underlie so many physiological and behavioral traits (Grillner *et al.*, 2013 and references therein). In conjunction with the changes to sensory and aerobic capabilities, this implies that VA was capable of sophisticated swimming maneuvers and responses. In other words, VA had the toolkit to change from being a benthic feeder to a free-swimming pursuit predator.

The sensory systems thus appear to have evolved out of step in chordates, beginning with vision, followed by otic and olfactory input, and finally taste. Intriguingly, Feinberg and Mallatt (2013) proposed that the ability to integrate sensory information from multiple sensory sources may have represented a major step forward in the evolution of consciousness – and that such an ability may in fact have arisen and been amplified in the basal vertebrates (beginning with *Haikouichthys*). So it is possible that VA was conscious of, at least, its sensory experiences. Studies on mice and people have shown that a family of four genes ('*Dlg*') responsible for shaping neurotransmitter receptors and enzyme complexes in the brain are intimately involved in learning, cognitive flexibility, and attention. Only one *Dlg* gene is found in lancelets, but four copies are present in vertebrates (another example of the importance of whole genome duplication early in vertebrate evolution) (Nithianantharajah *et al.*, 2013). So, not only was VA possibly conscious of its sensory experiences, but it might have been capable of flexible and sophisticated cognitive tasks (studies on basal chordates and vertebrates are needed to test this suggestion). Finally, based on the life histories of basal extant vertebrates and cephalochordates (tunicates are highly derived and strange creatures), VA probably had separate sexes. Given the further WGD-associated evolution of the pituitary gland (Dong *et al.*, 2013 and references therein), which is the master controller of hormone-mediated behaviors, coupled with the origin of the telencephalon, we can speculate that VA individuals engaged in courtship behavior – but based on which cues and what did courtship look like? To answer these questions, we must begin to unravel the genetic developmental pathways underlying such behaviors. Once we can do this, then VA will truly come alive in our minds.

We have had a complicated interaction with vertebrate species over the millennia. We have hunted, feared, worshiped, domesticated, tried to obliterate, and bonded with some of them. We began painting their images on cave walls more than 40 000 years ago, and ever since we have watched and studied them because they teach us about ourselves. Data are accumulating at an ever-increasing rate as technological advances give us the ability to uncover smaller and smaller pieces of this complicated evolutionary story. As of 2015 we know so much about some aspects of our vertebrate ancestor, and so little about others. That is what makes science so exciting!

See also: Amniotes, the Origin of. Land Vertebrates, the Origin and Evolution of

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Vicariance Biogeography

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Glossary

Area cladograms A representation of a relationship of areas resulting from substituting taxa for the areas where they occur.
Center of origin The supposed geographical area where a clade originated and from where it dispersed to subsequent areas.
Cladistics Phylogenetic systematics; the science that studies clades and their inference.
Cladogram A phylogenetic tree derived using cladistic techniques.
Model-based approaches In a biogeographical context, different events (vicariance and dispersal) receive different probabilities, but these values are difficult to estimate.
Molecular dating The methodology that infers the dates of cladogenesis or origins of taxonomic groups.
Node calibration The act of assigning an age to a node to calibrate other nodes in the tree.
Node dating A molecular dating technique that assigns ages to nodes without the need of prior analysis.
Panbiogeography An inference of a biogeographical relationship based on repeated occurrences in different areas, but without explicit methodologies.
Paraphyly/paraphyletic Terms applied to non-monophyletic groups including the common ancestor and some but not all the descendants of such ancestor, for example, dinosaurs are paraphyletic with respect to birds.
Parsimony-based approaches In a biogeographical context, parsimony-based approaches assume that vicariance often requires no cost, and dispersal has a cost associated (the methods favor vicariance over dispersal).
Phylogenetics The science that infers genealogies of higher taxa, or the so-called phylogenetic trees.
Taxon A group of organisms in the hierarchy of life; for example, a species, family, order, class, etc.
Total evidence dating A molecular dating technique that includes fossils (and their ages) into a phylogenetic analysis containing data on morphology and molecules for the extant taxa.
Vagility The degree to which a taxon moves.

After the introduction of the idea of continental drift (Wegener, 1912) and later its acceptance subsequent to the understanding of plate tectonics as the mechanism underlying the concept of continental drift, vicariance biogeography emerged as the favorite mechanism to explain disjunct distributions of ancient organisms. Vicariance has, for instance, been used to explain the modern distributions of ancestral Pangean animal and plant lineages (e.g., San Mauro *et al.*, 2005; Giribet *et al.*, 2012; Mao *et al.*, 2012; Murienne *et al.*, 2014). Perhaps one of the most paradigmatic case studies of vicariance is that of the breakup of Gondwana, with many studies focusing on a diversity of lineages (e.g., Sanmartín and Ronquist, 2004). Whether trying to explain the global distribution of ancient biotas through the reconfiguration of entire continental landmasses, or more local and recent events (e.g., the detachment of the Corso-Sardinian microplate from the Iberian Peninsula before the Miocene), vicariance biogeography assumes that the biotic distributions are due to drifting with landmasses and not due to subsequent dispersal, hypotheses that can be difficult to distinguish. For instance, a *trans*-Tasman distribution – of organisms found both in Eastern Australia and New Zealand – could be easily explained by vicariance, but also by dispersal as postulated by the West Wind Drift hypothesis (Sanmartín *et al.*, 2007), and an accurate estimate of the age of cladogenesis is a prerequisite to distinguish between hypotheses, as cladogenesis must predate the geological event supposedly causing vicariance in order to be considered more likely than dispersal. Only in well-sampled studies of diverse groups, the tree topology can help to infer the timing and directionality of dispersal (e.g., Sharma and Giribet, 2012). Vicariance, however, remains the paradigm against which one tests alternative hypotheses of dispersal, whether using parsimony-based approaches (where vicariance often requires no cost) (Ronquist, 1997) or model-based approaches (Ree and Sanmartin, 2009; Yu *et al.*, 2010).

A Brief History of 'Vicariance Biogeography'

The discipline of vicariance biogeography was a development of the 1980s, and a paradigm shift from previous biogeographic ideas that focused on dispersal from centers of origin, or other disciplines such as panbiogeography, which had been mostly 'story telling.' Vicariance biogeography really allowed the field of biogeography to move into the scientific domain, as it was hypothesis driven. According to Wiley (1988), vicariance biogeography developed from three key events: the emergence of plate tectonics as a major geological paradigm, the emergence of biological systematics, and the discovery of Croizat's works by Nelson (e.g., Nelson, 1974). Methodological developments followed (e.g., Nelson and Platnick, 1980, 1981). The implications of the new discipline were that current higher taxa were once species that diverged as a result of a vicariant event and that cladograms are able to reflect such events by transforming taxa into area cladograms (**Figure 1**). It is beyond this article to discuss the many specific methods for conducting analyses of area cladograms. A thorough discussion of the methods historically used to analyze vicariance biogeography can be found in Crisci *et al.* (2003). For the purpose of this article, it suffices to say that repeated

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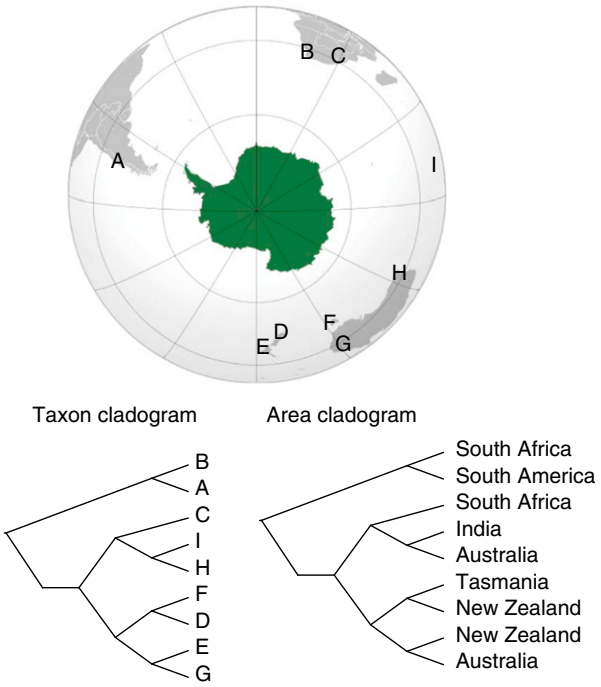

Figure 1 Example of the distribution of a temperate Gondwanan lineage found in modern circum-Antarctic terrains and their taxon cladogram and area cladogram derived from it.

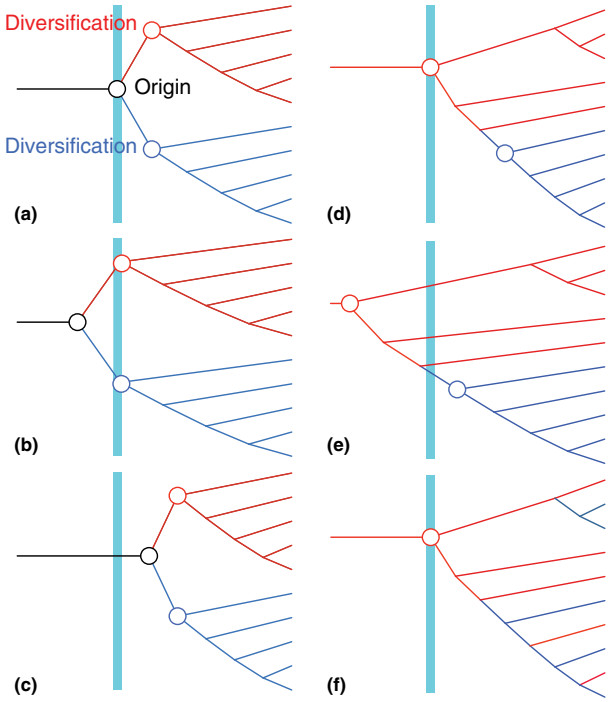

Figure 2 Alternative scenarios assuming a tectonic event (vertical cyan blue line) and accurately dated phylogenies. The different colors (red and blue) indicate modern distribution in two currently noncontiguous landmasses. (a) A scenario with the tectonic event coinciding with the cladogenetic event, and thus compatible with vicariance, with each descendant lineage diversifying in a different landmass. (b) Scenario with ancient cladogenesis preceding the tectonic event but compatible with vicariance. (c) Scenario where cladogenesis occurs after the tectonic event and thus incompatible with vicariance; this scenario assumes that one of the lineages (or both) achieved their current distributions through a mechanism of dispersal. (d) Scenario implying dispersal of the blue lineage, rendering paraphyly in the landmass of origin. (e) Scenario compatible with vicariance but with a deep cladogenetic history prior to the tectonic event. (f) Phylogenetic scenario with landmass paralogy, requiring multiple instances of dispersal.

(congruent) patterns – those found across taxa – may reflect common historical vicariance events, in the same sense that congruence among characters is used to derive phylogenetic hypotheses of taxa. Major problems with this approach are the assumptions that incongruent patterns are due to dispersal – they could also be explained by differences in the timing of the cladogenetic events – and that areas can be defined a priori. As an example, South Africa, Australia, and New Zealand are each non-monophyletic in **Figure 1** – because their inhabitant taxa do not form clades. Non-monophyly of these landmasses is, however, common in studies of groups that do not disperse well, if cladogenesis predated the rifting of Gondwana (e.g., Giribet *et al.*, 2012; Murienne *et al.*, 2014) (a scenario as in **Figure 2(e)**). In addition, assuming that South Africa or Australia are each an area of endemism for all types of organisms, it has been refuted by many studies based on short-range endemisms (e.g., Harvey, 2002; Giribet and Edgecombe, 2006). Interestingly, new methods for estimating areas without predefining them prior to biogeographical analysis – the so-called 'spatial analysis of vicariance' – are now being developed (Arias *et al.*, 2011). The method uses observed distributions as data, thus requiring neither predefined areas nor assumptions of hierarchical relations between areas.

Modern Approaches to Understanding Vicariance in Biogeography

Vicariance has played a key role in our understanding of the evolutionary process through large timescales, but in its original formulation, pattern alone, and not time (as it was difficult to obtain at the time), was used. Unfortunately, the timing of the cladogenetic events is key in biogeographical studies, as landmass or ocean configurations change drastically through time. This change of landmasses, emerging and disappearing through Earth's history, is beautifully illustrated in a recent biogeographic study of Sulawesi (Stelbrink *et al.*, 2012). However, few studies like this are available for other regions of the world. Examples of identical patterns with different timings, and thus different biogeographical explanations, are found in **Figure 2**. For example, **Figures 2(a)–2(c)** show the same pattern for three scenarios: In one case (a), the time of origin of two clades coincides exactly with the time of the 'event,' thus indicating vicariance; in another case (b), cladogenesis takes place earlier and diversification coincides with the timing of the 'event,' not necessarily constituting a case of vicariance; and in the third case (c), both the origin of the two groups and their diversification postdate the event – a clear case of dispersal between landmasses.

Problems with Assumptions of Vicariance Biogeography

A common assumption of supposing a strict correlation between a cladogenetic event and a vicariant event is that both more or less coincide in time (**Figure 2(a)**), and thus one is informative of the other. This is typically done by calibrating one or more nodes of a phylogeny with the geological time of separation of two landmasses, as was done, for example, by Boyer *et al.* (2007) for a group of arachnids and has been applied to many groups without a known fossil record – the so-called tectonic calibrations or vicariance-based calibrations (see a list of papers using tectonic-based calibrations in Kodandaramaiah, 2011). This practice has been legitimately criticized by Kodandaramaiah (2011), as this assumption does not allow for cladogenesis preceding the tectonic event (**Figure 2(b)**), while molecular evidence has shown in several cases that cladogenesis can indeed precede the geological event by vast amounts of time (e.g., Giribet *et al.*, 2012; Murienne *et al.*, 2014). Tectonic calibrations thus have the dual problem of not testing for vicariance (since it is assumed) and resulting in a push-toward-the-present effect (Giribet, 2015), as the approach equates time of cladogenesis with the tectonic event. Therefore, only those studies using independent calibration methods are now considered proper tests for vicariance.

While vicariance was originally invoked by observing organisms distributions (e.g., Brundin, 1965, 1966), dispersal could generate similar patterns, especially in the southern hemisphere with the circum-Antarctic current or the West Wind Drift hypothesis (Sanmartín *et al.*, 2007). The tempo of evolution is thus crucial for claiming vicariance. Dating phylogenies has progressed a great deal since the introduction of molecular data, especially recently, as researchers often use the fossil record to calibrate nodes in phylogenies instead of molecular clock approaches (Laurin, 2012). Fossil calibration is not without controversy, and two approaches currently exist: the so-called 'node calibration,' where a fossil is assigned to a particular node to constrain such node or a split, or the more recent 'total evidence dating' (Murienne *et al.*, 2010; Pyron, 2011; O'Leary *et al.*, 2013; Wood *et al.*, 2013), where fossils are positioned more precisely in a phylogeny using morphology in combination with molecular data (see a recent revision of the topic in Sharma and Giribet, 2014). But even when the same fossils are used for calibrating trees, dating methods are inherently different and few studies explore alternative outcomes (e.g., Mao *et al.*, 2012; Murienne *et al.*, 2014) and even fewer have studied the impact of the calibrations per se (Sauquet *et al.*, 2012). This latter study shows that estimates for the crown group age of *Nothofagus* – an emblematic tree for the study of Gondwanan vicariance biogeography which has more recently been found to show rampant dispersal – varied from 13 to 113 Ma across a full range of calibration scenarios (Sauquet *et al.*, 2012), in part due to inaccurate dating of the fossils and to the phylogenetic uncertainty of the fossils. Another recent example refers to the use of a Devonian harvestman fossil (Dunlop *et al.*, 2003), which has been interpreted as a member of the extant Phalangioidea and used as a node calibration in Opiliones trees (Giribet *et al.*, 2010; Hedin *et al.*, 2012). However, recent phylogenetic investigation of the fossil has shown it to be a member of a stem group Opiliones (Garwood *et al.*, 2014), contradicting prior node-based calibrations, and now used for total evidence dating (Sharma and Giribet, 2014).

To summarize, vicariance biogeography provides a powerful explanation for many biotic distributions, but these require proper dating to distinguish whether cladogenesis occurred prior to, during, or subsequent to a given tectonic event. Other ecological aspects, including range expansions and contractions, niche conservatism, secondary contacts with other biotas (e.g., the case of the Indian subcontinent; Ali and Aitchison, 2008), and competition, often play a role in preserving the vicariant biotas on both sides of a tectonic event. It is well known that this is not the case for many groups of organisms, once broadly distributed across Pangea, but now restricted to remote continental islands, such as monotremes of the Austro-Papuan region, or the tuatara and rheobatrachid frogs in New Zealand – all of them subjected to large continental extinctions.

Three premises are thus required to distinguish vicariance from dispersal:

1. The organism distribution requires a pattern consistent with continental rifting, and that the landmasses it inhabits are of continental origin, not islands of oceanic origin or Darwinian islands (*sensu* Gillespie and Roderick, 2002).
2. If continental in origin, the landmasses should not have undergone total submersion, as supposedly happened in New Caledonia (Grandcolas *et al.*, 2008; Murienne, 2009), or, less likely, in New Zealand (Waters and Craw, 2006; Giribet and Boyer, 2010; Sharma and Wheeler, 2013; Mildenhall *et al.*, 2014).
3. Although less commonly used, a phylogenetic pattern must also be concordant with the vicariant hypothesis, often represented by reciprocal monophyly of clades at opposite sides of the tectonic event (**Figures 2(a)** and **2(b)**), or paraphyly, in the case that the lineage has an old history in the contiguous landmass prior to the split (**Figure 2(d)**). However, multiple instances of paraphyly are difficult to reconcile with vicariance, even for lineages older than the tectonic event.

Recognizing vicariance is becoming more feasible, but this does not mean that biogeographic analyses should be restricted to this mode of cladogenesis. In fact, most biogeographic research focuses on island processes in Darwinian islands, based on the premise that the first colonizers of those islands must have been able to disperse, and that many were subject to different types of radiations, perhaps most prominent adaptive ones in places like Galapagos, Hawaii, the Canaries, or Cape Verde archipelagos (e.g., Arnedo *et al.*, 2001; Carranza *et al.*, 2001; Hormiga *et al.*, 2003; Gillespie, 2004; Parent and Crespi, 2006; Lerner *et al.*, 2011). Nonetheless, vicariance is still a much more parsimonious explanation for ancient distributions of organisms with low vagility and narrow ecological requirements – the latter condition often overlooked, but often tightly linked to low vagility – and is able to explain the distributions of disparate groups including amphibians (San Mauro *et al.*, 2005), velvet worms (Murienne *et al.*, 2014), arachnids (Giribet and Kury, 2007; Murienne *et al.*, 2013), myriapods (Giribet and Edgecombe, 2006;

Wesener and VandenSpiegel, 2009), insects (Krosch *et al.*, 2011), and plants (Mao *et al.*, 2012; Beaulieu *et al.*, 2013), to mention just a few of the most charismatic groups.

See also: Dispersal Biogeography. Paleobiogeography and Fossils. Parsimony Methods in Phylogenetics

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W

Waddington's Epigenetic Landscape, History of

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Glossary

Spineless-aristapedia (ssa) Regulatory gene in Drosophila melanogaster, encoding a basic-helix-loop-helix-PAS transcription factor that is required for the proper specification of distal antennal (arista) tissue. Loss-of-function mutations transform the arista cells into distal leg (tarsus) tissue ('aristopedia') and the reduction of bristle size ('spinelessness').

Introduction: Conrad H. Waddington as Systems Integrator

Conrad Hal Waddington (1905–75) began to follow the unusual developmental pathway of integrating embryology, evolution, and genetics early in his professional career. Although his epigenetic landscape model was first presented more than 50 years ago, its brilliance and elegant originality have enabled it to add useful explanatory and aesthetic dimensions to important current discoveries in the emerging fields of epigenetics and developmental biology. New research on Waddington's epigenetic landscapes (Gilbert, 1991a,b; Bard, 2008; Fagan, 2012; Baedke, 2013; Fusco *et al.*, 2014) has shown their heuristic value for several fields, some of them outside biology. The significance of Waddington's "epigenetic landscape" diagrams becomes clearest when placed in the detailed context of his life and work.

Although Waddington designed the "epigenetic landscape" to show the individuation of a pluripotent population of cells into distinct and different pathways of differentiation, Waddington's own life shows a quite different pattern of individuation. What is remarkable about Waddington's individuation is the way in which multiple options, talents and interests evident in his early university years are not foreclosed, but kept open throughout his life. Followed passionately over many decades, these talents and interests cross-fertilize, leading to the creative synergy seen in his epigenetic landscape model. He spent his early years in India where his father was a tea planter. Returning to England at the age of four, and following preparatory school, he won a scholarship to Cambridge University. After taking a First Class Honors degree in geology in 1926, Waddington pursued graduate work in paleontology with a focus on the systematics of ammonites, an extinct group of cephalopods. Reflecting his diverse interests, he held at the same time an 1851 studentship in paleontology and an Arnold Gerstenberg studentship in philosophy (1927).

Whether speaking of Waddington's early work in paleontology or his subsequent work in fields as diverse as embryology, genetics, ethics, and interconnections in the fields of science and art history, a common theme is that all reflect the process philosophy of Alfred North Whitehead (Robertson, 1977). For Whitehead, no thing exists except in relation to other things, and the relationships thus created are always in a process of becoming. As Waddington would later write of his early work in paleontology, it "forces on one's attention the Whiteheadian point that the organisms undergoing the process of evolution are themselves processes … The whole developmental process is preserved so that one cannot avoid examining it" (Waddington, 1975). In this early work, one can see him already viewing the fossil record with the developmental eye of an embryologist.

While pursuing his graduate work in paleontology, Waddington's interest began to move from paleontology toward genetics. Yet, he had also been reading the work of Hans Spemann, the German embryologist, who discovered the organizer. After a meeting with Dame Honor Fell, director of the Strangeways Research Laboratory, Cambridge, he was encouraged to begin work there in the field of embryology. Using techniques he himself developed for growing chick embryos *in vitro*, he successfully manipulated and transplanted different embryonic regions from one early chick embryo to another. Among his discoveries he could show that the elongation of the primitive streak was directed by the underlying hypoblast. His photographs of twin chick embryos resulting from the transplanting of an exogenous node beneath the ectoderm constituted an important demonstration of primary embryonic induction in a warm-blooded animal: the earlier discovery of the organizer by Spemann and Mangold (1924) had been with amphibians.

Waddington was able to present his first results on culturing chick embryos in 1930 at the International Congress of Experimental Cytology in Amsterdam. His presentations were extremely well received; he soon received several invitations to come to work in Germany. From 1932 to 1938, Waddington studied amphibian neural induction, attempting to learn and transfer the techniques of amphibian experimental embryology to the study of chick development. Approximately 14 single-authored papers would appear between 1932 and 1938; an additional four were the product of joint authorship with distinguished colleagues in the field. The budding paleontologist who saw the world at least in part through the eyes of Whiteheadian process philosophy had undergone his own metamorphosis. By the last years of the 1930s he had become a skilled research scientist, a respected contributor to embryology whose careful work was widely viewed as having made important contributions to the field.

The Split between Embryology and Genetics: A Brief Review

A disciplinary split between genetics and embryology was fundamental and formative to all working in those fields at this time. Waddington's lifelong commitment to reconciling this split provides an essential key for understanding the work that led him to formulate the epigenetic landscape model. We must take a brief look at the history of those disciplines as they unfolded during this period.

A central question for the experimental embryology that had emerged in the early years of the twentieth century was this: Which compartment of the zygote – the nucleus or the cytoplasm – directs heredity and development? (Gilbert, 1988, pp. 312–314). A critical finding emerged from *Drosophila* studies of Thomas Hunt Morgan and coworkers at Columbia University: factors for eye color, body color, wing shape, and sex determination, all segregated with the X chromosome within the nucleus. In the years following 1910, Morgan's work – which had become a primary driving force in American biological science – drove a wedge into embryology, splitting it into two divisions, the embryologists and the new geneticists. Soon after 1911, genetics began to arise as a separate discipline, complete with its own techniques, favored organisms, rules of evidence, journals, and vocabulary (Gilbert, 1991a, pp. 182–183).

In the 1920s and 1930s, the remarkable success of genetics in applied breeding techniques in both animal husbandry and plant breeding led it to be seen by many as the preeminent biological science in America (Gilbert, 1996a, pp. 101–106). Geneticists began to encroach upon what had been the traditional territories of embryology. If cytoplasmic development was little more than an epiphenomenon of genetic control (as many geneticists argued), then perhaps it was the research methods of genetics that could best obtain the answers to the developmental questions that embryologists had been asking (Gilbert *et al.*, 1996b).

Many embryologists reacted strongly to this territorial push by geneticists. Prominent figures such as Lillie, Spemann, and Just argued that there could be no 'genetic theory of development' until geneticists could meet at least two major challenges:

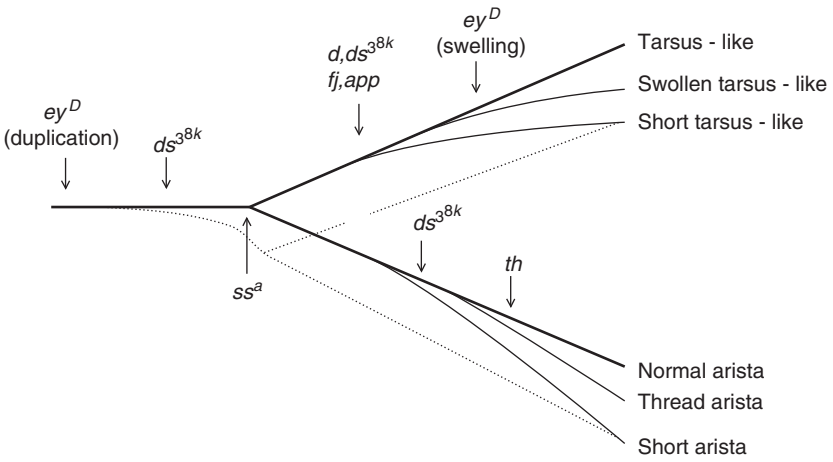
1. they needed to explain how chromosomes – thought to be identical in every cell of the organism – could produce different and changing types of cell cytoplasms; and
2. they had to provide evidence that genes control the early stages of embryogenesis (almost all the genes known at the time affected the final steps in development, such as eye color, bristle shape, and wing venation in *Drosophila*).

As E.E. Just summed it up, embryologists were interested not so much in the number of bristles a fly has on its back, but in understanding how a fly forms its back in the first place.

This brief sketch gives something of the general climate at the time. Geneticists and embryologists were for the most part professionalizing their respective disciplines, focusing on either genes or organizers. From the beginning, Waddington was a consistent and conspicuous exception to this pattern of disciplinary exceptionalism, and the words in the title of his book, *Organisers and Genes*, should be recognized as being metonymous for embryology and genetics, respectively. He would argue consistently in a large body of work over four decades that the phenomena investigated by genetics and embryology were not separate. They represented two sides of the same coin. What was desperately needed was a form of synthesis and integration that would demonstrate this underlying unity. The synthesis as envisioned by Waddington would involve not only these two disciplines but the broader study of evolutionary change revealed through paleontology. Waddington firmly believed that epigenetics, or 'the science concerned with the causal analysis of development' would provide the key for the needed synthesis. The preceding definition appears on the title page of *The Epigenetics of Birds* (Waddington, 1952), but it is only a variation on a theme that appears much earlier and is consistently reintroduced in all of his subsequent work.

'Development as an Epigenetic Process': Steps Toward the Epigenetic Landscape

After an initial decade of research in embryology, the topic of Waddington's first book was, surprisingly, not embryology but genetics. Moreover, when *An Introduction to Modern Genetics* (Waddington, 1939) appeared, he was only 33 years old, and had not published a single academic paper in genetics. He states in the Preface "a word of explanation, and perhaps of apology, for having written a text-book of genetics although the field of my own research is usually classified as the separate science of embryology." He explains that the need to do so has arisen "primarily because the different kinds of biological cobblers have in the past stuck to their own lasts." He then suggests that "the connection between genetics and the other branches of biology, such as cytology, embryology, the study of evolution and of the biochemical nature of cell constituents, is much closer than is often admitted, and that the boundaries between these subjects deserve less attention than is usually paid to them." It is, in typical Waddington fashion, an apology that does not sound much like an apology.



The aristapedia developmental track system. The developmental process runs from left to right, either into the tarsus track above or the arista track below. Both these tracks are affected by various genes. The dotted lines represented one interpretation of the actions of ds38k, which affect both normal and tarsus-like antennae. Only the mutant genes have been inserted; their normal allelomorphs would act in the opposite sense.

Figure 1 The aristapedia mutation. Reproduced from Waddington, C.H., 1940. Organisers and Genes. Cambridge: Cambridge University Press.

In this book, Waddington introduces an area of theoretical focus in which he will make major contributions for the rest of his professional life. The section "Development as an Epigenetic Process" (Waddington, 1939, pp. 154–155) begins by reviewing the classical controversy in embryology "between the preformationists and the epigenisists." Perhaps playing the role of Devil's advocate, the author, although an embryologist, sees light on both sides. He finds on the one hand that the modern view of development necessitates an admission that "development proceeds on the basis of the 'preformed' qualities of the fertilized egg." Nevertheless, this must be balanced by the understanding that "the interactions of these [the egg's] constituents gives rise to new types of tissue and organ which were not present originally, and in so far development must be considered as 'epigenetic.'" Waddington, who is generally given credit for coining the term 'epigenetics' (Goldberg *et al.*, 2007), was not only the first to use the term 'epigenetic' at this systems level, but in a short paper published almost at the same time (Waddington, 1942) he introduced the term "epigenotype" to capture the interactions between genes, their products, and the environment. It has been suggested that in today's language "we might think of the epigenotype as the networks of transcription factors, paracrine factors, and environmental influences that allow the genotype to realize the phenotype" (Gilbert, 2000, 2011). What a gene does or does not do, then, can depend upon its context.

Pursuing the theme of integration, in *Organizers and Genes* Waddington calls himself a student of 'diachronic biology,' a science, in his own words, of "embryology-genetics-evolution which again form a group whose interconnections are obvious and unavoidable" (Waddington, 1940, p. 1). Its conclusion (1940, p. 148) seems to go one step further: "the developmental side of biology – embryology, genetics and evolution – seems to be reaching a point where radically new types of thinking are called for."

Waddington became a contributor to this effort to find new types of thinking over the following decades. Yet the first steps toward this can be seen even in this early work. First, he presents (1940, pp. 77, 83) an important thought predecessor of the epigenetic landscape diagram, 'the branching-track system.' Its purpose is to represent a sequence of reactions constituting the developmental pathway leading from the gene for a particular trait to the adult morphogenetic structure of that trait. Choosing the example of the genetic control of the development of the antenna in *Drosophila melanogaster*, he compares the development of this organ in the wild type fly with that caused by the recessive mutation aristapedia ssa, in which 'the arista is transformed into a more or less leg-like organ.' After showing the different end stages, the developmental process that gives rise to these allelomorphs is represented in this 'branching-track diagram' shown in **Figure 1**.

The 'branching-track' model is already a significant departure from the customary forms of presentation in the field, in which successive compounds would be listed in a linear array and referred to as "substances in a chain reaction" (e.g., Beadle and Ephrussi, cited in Gilbert, 1991b, p. 142). With only a little imagination, one can see the same developmental patterns represented by a true epigenetic landscape diagram. And in fact, the first schematic representation of an epigenetic landscape appears as the frontispiece of this 1940 volume (an actual landscape, drawn by an artist friend John Piper). A brief note below guides the reader to a later section: 'The Epigenetic Landscape.' Here the following (1940, pp. 91–92) appears:

> In embryonic development we are confronted with alternative modes of development … The system of developmental paths has been symbolized in two dimensions as a set of branching lines. Perhaps a fuller picture would be given by a system of valleys diverging down an inclined plane.

As this suggests, the frontispiece illustration is only a preliminary version. The 'fuller picture' representing the

epigenetic landscape model appeared in subsequent major publications (1954, 1956, 1957).

The Evolution of the Epigenetic Landscape

In order to extend, illustrate, and clarify his concept of the epigenetic system, Waddington began to experiment with a new form of symbolic representation of the entire developmental process. A key chapter in *Principles of Embryology* (1956, pp. 329–347) is entitled 'The role of genes in the epigenetic system.' This chapter begins with a discussion of developmental pathways and their genetic control. Citing Waddington in close paraphrase to best follow both his thinking and terminology, the pathways are analyzed as follows:

1. The development of an organ or complex tissue takes place in a series of steps, each of which is affected by genes.
2. At each step, there are several genes acting and actual development is the result of a balance between opposing gene programs.
3. An organ or tissue is formed by a sequence of changes which can be called 'the epigenetic path' leading to it.
4. In a normal or 'wild type' egg these paths are usually quite distinct, so that a specific embryonic tissue can turn either into a leg or into a wing, but only with great difficulty into something else.
5. Development produces not merely 'substances' or 'tissues,' but organs showing altered pattern and three dimensional shape. For example, a neural plate rolls up into a neural tube through morphogenesis (1956, p. 415).
6. Once a piece of tissue has entered on a path of development, its final condition can be affected by all the genes which are concerned in that path (1956, p. 340).
7. Finally, each path is 'canalized,' or buffered, protected by threshold reactions, so that even if it is mildly disturbed it will be effectively regulated back to the normal end result, which is the cell fate.

The synergistic action of many genes "whose operations interact in such a way to define a pathway of change in a multidimensional phase space" has a stability to which the developing system tends to return after disturbance (Waddington, 1973, p. 502). Such stabilized pathways are to be called "chreods" (Waddington, 1957).

The influence of Whitehead's philosophy is clear. In Waddington's autobiographical sketch, he writes that the lessons scientists can learn from Whitehead are "his replacement of 'things' by processes which have an individual character which depends upon the 'concrescence' into a unity of very many relations with other processes" (Waddington, 1975, p. 3). Waddington wrote that he used the term chreods as a substitute for Whitehead's 'concrescence' to indicate a stabilized or buffered pathway of change.

The term "chreods" (Waddington 1957) received much theoretical attention when the idea first appeared. The following citation from Piaget (1971, p. 47), quoted here at length, is illustrative. It not only explains Waddington's distinction between 'homeostasis' and 'homeorhesis,' but clarifies how this distinction contributes to Waddington's proposed synthesis of genetics and embryology (see also the discussion of Piaget and Waddington in Gilbert and Borish (1997)):

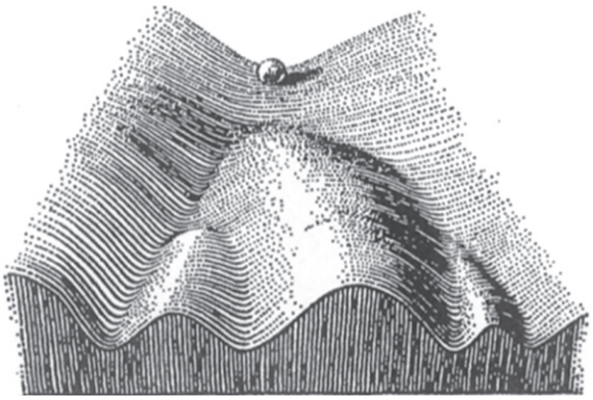
> Waddington has suggested the name 'chreods' (necessary routes) to describe developments particular to an organ or part of an embryo, and he applies the term 'epigenetic system' … to the sum of the chreods, taken as being – to a greater or lesser degree – channeled. But the main interest of this idea is not just in the names he gives things (or in the symbolic patterns thereby presented to us, of channels, some wide, some narrow, that the processes must follow). It is, rather, in a new kind of equilibrium as something which is, as it were, kinematic and which, in determining such processes, is nevertheless quite different from homeostasis: there is a kind of 'homeorhesis' when the formatory process, deviating from its course under outside influence, is brought back on course by the interplay of coercive compensations. In Waddington's opinion, such a mechanism is dependent upon a network of interactions rather than upon the action of individual genes …

Piaget recognizes that in 'homeorhesis', Waddington has invented a whole new term for discussing homeostasis in movement, as when embryonic precursors follow along the canalyzed tracks. It is important to see that behind this new terminology is Waddington's attempt to integrate the competing perspectives of genetics and embryology. It enables him to develop his view that the system of tracks and their genetic control, rather than the simple action of genes, is fundamental in embryonic development.

Waddington also believed that natural selection could work on aspects of development. If such a developmentally controlled pathway is advantageous to the survival and functioning of an organism, the path that leads from one state to another (e.g., from ectoderm to neural tube) becomes canalyzed by natural selection. His synthesis would therefore include both micro- and macroevolutionary changes, thus addressing the 'diachronic' perspective that he had pointed to in his early work.

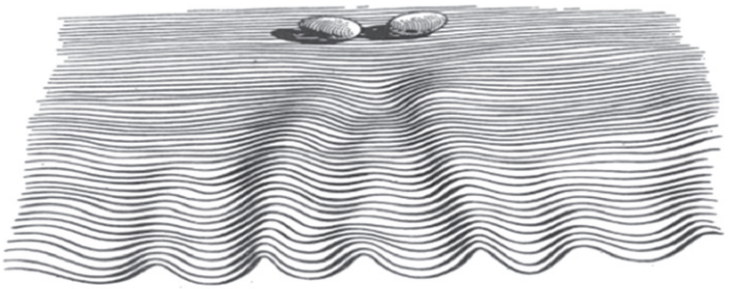
Ever interested in art and the visual representation of scientific ideas and data (he would later try using Japanese sumi-e-techniques to data splay rather than error bars), Waddington saw that aspects of the organismic developmental process could lend themselves to visual, pictorial representation. Going beyond the simpler branching-track diagram, which was itself a departure from the convention of strict linear representation of change, he began to experiment with what he called "a symbolic representation of the developmental potentialities of a genotype in terms of surface" (Waddington, 1956).

The establishment of cell fate can be visually represented by a ball rolling down the valleys of the epigenetic landscape. The resulting image of the epigenetic landscape is "a set of valleys or channels separated from one another by hills. The inclined plane of the landscape represents the tendency of the cells to pass from an immature to adult condition" (Gilbert, 1991b, p. 148). At certain times two downhill paths are possible, and the cell or tissue can be deflected into one or the other path. Regulation normally takes place only at specific branch points, where development has led the embryo into a valley with 'gently sloping sides.' As development proceeds, the original wide and gently sloping valley branches into subdivisions having far steeper sides. Once pushed down one path, it would be difficult to get to another. The further the ball rolls, the steeper the sides get. Three alternative, but complementary, representations of the Epigenetic Landscape – all proposed by Waddington – are shown in **Figures 2(a)–2(c)**.



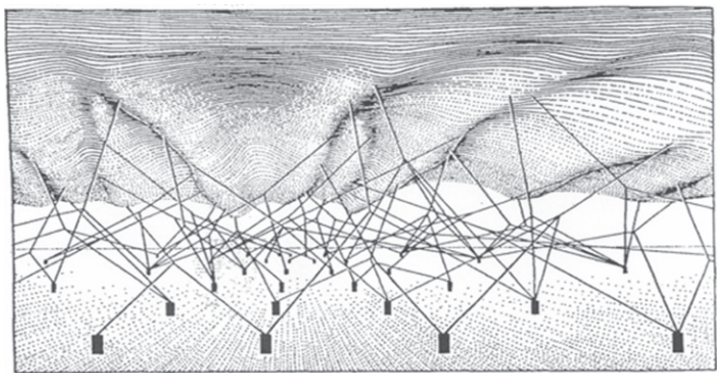
Waddington's image of the epigenetic landscape. The path of the ball represents cell fate. Depending on what happens at the different bifurcating branch points, each valley leads to the specific fates the embryonic cell might become if it rolls down that path. At the beginning of its journey, development is plastic, and a cell has many potential fates. However, as development proceeds, certain decisions cannot be reversed. (For more details on the use of these epigenetic landscapes to synthesize genetics, embryology and evolution - see Gilbert 1991b)

(a)



The 'epigenetic landscape." A symbolic representation of the developmental potentialities of a genotype in terms of a surface, sloping towards the observer, down which there run balls each of which has a bias corresponding to the particular initial conditions in some part of the newly fertilized egg. The sloping surface is grooved, and the balls will run into one or other of these channels, finishing at a point corresponding to some typical organ

(b)



Waddington's representation of how genes interact with the epigenetic landscape. The pegs in the ground represent genes or complexes of genes; the valleys are formed by tension acting on the guy ropes. The action of genes thus shapes the different pathways that canalize development toward specific cell fates.

(c)

Figure 2 (a) Cell fates in Waddington's epigenetic landscape. Reproduced from Waddington, C.H., 1957. The Strategy of the Genes. London: George Allen & Unwin Ltd. (b) Developmental potentialities of a Genotype in Waddington's epigenetic landscape. Reproduced from Waddington, C. H., 1954. The integration of gene-controlled processes and its bearing on evolution. In: Proceedings of the 9th International Congress of Genetics (Caryologia, Suppl. Vol.), p. 132; Waddington, C.H., 1956. Principles of Embryology. London: George Allen & Unwin. (c) Interactions underlying the epigenetic landscape. Reproduced from Waddington, C.H., 1956. Principles of Embryology. London: George Allen & Unwin.

The steepness of the valley walls indicates the changing equilibrium state of the track. It is a depiction of how canalyzed the reaction is. If the walls are steep, it would be difficult to escape from that valley once inside it. However, if the hills representing the two valleys are small, it would be possible for the cell fate to change from one cell type to another. The long road of discovery and exploration that lay behind Waddington's epigenetic landscape diagrams can recall these lines by the poet Robert Frost:

> Two roads diverged in a wood, and I—
> I took the one less traveled by,
> And that has made all the difference.

The Hidden Potential of the Epigenetic Landscape Diagrams

How were Waddington's epigenetic landscape diagrams received at the time they were first proposed? Although there were notable exceptions such as Piaget and the French mathematician Thom (1970), the concept of the epigenetic landscape did not receive an enthusiastic reception from either the geneticists or the embryologists for whom they were primarily meant. Whatever he may have thought about these developments, Waddington did not actively defend, or attempt to make converts for the epigenetic landscape diagrams he had spent so much time working toward. In fact, he was even instrumental in promoting their disuse.

When Jacob and Monod proposed the bacterial lac operon, in 1961, Waddington almost immediately introduced it into developmental biology as a useful model for embryonic induction. He devoted most of the first chapter of his *New Patterns in Genetics and Development* (Waddington, 1962) to a detailed and favorable explication of Jacob and Monod's work. Nowhere in this book is the epigenetic landscape even mentioned. Indeed, as developmental genetics supplanted Waddington's 'epigenetics,' the 'wiring diagrams,' pioneered by Jacob and Monod and extended to eukaryotes (see, for instance, Britten and Davidson, 1969), supplanted the more physical epigenetic landscape. Bacteria could be used to explain differential protein synthesis, but they were not envisioned to generate multiple cell types in a multicellular organism. The epigenetic landscape was eclipsed for nearly 50 years, until the study of stem cells in regeneration refocused attention on the alternative paths that multipotential cells could travel (see below). The potential of the epigenetic landscape diagrams can be described as follows:

1. They present a view of development as a four-dimensional dynamic process, a movement, an unfolding, a becoming.
2. They illustrate that this process of becoming involves movement along alternate and competing developmental pathways, each of which leads to a specific outcome. They impel recognition of the critical fact that the choice among pathways is not made at all points along the pathway, but at a succession of critical branch points.
3. They encourage a critical focus on the challenging task of locating, among a complex series of developmental events, those critical branch points along developmental pathways where the fate of a cell (or perhaps an individual, an institution, a society or ecosystem) is potentially in one or more directions up to the branch point, but where choice is made, development after the branch point is constrained to go in only one direction. They strongly imply that factors relating to the branch points, once located, should become serious targets of study.
4. They demand giving serious attention to identifying the nature of the underlying competence, or in Waddington's terms, the "period of competence," which is required to allow or "attract" development to proceed along the canalized pathways in the valleys below the contoured hillsides.
5. Once the decision has been made to move down one of the pathways beyond a branch point, leading toward a specific outcome, the representation of the terrain or contour lines between diverging pathways provides a useful representation of the difficulty of undoing an earlier decision, of moving over a higher contour line to get back to another pathway not chosen at the earlier branch point.
6. They thus suggest a framework for dealing with different possibilities of intervention in whatever developmental system is under discussion: how can we most effectively modify the height of the hills, providing the impetus to enter a different and perhaps more positive path than would otherwise have been accessible?

Waddington (1957, p. 167) would use these landscape models in his discussions of genetic assimilation, wherein some of the ridges between the valleys would be lowered by mutation or environmental perturbation.

The many new associated developments in contemporary biology in the last 20 years have brought about a marked resurgence of interest in the concept of the epigenetic landscape, thus fulfilling Slack's (2002, p. 894) prediction that Waddington's ideas would doubtless resurface to help meet new challenges facing theoretical biology. Indeed, the epigenetic landscape is still used to represent mechanisms for genetic assimilation (see Landecker, 2011; Paaby and Rockman, 2014), and the alteration of one cell type into another, as in cell fate specification (Bhattacharya *et al.*, 2011), in reprogramming (Wang *et al.*, 2011) or cancer (Huang *et al.*, 2009; Pujadas and Feinberg (2012). But its major comeback has been in studies of regeneration and embryonic stem cells (ES).

One of the most exciting developments in contemporary epigenetics was made by Japanese biologists Takahashi and Yamanaka (2006), who were able to demonstrate that when mouse embryonic fibroblasts were made to carry viruses carrying four specific active genes (Oct4, Sox2, Klf4, and c-Myc), the cells could be caused to return to their earlier state of ES. The newly reprogrammed 'induced pluripotential stem cells' (iPSC) could both change shape and become transformed into the three different tissue types of ectoderm, mesoderm, and endoderm. From here, the stem cells would follow Waddington's epigenetic landscape to their various cell fates. The important program for science, then, was to determine what chemical and physical factors would direct a stem cell to a particular fate. What factors would cause the pluripotent cell to travel down the pathway to make cardiac myocytes (that might cure heart disease), while what other factors could direct the pluripotent cell into the pathway of

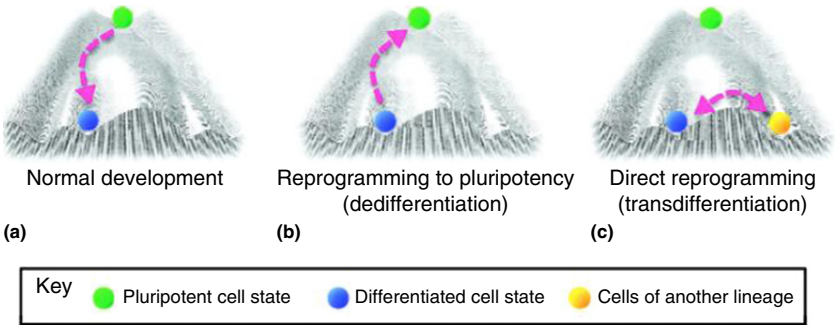


Figure 3 Changing cell fates on Waddington's epigenetic landscape (Takahashi, 2012).

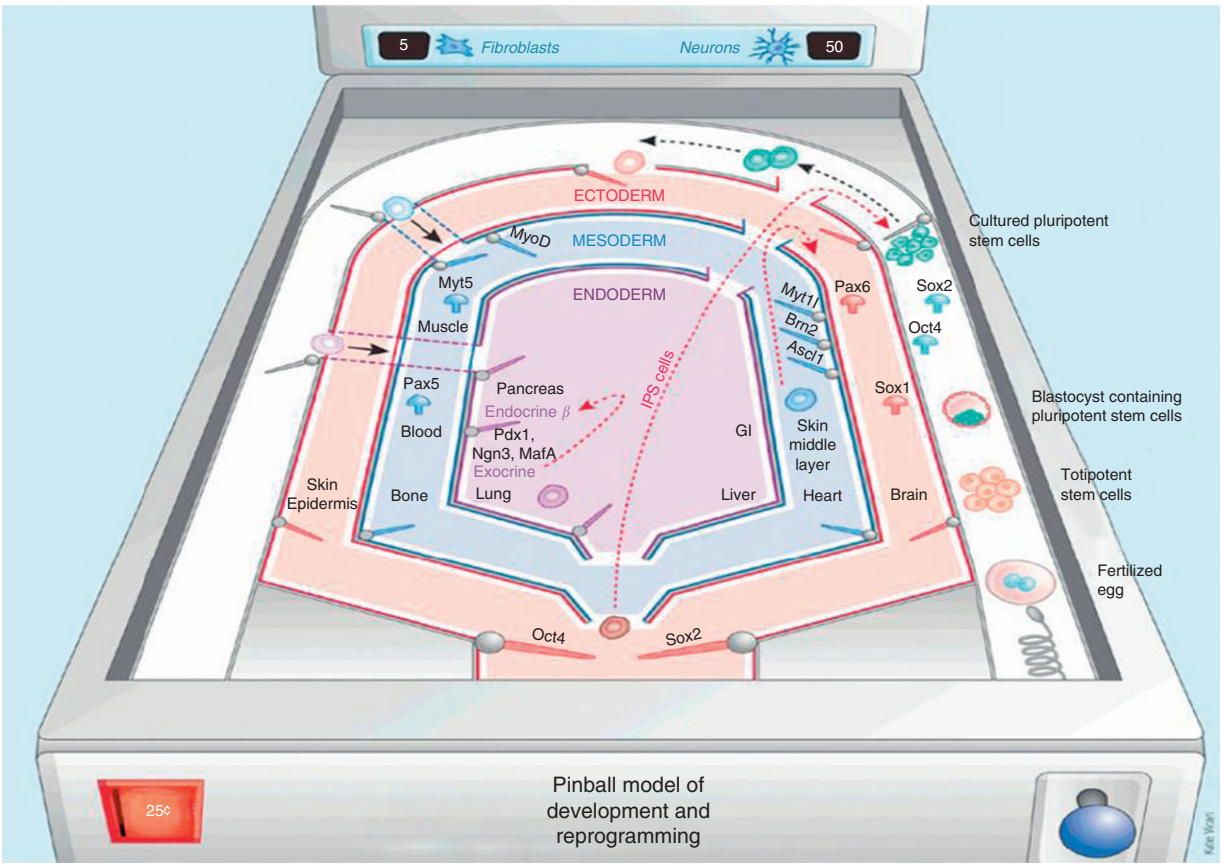


Figure 4 Pinball model of development and reprogramming. Reproduced from Sareen, D., Svendsen, C.N., 2010. Stem cell biologists sure play a mean pinball. Nature Biotechnology 28 (4), 333–335.

neuroblasts (to regenerate lost brain cells due to neurodegenerative diseases)?

More than a half-century after they were first proposed, stem cell pioneer Kazutoshi Takahashi (2012) chose to use Waddington's epigenetic landscape diagrams to represent not only his own team's path-breaking discoveries (See Figure 3(b)), but all of the newer work on cellular reprogramming (See Figure 3(c)).

With the ability to choose from all previous work in genetics and embryology or any desired image from art of science outside of biology, why did Takahashi choose to represent his team's work in this form? The answer may be that the epigenetic landscape diagrams have a dynamic quality which makes it possible to represent a complex four-dimensional process involving development over time on a two-dimensional surface. This must be recognized as an unusual achievement in and of itself. It also starkly represented the new 'explananda' for the field of regenerative medicine: What controlled each of these branch points on the epigenetic landscape?

Initially formulated by Waddington to represent possible cell fates in embryonic development over time, could the epigenetic landscape diagrams have a usefulness beyond that originally envisioned by Waddington? Many in the field are pursuing this. Altered versions of Waddington's original

landscapes using other images have been proposed, such as a saddle-node bifurcation (Ferrell, 2012), a nonhierarchical 'epigenetic disc' model (Ladewig *et al.*, 2013), or 'cusp catastrophes,' attractor surfaces in a multidimensional space (Thom, 1970). Building on Takahashi's creative use above, Waddington's landscapes have even been transformed into 'Epigenetic Pinball' games (Sareen and Svendsen, 2010) wherein the balls rolling down the hill could be shot back up the hill again by the transcription factors encoded by the genes encoded by the Takahashi virus mix (See **Figure 4**).

Waddington saw science and art as changing together. The biology of the 1960s, as well as the art of the 1960s, were entering a new era, eschewing representation for mechanism. The title of Waddington's book of art criticism, *Beyond Appearance* (Waddington, 1970), expresses this well. His quotation of Dryden to begin this book shows his views that the old world is changing and that he is impatient to get into the new one.

> All, all of a piece without.
> Thy chase had a beast in view,
> Thy wars brought nothing about,
> Thy lovers were all untrue:
> T'is well an old age is out
> And time to begin a new.

Waddington's concerns embraced biological science but also went beyond them in his multiple explorations of so many different areas of human knowledge and experience. Nevertheless, both in art and in science he always came back from the search for details to the search for pattern, and then one step further for, in the language of his friend and colleague Gregory Bateson (1979, p. 12) "the pattern that connects." Perhaps one of his major contributions, the epigenetic landscape, draws some of its strength and cogency from Waddington's persistent and lifelong inquiry into this seminal question. It is the pattern of processes that mattered. Waddington's epigenetic landscape added no new pieces of data. Rather, these diagrams provided a Gestalt, a framework into which one could place one's new data and give that data meaning. Thus, on the last page of *Principles of Embryology* (1956), he quotes T.S. Eliot:

> And to make an end is to make a beginning.
> The end is where we start from…
> We shall not cease from exploration
> And the end of all our exploring
> Will be to arrive where we started
> And know the place for the first time
> –From 'Little Gidding' by T.S. Eliot

These seem all the more apt for the new uses of epigenetic landscape diagrams in regenerative medicine.

See also: Epigenetic Inheritance. Epigenetics and Genome Evolution

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Water Transport, the Role in Plant Diversification of

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Glossary

Cavitation (xylem) The breaking of the xylem water column under tension, most often by the entry of air through pit membranes.
Embolism (xylem) The consequence of a cavitation event that leads to the formation of an air – vapor void, which fills the conduit such that it is unable to transport water.
Euphyllophytes Plants with true leaves.
Hydroids Present in some mosses, hydroids are functional analogues of xylem, but with little to no lignin in the conduit walls.
Lagerstätten A deposit of sedimentary rock that preserves fossils in exceptional detail. Examples include the Burgess Shale in Canada, which preserves early animals from the Cambrian Period, the Solnhofen Limestone in Germany, which contains exceptionally preserved fossils of the early flying dinosaur Archaeopteryx, and the Rhynie Chert from Scotland, among many others.
Mesophyll Photosynthetic parenchyma tissue that lies between the upper and lower leaf epidermal layers.
Microphylls Small plant leaves with a single, unbranched vascular trace.
Parenchyma The most common soft tissue in plants, parenchyma cells are characterized by their large size and thin walls. They may be specialized for mechanical support, storage, and photosynthesis.
Perforation plates Digested cell wall openings on one or both ends of a vessel element. Water moves through perforation plates as it flows through multiple vessel elements within a vessel. Perforation plates may be foraminate (comprising several round openings), scalariform (comprising several elongated, ladder-like openings), and simple (a single unobstructed opening).
Phloem Adjacent to xylem, phloem is metabolically active vascular tissue specialized for the transport of sugars, small proteins, and signaling molecules.
Pit membranes Partially digested and non-lignified regions in the radial walls of tracheids and vessels that allow water to move from one conduit to another. Pit membranes may be homogeneous, as in angiosperms or they may have variably thickened regions as in the torus-margo type found in conifers.
Scalariform pit membranes Homogeneous pit membranes arranged in a ladder-like manner.
Secondary xylem Originating from the vascular cambium, secondary xylem comprises at least one growth ring and imparts woodiness to stems and roots.
Specific conductivity The volume flow rate of water through a segment such as a stem, standardized for the pressure driving the flow, the length of the segment, and the functional xylem area.
Stomata Small apertures most commonly found on the underside of leaves comprising a pore surrounded by two elongated guard cells, which open and close to regulate the size of the pore. Open stomata allow CO_2 entry for photosynthesis but at the cost of water escaping from the leaf.
Thallus In ancestral taxa such as liverworts, a thallus is a flat, weakly differentiated vegetative structure only a few millimeters thick, that combines photosynthetic parenchyma on the top side with root-like cells on the bottom.
Torus-margo pit membranes Found primarily in tracheids of extant conifers, these membranes are composed of a thickened, central torus structure surrounded by a highly porous margo region, through which water moves from conduit to conduit. Should some tracheids become embolized, the torus acts as a seal, protecting the neighboring water-filled conduits from air entry.
Tracheids Single, elongated xylem cells. Conifer tracheids rarely exceed 80 μm in diameter and a few millimeters in length, whereas in ferns and some extinct taxa, tracheid size can greatly exceed these dimensions. Conifer tracheids have torus-margo pit membranes, whereas homogeneous pitting occurs in the tracheids of seed-free vascular plants.
Tracheophytes Plants with well-developed and lignified xylem tissue.
Vessels Found primarily in angiosperm xylem, vessels are composed of vessel elements (single cells with digested end-walls) stacked to form conduits that in some lineages may be up to 500 μm in diameter and several meters in length.

Introduction

The evolution of xylem tissue transformed plant life on land. Without efficient water transport, plant hydration was a function of habitat water availability, first relegating plants to simple photosynthetic thalli appressed to wet substrates, with more derived taxa reaching heights of only a few centimeters, much like modern bryophytes. The appearance of vascular tissue, and the subsequent evolution of hydraulically adaptive traits changed all this (Figure 1). Equipped with the ability to move water from roots to shoots through dedicated conduits, plants decoupled leaf hydration from their environment, growing progressively taller, and better at competing for light and effective spore dispersal. The simultaneous evolution of stomata ensured that plants were able to balance CO_2 uptake for

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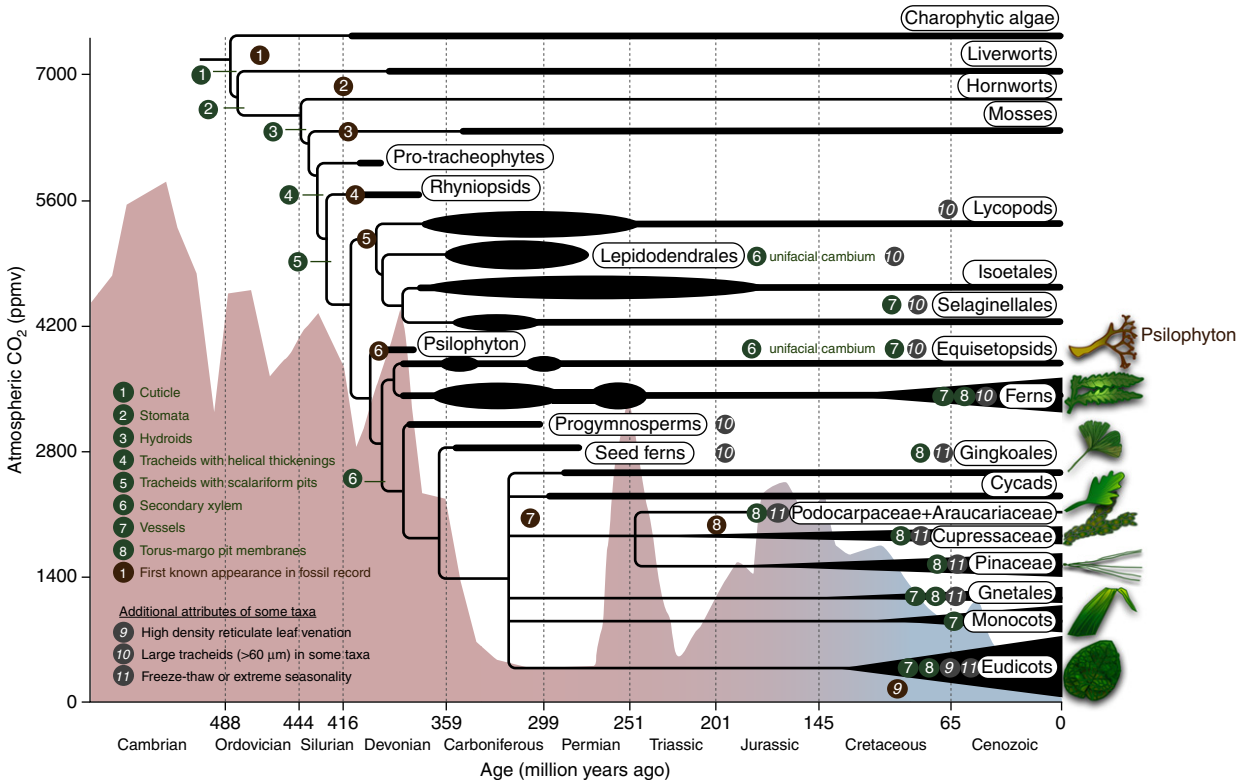

Figure 1 A simplified phylogeny of extinct and extant groups of land plants versus geologic age (modified from Sperry, 2003 and Pittermann, 2010), plotted against a backdrop of estimated atmospheric CO_2 levels (Berner, 2006). Heavy lines indicate presence in fossil record, with branch thickenings informally indicating estimated abundance and/or taxon diversity. Green labels indicate synapomorphies or independently evolved xylem traits, while gray labels mark relevant environmental or vascular attributes. Brown labels show first appearance in the fossil record; vessels (7) and torus-margo pit membranes (8) first appeared in lineages that have closest affiliation with conifers. Cartoon leaves represent the characteristic leaf type of select euphyllophytes (taxa with true leaves), of which *Psilophyton* is the ancestral euphyllophyte. Basal taxa vary with respect to the presence of microphylls. See Kenrick and Crane (1997), Sperry (2003), Feild *et al.* (2011) and Wilson (2013), and references therein for more detailed information.

photosynthesis in the leaves against the inevitable cost of water loss through the stomatal pore.

Despite the evolutionary leaps made possible by transport tissue, life on land presents considerable challenges for plants. Of these, hydraulic failure by drought-induced air embolism in the xylem is perhaps the most pressing, so plants evolved means to either cope with this problem, or avoid it altogether. This of course involves functional trade-offs, which drive fitness, shape the evolutionary direction of plant morphospace, and influence plant diversity and abundance. This review examines the general principles by which the structure and function of xylem tissue propelled plant evolution, borrowing examples from ancient, extinct flora as well as modern-day angiosperms and conifers. The xylem is but one of many drivers of plant diversity, but it is central to the physiological shifts that occur during large-scale radiation events, and it bears on past, present, and future patterns in plant biogeography.

Plant Water Transport

Xylem tissue comprises a network of dead, hollow elongated conduits such as tracheids or vessels, which may be contact with living phloem or parenchyma tissue. Extending from the roots to the leaves, the xylem is the central component of the soil – plant – atmosphere continuum (SPAC), and is responsible for moving water throughout the plant body from regions of high water availability (roots) to areas of low water availability (leaves). In a transpiring plant, water evaporates from the leaf mesophyll tissue to the atmosphere through open stomata, with drier atmospheres driving higher rates of water loss. As water evaporates from cell wall pores, the menisci within those pores curve, and their high surface tension places hydrogen bonds in an energetically unfavorable state, as compared to those that are flat during full hydration. Effective water transport ensures that the curvature of the menisci is reduced by continual replacement of lost water, which ultimately arrives through cell wall contact with xylem conduits. Hydrogen bonds sustain this dynamic wicking process by cohesion, allowing maintenance of hydraulic continuity over long distances. However, water movement along the SPAC generates tension (negative pressure) because opposite to the transpiring leaves, water is bound to soil particles and is subject to frictional forces as it moves into the root and through the xylem. Plant water transport has thus come to be known as the Cohesion – Tension theory (Tyree and Zimmermann, 2002; Pickard, 1981). It is an energetically free process that is powered primarily by the sun, with efficiency

being mostly a function of conduit diameter raised to the fourth power, a basic physical law of fluid flow through tubes. To optimize water transport, selection favors conduits that are much longer than they are wide (Sperry *et al.*, 2006). Reliable xylem function is critical to canopy performance: without sufficient replacement of lost water, stomata close, the leaves lose turgor, wilt and possibly die.

If the demand for water by the leaves exceeds that which can be supplied by the roots, such as during a drought, the negative pressure of the xylem sap can exceed a species' specific threshold, which allows air to enter water-filled conduits and can cause the water column to rupture. This phenomenon, known as cavitation, occurs when surrounding air is pulled into the conduit through the largest pores in the interconduit pit membranes. The resulting air embolism blocks the flow of water and increases the friction of the xylem network, impeding flow (Tyree and Zimmermann, 2002). Experimental and comparative work suggests that conduit dimensions, which influence the extent of pitting per conduit, along with adjustments to pit membrane porosity, and conduit arrangement, play a role in the entry and spread of air such that species with conduits that are smaller, spatially separated from one another or have less permeable pit membranes, are most resistant to cavitation (Brodersen *et al.*, 2012, 2014; Christman *et al.*, 2009; Lens *et al.*, 2011; Wheeler *et al.*, 2005). Arising from this is the axiom that embolism resistance comes at the cost of hydraulic efficiency because resistant xylem has higher frictional resistance. Such trade-offs are variable, but they are apparent in north-temperate plants at the level of the pit membrane, the conduit, and the xylem tissue (Jacobsen *et al.*, 2007; Pittermann *et al.*, 2006a,b, 2010, 2012), and may be related to the overall size, architecture, and phenology of woody plants (Domec *et al.*, 2008; Hacke *et al.*, 2015; Pittermann *et al.*, 2012).

The Early Evolution of Plant Water Transport

The evolution of water transport tissue and hydraulic capability in early land plants facilitated a diverse suite of anatomical and morphological features at the beginning of land colonization. The best-known early vascular plants are found in association with a number of nonvascular plants in an Early Devonian Period lagerstätten from Scotland, known as the Rhynie Chert. This locality preserved a diverse ~405 million year old hot spring ecosystem, and includes nonvascular plants like the protracheophyte *Aglaophyton major* (**Figure 2(a)**), once thought to be a vascular plant for many decades because of its close resemblance to another Rhynie Chert vascular plant, *Rhynia major*. Additional rhyniophytes include partially vascularized stem group bryophytes (*Horneophyton*), and a completely vascularized stem group lycophyte, *Asteroxylon mackiei* (**Figures 1** and **2(b)**; Taylor *et al.*, 2009). Of these, only the xylem-bearing *A. mackiei* could support

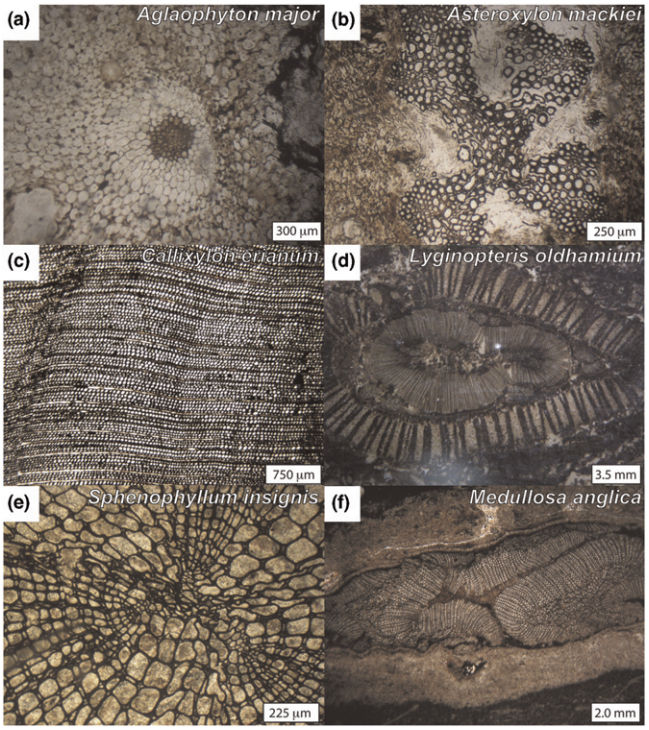

Figure 2 Cross-sections of fossil plant xylem. (a) Cross-section of the nonvascular plant *Aglaophyton major* from the Rhynie Chert. Note the dark central strand: these cells contain proto-tracheids. (b) Cross-section of the stem group lycophyte *Asteroxylon mackiei* from the Rhynie Chert with xylem cells arranged in the shape of an X. (c) Cross-section of secondary xylem (wood) from *Archaeopteris*, assigned the specific name *Callixylon erianum*. (d) A stem of the stem group seed plant *Lyginopteris oldhamium* showing xylem and its diagnostic cortical tissue. (e) Transverse view of xylem from the extinct sphenopsid *Sphenophyllum insignis*, showing its large tracheids. (f) Transverse view of the stem group seed plant *Medullosa anglica*, notable for its large secondary xylem tracheids.

microphylls. Although early land plants had much in common, including relatively simple reproductive systems and photosynthetic stems, their differential water transport capability, among other adaptations, facilitated the partitioning of the Rhynie Chert ecosystem into a series of distinct ecological niches, in which *A. mackiei* may have occupied a drier, upslope habitat, and regrown aerial stems from a subterranean rhizome in favorable conditions.

Rhynie Chert plants possessed xylem with a number of curious anatomical features, including conduits with complex ornamentation, isolated conduits, or alternatively, conduits connected by large holes. A likely explanation for the diversity of xylem cell features found in these Early and Middle Devonian taxa is that some plants contained xylem adapted for drought resistance and diversified into infrequently wet ecosystems, whereas other plants evolved xylem adapted for efficient water transport and specialized in disposable aboveground structures in everwet environments.

The major constituents of the Middle Devonian flora are plants with small stems that produced secondary (woody) xylem such as *Psilophyton* (**Figure 1**); given the limits of current data, it appears that wood first evolved within small-stemmed plants (<1 cm diameter) with low cavitation resistance (Gerrienne *et al.*, 2011). The ability to produce woody xylem has evolved at least four times, and appears to have enabled and driven a series of biomechanical and functional innovations that led to an explosion of ecological and taxonomic diversity of seed-free vascular plants, as well as seed ferns during the Devonian, Carboniferous, and Permian Periods (**Figure 1**). By the end of the Devonian Period, forests were dominated by large, spore-bearing *Archaeopteris* progymnosperms, which contained xylem that functionally and morphologically resembles the wood found within living conifers (e.g., *Callixylon erianum*, **Figure 2(c)**; Meyer-Berthaud *et al.*, 2000). Early seed plants, in particular, evolved a series of experiments in xylem structure and architecture that facilitated ecological and evolutionary expansion into new niches (Wilson and Knoll, 2010). A Carboniferous seed fern from Western Europe and North America, *Lyginopteris oldhamium*, evolved one of the earliest scrambling and climbing morphologies by coupling highly conductive woody xylem with a distinctive striped cortex that acted as a mechanical collar (**Figure 2(d)**), allowing its stems to bend and flex under mechanical stress (Masselter *et al.*, 2007). Two other plants with diverse biomechanical strategies, *Sphenophyllum* (Equisetopsida) and the seed plant *Medullosa* (**Figure 2(f)**) independently evolved quasi-vessels, individual cells more than 200 microns in diameter and more than 2.0 cm long, which functionally served as vessels in their stems. These giant conduits supported large leaf areas per individual stem in *Sphenophyllum* and *Medullosa* and expanded the niches that both spore-bearing and early seed plants, respectively, could occupy (Wilson, 2013).

Many of these anatomical experiments in high-conductivity xylem tied Paleozoic Era plants to nearby water sources. During the assembly of the supercontinent Pangaea in the Permian, the broad, tropical, everwet swamps that had been the loci of plant diversification were uplifted and dried, causing the extinction of many of the unusual Paleozoic plant taxa and their xylem experiments. The radiations of conifers, most ferns, and angiosperms later in the Mesozoic Era opened new functional niches that had previously been occupied by some of these physiological and biomechanical novelties, and facilitated a diversification of new anatomical forms, including highly productive leaves.

The Evolution of Xylem in Leaves

Research into the evolution in vascular water transport in plants has traditionally focused on improvements in root-to-shoot flow derived from the production of larger and longer conduits such as those found in wood, but the most significant gains may have occurred in leaves. The earliest angiosperms occupied understory habitats (Feild *et al.*, 2004), lacked vessels, had relatively low leaf vein density, and were neither hydraulically efficient nor cavitation resistant (Feild and Arens, 2007; Hacke *et al.*, 2007). Whilst large tracheids occurred in many extinct seed-free vascular plants, the combination of long, multi-cellular conduits coupled with a morphologically adaptive body plan eventually transformed the physiological capacity of angiosperms, with profound implications for downstream leaf hydraulic function and gas-exchange. Two key factors make leaf vein networks highly informative about the broader physiology of an individual or plant species:

1. The leaf is a major resistor in the hydraulic continuum, comprising over >30% of whole plant resistance (Sack and Holbrook, 2006), so adaptive improvements in leaf water transport will likely increase photosynthesis.
2. The efficiency of leaf water transport is highly dependent upon the spacing of the leaf venation (the vein density), a feature that is easily measured and frequently well preserved during fossilization.

Leaf vein density in living plants is a reliable indicator of photosynthetic performance, with higher densities of veins found to deliver water more efficiently to mesophyll tissues (Sack and Frole, 2006). More effective delivery of water by veins to sites of evaporation in leaves allows greater densities of stomata to be produced on the leaf surface (Fiorin *et al.*, 2015), and higher photosynthetic rates to be achieved (Brodribb *et al.*, 2007). Leaf venation thus provides a sensitive proxy for both transpiration and CO_2 uptake in leaves that can be used to reconstruct the performance of fossil species.

Following the emergence of single-veined, microphyllous vascular plants onto land, the early evolution of leaf venation in euphyllophytes was associated with changes in architecture to distribute water across the 2D surface of the leaf blade. Branched networks, such as in ferns, evolved soon after colonization of the land by vascular plants, followed about 100 million years later by multiple origins of internally reticulate vein systems (**Figures 1** and **3**; Boyce, 2005). These looped networks are believed to better accommodate damage (Katifori *et al.*, 2010) than branched vein architectures, and also yield a more optimal distribution of water under variable evaporative loads on the lamina (Corson, 2010). During this period of architectural evolution in leaf venation, the density of venation in the blade (measured as the length of veins per area of leaf tissue: mm/mm^2) of fossil species appears to have remained in a low and stable range, a feature that still characterizes the living relatives

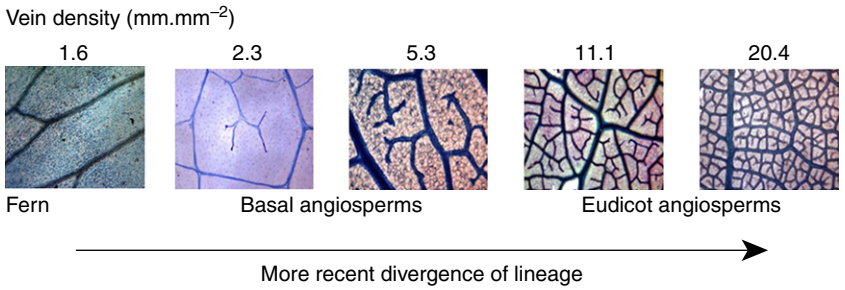

Figure 3 A clear trajectory in the density of veins in the leaves of vascular plants can be seen over time. Early-diverging lineages such as ferns (far left) all have low densities typically <4 mm mm^{-2}. Maximum vein densities climbed rapidly as angiosperms radiated, but the early clades remained at low density (such as *Amborella trichopoda*, second panel). Modern Eudicots (right) have maximum vein densities >10 mm mm^{-2} giving them the ability to photosynthesize and transpire rapidly.

of early-diverging lineages such as lycophyte, fern, and gymnosperm species (Boyce *et al.*, 2009).

In one of the most remarkable functional shifts in plant evolution, an abrupt end to the extended period of stasis in leaf vein density appears to coincide with the diversification of angiosperms, whereby vein density in this group rose sharply (Boyce *et al.*, 2009; Brodribb and Feild, 2010; **Figure 3**). Although this jump in vein density is unique to angiosperms, it is not synonymous with angiospermy, because vein density did not rise immediately after the appearance of angiosperms, but rather appears to have remained in the same range as ferns and lycophytes (2–4 mm/mm^2) throughout the earliest branches of angiosperm evolution (up to $\sim$100 million years) (Brodribb and Feild, 2010). This delayed evolution of high vein density in angiosperms is evident from fossil assemblages (Feild *et al.*, 2011) and when examining living representatives of early angiosperm branches, with all living relatives from the first five extant branches of the angiosperm phylogeny found to possess fern-like vein densities and corresponding low rates of photosynthesis (Feild *et al.*, 2011). The impact of high vein density evolution in angiosperms must have been dramatic, with suggestions that the accompanying rise in species productivity may explain the rise to dominance of angiosperms during the late Cretaceous. Models of rainfall patterns also imply that the increase in evaporation made possible by high vein density leaves may have led to the expansion of lowland rainforest in the equatorial zone (Boyce *et al.*, 2010).

The trigger for high vein density evolution in angiosperms is debatable. Extrinsic forcing by falling CO_2 concentration has been proposed, but more recent analysis suggested that intrinsic factors unique to angiosperm development are also required (Zwieniecki and Boyce, 2014). The most plausible explanation is that increased efficiency of primary xylem allowed leaf veins to become much narrower and more densely packed in the leaves of the more derived angiosperm clades (Feild and Brodribb, 2013). There is a clear trend toward increasing porosity of xylem perforation plates in the angiosperm phylogeny, with the most porous (simple) perforation plates found only in the relatively derived Magnoliid, Monocot, and Eudicot clades. This adaptation, providing greater efficiency in leaf vein water transport, would have preadapted angiosperms to benefit from the declining atmospheric CO_2 concentrations that occurred during the Cenozoic Era (Berner, 2006; Zachos *et al.*, 2008).

The radiation of broad-leaved angiosperms had a profound effect on the diversity of low-latitude forests, with the extinction of some gymnosperm clades and the contraction of other groups to higher latitudes (Crane and Lidgard, 1989; Millar, 1998). One family of conifers that has been successful in the shadow of angiosperms is the Podocarpaceae, which appears to have radiated in the tropics during the Cenozoic period producing multiple origins of shoot flattening. Like the Polypod ferns (Schuettpelz and Pryer, 2009), the Podocarpaceae appear to have radiated in the sub-canopy of angiosperm forests where competition for light is paramount. As a result, many podocarp species exhibit leaf flattening associated with reduced self-shading (Brodribb and Hill, 1997) and lower leaf mass per unit area. This is an unusual evolutionary trajectory for a conifer group, particularly considering that most conifers are characterized by needle leaves. At least nine independent origins of leaf flattening can be seen in Podocarpaceae (Biffin *et al.*, 2012). The most successful of these is the genus *Podocarpus*, the most speciose of all conifer genera after *Pinus*, with species distributed mostly in wet tropical regions. *Podocarpus* leaves can reach 300 mm in length and 50 mm in diameter despite the fact that they are supplied by water from a single midrib – a feat achieved by tracheid-like tubes that distribute water radially from the xylem into the mesophyll (Brodribb and Holbrook, 2005). Taken together, the post-Cretaceous dominance of angiosperms created physiological challenges for competing plants such as conifers, but as the next section will show, new niches and Cenozoic climate change also created opportunities for the radiation and diversification of several important clades.

Cenozoic Climate Change, Hydraulic Efficiency and the Evolution of Cavitation Resistance

The deep-time evolution of xylem shows trends toward increased axial and leaf hydraulic capacity, but there is reason to suspect that drops in atmospheric CO_2 levels ($[CO_{2atm}]$) coupled with both cooler and drier climates in the Cenozoic challenged xylem function and plant physiology as a whole (**Figure 1**; Berner, 2006; Pittermann, 2010; Ward *et al.*, 2005; Zachos *et al.*, 2008). Recent experiments have shown that plants grown at sub-ambient $[CO_{2atm}]$ levels that are representative of the Pleistocene have higher xylem conductivities than plants grown at ambient and elevated $[CO_{2atm}]$ (Rico *et al.*, 2013;

Medeiros and Ward, 2013). This shift toward increased water transport was mirrored in higher leaf stomatal densities, and by extension, higher stomatal conductance, both interpreted to compensate for reduced mesophyll CO_2 levels (Rico *et al.*, 2013 and references therein). Interestingly, stems of *Helianthus annuus* were more vulnerable to cavitation when grown at low [CO_{2atm}] due to inherently weaker xylem; it may be that wider vessels with thinner walls were predisposed to increased occurrence of air seeding (Rico *et al.*, 2013, but see Medeiros and Ward, 2013). How large-scale shifts in xylem traits bear out in the Cenozoic fossil record has not been studied.

Carbon stress may have altered xylem physiology, but increasing aridity in some parts of the globe almost certainly selected for improved water-use efficiency, as seen in the low-latitude Oligocene expansion of C4 plants and succulent lineages (Arakaki *et al.*, 2011; Edwards *et al.*, 2010). However, along with Cenozoic climate change, the expansion of tropical angiosperm forests may have also left its mark on xylem in lineages such as ferns. Competition for light pushed terrestrial ferns into the canopy (Schuettpelz and Pryer, 2009), but vascular adaptations to the increased evapo-transpirative demands this habitat entails, including cavitation resistance, greater water-use efficiency, and desiccation tolerance must have kept them there (Watkins *et al.*, 2010; Pittermann *et al.*, 2013; Schuettpelz and Pryer, 2009; gametophytes are discussed in Watkins *et al.*, 2007 and Watkins and Cardelús, 2012). Extant ferns have only primary xylem, so adaptations to water deficit include spatially separated vascular bundles to reduce the spread of embolism, along with smaller tracheids and less permeable pit membranes (Brodersen *et al.*, 2012, 2014; Pittermann *et al.*, 2015). Passive, leaf turgor-mediated stomatal closure supports a rapid response to water stress that is consistent with the humid habitats to which most ferns are constrained (McAdam and Brodribb, 2013). Further studies on the directionality and tempo of xylem evolution in ferns are currently under way (Pittermann *et al.*, 2015).

In climates with high water availability, angiosperms, ferns, and a small number of conifers benefit from the hydraulic gains that large conduits afford, but in habitats where drought and freezing are common, wide conduits can pose some problems. Temperate angiosperms such as deciduous oaks have large vessels, but their growing season is abruptly abbreviated following the first hard freeze. The problem is that freeze – thaw cycles can impede water transport if coalesced gas bubbles that are pushed out of the ice front in the conduit sap expand during a thaw, and nucleate cavitation (Sperry and Robson, 2001). Cavitation risk scales largely with conduit width: the wider the conduit, the larger the bubble, and the greater the probability that the bubble will expand under tension (Pittermann and Sperry, 2003, 2006). Safe, narrow tracheids are an ancestral character, so the conifers' conserved xylem structure (and that of the vesselless angiosperms, Feild *et al.*, 2002) may have preadapted them to the challenging climates of the Cenozoic; this idea is consistent with the high-latitude Mesozoic origins of *Pinus*, and pine expansion during the Oligocene (Millar, 1998). Similar safety versus efficiency trade-offs played out within the Cupressaceae conifers, in which narrower conduits and smaller pit membrane apertures supported the radiation of *Juniperus*, *Cupressus*, and *Callitris* lineages into drier, cooler habitats, relegating ancestral taxa such as *Cryptomeria*, *Taxodium*, *Sequoia*, and *Metasequoia* to refugia with higher water availability (Pittermann *et al.*, 2010, 2012). In the north-temperate Cupressaceae, drought resistance came at a cost however: narrower conduits with thicker walls lead to denser wood and thus a substantial carbon investment in xylem tissue (Hacke *et al.*, 2001; Pittermann *et al.*, 2012; see also Jacobsen *et al.*, 2005 who find similar trends in angiosperm fibers). Parallel costs included reduced hydraulic efficiency, which compromised photosynthetic capacity and growth (Pittermann *et al.*, 2012). Unable to produce very wide tracheids, conifers have traditionally been viewed as physiologically inferior to angiosperms, but the evolution of low-resistance torus-margo pit membranes has compensated for their narrow tracheids (Pittermann *et al.*, 2005; Brodribb *et al.*, 2012), and allowed them to remain competitive even in the suboptimal habitats of Cenozoic climates.

Conclusion

A variety of xylem adaptations at scales ranging from pit membranes to conduits, and from stems to leaves have facilitated the diversification and radiation of numerous plant groups over evolutionary time. As plants competed for light, increased stem and leaf efficiency supported the transformation of the planet's surface as plants came to occupy nearly every continent, adapting to climate change and competition. This review has focused exclusively on how reliable and efficient water transport shaped the evolution of plants, omitting other critical and equally important functions such as mechanical support (Rowe and Speck, 2005; Couvreur *et al.*, 2014), and general plant architecture, both of which contribute to clade diversification and resistance to drought stress (Schenk *et al.*, 2008). In these and other respects, natural selection demands compromise because the appearance of one physiologically adaptive trait may arrive at the cost of another. It is this complex interplay of anatomy, physiology, biochemistry, and biomechanics that supports plant diversification, and places the structure and function of xylem right at the center of this history.

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See also: Plant–Pollinator Interactions and Flower Diversification. Seedless Land Plants, Evolution and Diversification of

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Note
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Cross-reference terms in italics are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space).

Location references refer to the volume number, in bold, followed by the page number.

Major discussion of a subject is indicated by bold page numbers; page numbers suffixed by '*F*' and '*T*' refer to Figures and Tables, respectively.

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